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Use of Azolla as Biofertilizer for Cultivation of BR 26 Rice in Aus Season

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Abstract: An experiment was conducted in silt loam soil (pH 6.52) to compare the effect of the recommended dose of N (80 kg N ha^{-1}) with that of incorporated two layers of Azolla plus 40 kg N ha^{-1} or Azolla left floating (no incorporation) plus 40 kg N ha^{-1} on the yield and NPS uptake by BR 26 rice. The highest grain yield of 3.95 t ha^{-1} with the highest total (grain + straw) uptake of 75.4 kg N ha^{-1}, 32.7 kg P ha^{-1} and 8.5 kg S ha^{-1} were recorded for incorporations of two layers of Azolla (grown from 0.2 kg m^{-2} inoculum) at 25 and 40 DAT plus application of 40 kg N ha^{-1} at 15 and 60 DAT- all of which remained statistically at par with their counterparts of another similar treatment but using 0.1 kg m^{-2} inoculum instead. All these data differed significantly with those that were recorded against the recommended dose of N as urea. Azolla left growing after inoculation was found significantly beneficial for rice growth. Azolla contributed to the increased uptake of N, P and S. Incorporation of two layers of Azolla biomass showed slight increases in soil organic matter status (from 1.85% initial to 1.88% after crop harvest).

Key words: Azolla, rice, NPS uptake, organic matter

Introduction

The application of nitrogenous fertilizers has become an essential practice to increase per unit yields of any crop since most soils are deficient in N. But the continuous use of chemical fertilizers inflicted deleterious effects on soil organic matter reserves thereby creating further deficiency in N. That is why global attention has been drawn to find out the alternatives and supplements to chemical nitrogenous fertilizers. The addition of biofertilizers and organic manures could be a priority to address this problem. The use of Azolla as biofertilizer for irrigated rice cultivation has already been found successful in many countries of the world (Lumpkin and Plucknett, 1982; Mian, 1993). Arvadia *et al*. (1989) reported that Azolla grown dual with rice could fulfil the entire requirement of N by rice. Few other such reports are also available (Singh and Singh, 1995; Singh, 1998a; 1998b). The benefits of enriching soil organic matter status by incorporated Azolla biomass have also been reported elsewhere (Singh and Singh, 1987b; Hoque, 1998; Mamun, 2000). The present investigation was planned to test the effects of simultaneously growing Azolla (incorporated and not incorporated) plus the application of 50% of the recommended dose of urea compared to applying 100% of the recommended dose of urea, on the yield of BR 26 rice in aus season.

Materials and Methods

An experiment was carried out in the Field Laboratory of the Department of Soil Science of Bangladesh Agricultural University, Mymensingh, during the 15th March to 12th July of 1995 with six different treatments comprising of Azolla and urea using BR 26 rice as the test crop. The soil was silt loam having pH 6.52, organic matter 1.07%, total nitrogen 0.093%, exchangeable phosphorus 12.0 ppm and available sulphur 11.0 ppm. The content of total nitrogen, available phosphorus and sulphur of the supplied Azolla were 0.21, 0.09 and 0.034%, respectively. The treatments were, T_0= control, T_1= Recommended dose of N (80 kg N ha^{-1}), T_2= 0.1 kg m^{-2} Azolla inoculum (Azolla incorporation at 25 and 40 DAT) + 40 kg N ha^{-1}, T_3= 0.1 kg m^{-2} Azolla inoculum left growing + 40 kg N ha^{-1}, T_4= 0.2 kg m^{-2} Azolla inoculum (Azolla incorporation at 25 and 40 DAT) + 40 kg N ha^{-1} and T_5= 0.2 kg m^{-2} Azolla inoculum left growing + 40 kg N ha^{-1}. The trial was laid out in randomized block design using three replications having unit plot size of 4.0 x 2.5m^2, 20 cm row to row distance and 15 cm plant to plant distance. Basal application of P_2O_5, K_2O and S were made @ 60, 50 and 20 kg ha^{-1} to all treatments including the control. The sources of fertilizers used were urea, muriate of potash and gypsum. Thirty days old seedlings of BR 26 were transplanted in the experimental plots. Each plot was filled with water up to 4-6 cm depth and Azolla was inoculated at 7 DAT. Intercultural operations were done whenever necessary. After the formation of a mat at 25 DAT first incorporation was done and the second layer was grown thereafter to incorporate at 40 DAT. Ten plants were randomly collected from each plot before harvesting to record the data on yield contributing parameters. Grain and straw yield were recorded plot wise. The plant samples were oven dried at 65°C for 48 h and ground. Plant samples were chemically analyzed to determine N, P and S contents following standard analytical method (Page *et al*., 1982). Initial and post harvest soil samples were also analyzed for soil organic matter content (Walkly and Black, 1934). Statistical analysis of the results were done in an MSTAT and the comparison of the treatment effects were computed as per Duncan's multiple range test (Gomez and Gomez, 1984).

Results

Yield contributing parameters: The plant height of BR 26 rice was significantly influenced by Azolla and N-fertilizer (Table 1). Which ranged from 59.0 to 68.4 cm. The highest height was obtained with T_4 treatment (incorporation of 2 layers of Azolla grown from 0.2 kg m^{-2} inoculum + N_{40} ha^{-1}) which was statistically identical to T_2 and significantly different from that of other treatments. The lowest plant height was found in the control treatment. It was observed that Azolla in combination with N-fertilizer showed significant positive effect on this parameter. The highest total tillers/hill (18.2) was obtained with the treatment T_4 (incorporation of 2 layers of Azolla grown from 0.2 kg m^{-2} inoculum + N_{40} ha^{-1}) which was statistically similar to T_1, T_2 and T_3 treatments but statistically different from T_0 and T_5 treatments (Table 1). The lowest total tillers/hill (14.0) was recorded in the control. Application of Azolla alone with N-fertilizer

Table 1: Effect of Azolla and urea application on different growth parameters of BR 26 rice in aus season

Treatment	Plant height (cm)	Total tillers/ hill (No.)	Effective tillers/ Hill (No.)	Filled grain panicle (No.)	1000 grain wt. (g)
T0	59.0d	14.0c	10.2c	55.4	21.2
T1	65.0b	16.7ab	12.3b	62.0b	21.9
T2	68.2a	18.1a	14.1a	69.2a	22.8
T3	63.0c	17.0ab	12.0b	61.26b	21.6
T4	68.4a	18.2a	14.4a	70.0a	22.1
T5	64.0bc	15.0bc	12.5b	62.3b	21.5
SE(±)	0.24	0.53	0.30	0.44	0.71

The figures in a column having common letter(s) do not differ significantly at P = 0.01 as per DMRT

Table 2: Effect of Azolla and urea application on the grain and straw yields of BR 26 rice in aus season

Treatment	Grain yield		Straw yield	
	t ha^{-1}	% increase over control	t ha^{-1}	% increase over control
T0	2.64e	-	2.29c	-
T1	3.53bc	34	4.00b	37
T2	3.83ab	45	4.49ab	54
T3	2.99d	13	3.87b	33
T4	3.95a	49	4.80a	65
T5	3.35c	27	3.93b	35
SE (±)	0.075		0.169	

The figures in a column having common letter(s) do not differ significantly at P = 0.01 as per DMKT

Table 3: Effect of Azolla and urea application on N, P and S uptake in grain and straw of BR 26 rice in aus season

Treatments	N-uptake		P-uptake		S-uptake	
	Grain	Straw	Grain	Straw	Grain	Straw
T0	24.3c	15.8c	10.8d	6.1b	2.4d	2.0b
T1	37.5ab	24.0b	13.8c	10.8a	3.3c	3.1ab
T2	44.2a	28.3ab	18.8a	11.7a	4.4ab	3.7a
T3	32.5b	24.4b	15.1bc	11.0a	3.7bc	3.6a
T4	44.6a	30.7a	19.8a	13.0a	4.6a	3.9a
T5	36.5ab	23.7b	16.26a	11.4a	4.2ab	3.6a
SE(±)	1.83	1.03	0.33	0.55	0.22	0.38

The figures in a column having common letter(s) do not differ significantly at P = 0.01 as per DMKT

Table 4: Effect of Azolla and urea application on organic matter status initial and after crop harvest of BR 26 rice in aus season

Treatments	Organic matter content		
	initial (%) Increase/decrease over control(%)	Post harvest (%)	Increase/decrease over initial (%)
T0		1.84	-0.54 -
T1		1.83	-1.08 -0.54
T2	1.85	1.88	1.62 2.17
T3		1.86	0.54 1.07
T4		1.88	1.62 2.17
T5		1.87	1.08 1.63

Table 5: Correlation matrix (n-4)

Characters	Grain yield	Straw yield	Plant height	Tiller /hill	Effective tiller/hill	Filled grain	1000 grain grain wt.
Grain yield	1.000						
Straw yield	0.920**	1.000					
Plant height	0.982**	0.959**	1.000				
Tiller/hill	0.810*	0.882*	0.889	1.000			
Effective tiller/hill	0.959**	0.957**	0.987**	0.871*	1.000		
Filled grain	0.949**	0.935**	0.982**	0.886*	0.996**	1.000	
1000-grain wt.	0.860*	0.813*	0.908*	0.869*	0.872*	0.887*	1.000

* = P<0.05; ** = P<0.01

significantly influenced the number of effective tillers/hill. The effective tillers/hill ranged from 10.2 to 14.4. The treatment T_4 produced the highest number of effective tillers/hill followed by the T_2 treatment. The lowest effective tillers/hill was recorded in the control. Treatments T_1, T_3 and T_5 were found statistically similar. All the treatments apparently produced higher number of effective tillers/hill compared to the control.
The filled grain/panicle ranged from 55.4 to 70.0 with the highest recorded by T_4, which was statistically similar to T_2 treatment. Treatments T_1, T_3 and T_5 were statistically similar. All the treatments produced higher filled grain/panicle compared to the control.
1000 grain weight showed statistically insignificant of different treatments (Table 1). The 1000 grain weight varied from 21.2 to 22.8 g. The highest 1000 grain weight was recorded in T2 treatment which was not significant different with all other treatments. The lowest 1000 grain weight (21.2 g) was obtained in control treatment.

Grain and straw yield: Grain yield of BR 26 rice was influenced significantly by different treatments of Azolla and urea (Table 2). The grain yield ranged from 2.64 to 3.95 t ha^{-1} over the treatments. The highest grain yield was observed in T_4 (incorporation of 2 layers of Azolla grown from 0.2 kg m^{-2} inoculum + N_{40} ha^{-1}) and it was statistically similar to T_2 treatment. All the treatments gave significantly higher yield over the control. The grain yield of T_2, however, did not differ significantly with that of the recommended dose of fertilizers i.e., T_1 (3.53 t ha^{-1}). The yield of T_5 (3.35 t ha^{-1}) was significantly higher over the yield of T_3 (2.99 t ha^{-1}).
The percent increase in grain yield due to different treatments over control ranged from 13 to 49. Incorporation of 2 layers of Azolla (0.2 kg m^{-2} inoculum) + N_{40} ha^{-1} was found as the superior treatment in the present study. The application of Azolla and urea also markedly influenced the straw yield of BR 26 rice. The straw yield ranged from 2.29 to 4.80 t ha^{-1}. The treatment T_4 produced the highest straw yield (4.8 t ha^{-1}) which was followed by T_2 (incorporation of 2 layers of Azolla grown from 0.1 kg m^{-2} inoculum + N_{40} ha^{-1}).

Nitrogen, phosphorus and sulphur uptake: The nitrogen uptake of grain ranged from 24.3 to 44.6 kg ha^{-1} with the highest uptake recorded by T_4 treatment followed by T_3 treatment. This T_4 treatment was statistically similar to T_1 and T_5. All the treatments registered higher N uptake in comparison to the control. The N uptake in straw was also statistically significant. The highest nitrogen uptake was recorded by T_4 treatment which was statistically similar to T_2 treatment. The lowest N uptake was recorded in the control. Treatments T_1, T_3 and T_5 were statistically identical (Table 3). The treatment T_4 produced the highest P uptake of grain which was followed T_2 (18.8 kg ha^{-1}) treatment. Treatment T_4 recorded the highest P uptake in straw (13.0 kg ha^{-1}) followed by T_2, T_5, T_3 and T_1 having the P uptake of 11.7, 11.4, 11.0 and 10.8 kg ha^{-1}, respectively.
The S uptake in BR 26 rice was significantly influenced by Azolla and urea application (Table 3). It ranged from 2.4 to 4.6 kg ha^{-1}, the highest value was obtained with T_4 which was statistically different from T_3, T_1 and T_0 treatments. The highest S uptake (3.9 kg ha^{-1}) in straw was also recorded by the treatment T_4, which was significantly superior to the control only.
Organic matter status: There was slight increase in organic matter contents of the soils where Azolla biomass was incorporated (Table 4). The organic matter content was found to be 1.88% due to incorporation of two layers of Azolla and 1.86-1.87% when Azolla was not incorporated (left growing). The organic matter status decreased slightly in urea-treated soils and in the control soil.
To examine the interrelationship among the yield and yield components, correlation statistics was done (Table 5). The values of correlation coefficient indicated that grain yield was dependent on all parameters such as plant height ($r = 0.982^{**}$; $P = 0.01$), tiller per hill ($r = 0.810^{*}$; $P = 0.05$), effective tiller per hill ($r = 0.959^{**}$; $P = 0.01$), filled grain ($r = 0.949^{**}$; $P = 0.01$) and 1000 seed weight ($r = 0.860^{*}$; $P = 0.05$). Grain yield was also significantly correlated with straw yield ($r = 0.920^{**}$; $P = 0.01$).

Discussion

Results indicated that the highest amount of grain yield of 3.95 t ha^{-1} was recorded for the treatment T_4 I. e., the incorporation of two layers of Azolla (25 and 40 DAT) grown from 0.2 kg m^{-2} inoculum and 40 kg N ha^{-1} in 2-equal splits (15 and 60 DAT), receiving the highest amounts of total (grain + straw) N (75.4 kg ha^{-1}), P (32.7 kg ha^{-1}) and S (8.5 kg ha^{-1}), respectively. These data were in comparison to 3.53 t ha^{-1} grain yield receiving, respectively, 61.5, 24.6 and 6.4 kg total N, P and S due to the application of the recommended dose of fertilizers i.e. T_1. Thus, the performance of two incorporations of Azolla plus 40 kg N ha^{-1} (T_4) was found significantly better over the recommended dose of fertilizers (T_1). Incorporation of two layers of Azolla grown from 0.1 kg m^{-2} inoculum plus 40 kg N ha^{-1} (T_2) produced the second highest amount of grain (3.83 t ha^{-1}). However, the effect of T_2 and T_4 in respect of grain yield, total N, P, and S uptake was statistically at par. The overall performance of T_3 (Azolla layer from 0.1 kg m^{-2} inoculum left growing + 40 kg N ha^{-1}) and T_1 (the recommended dose of fertilizers) were found statistically similar. Rahman *et al*. (1994) in an experiment found that the grain yield of BR 3 rice in Boro season was 3.44 t ha^{-1} for using 100 kg N ha^{-1} as urea compared to 3.37 t ha^{-1} due to incorporation of two layers of Azolla alone with 40 kg N ha^{-1} as urea which, however, was not significantly different. In another experiment, Islam *et al*. (1996) observed 5.16 t ha^{-1} grain yield of BR 2 rice in Boro season for using 2 layers of Azolla plus 40 kg N ha^{-1} which was significantly higher over the yield (4.05 t ha^{-1}) obtained with the 80 kg N ha^{-1} as urea. Similar other related results have been reported earlier (Mian, 1990; Mian, 1992; Singh and Singh, 1987a; Singh, 1998a & 1998b). Rahman *et al*. (1994) reported 10.3, 2.0 and 0.55 kg ha^{-1} more N, P and S uptake due to two incorporations of Azolla along with 40 kg N ha^{-1}, respectively, over using 100 kg N ha^{-1} as urea. Islam *et al*. (1996) reported more N uptake of 11 kg ha^{-1} for using 40 kg N ha^{-1} as Azolla plus 40 kg N ha^{-1} as urea over using only 80 kg N ha^{-1} as urea. Similar other reports are also available (Mian, 1991; Singh and Singh, 1987b). The transfer of N from Azolla to rice has been proved unequivocally, using ^{15}N as the tracer, in several other studies (Mian and Stewart, 1985a & 1985b; Mian, 1990; Mian, 1992). The availability of N from floating Azolla (not incorporated) to rice plants have also been reported (Ito and Watanabe, 1985; Singh and Singh, 1987b; Mian and Kashem, 1995).
The organic matter content in soils increased slightly due to incorporation of two layers of Azolla plus 40 kg N ha^{-1} as urea (1.88% at harvest in place of 1.85% initial) compared to 1.83% in case of using the recommended dose of N (80 kg N ha^{-1} as urea). Such increases in organic matter contents of soils due to incorporation of Azolla biomass have also been reported by others (Singh and Singh, 1987b; Solaiman *et al*., 1990; Hoque, 1998; Mamun, 2000). It may be inferred from

overall results that if Azolla can be grown with rice for incorporation may reduce the use of urea in addition to maintaining soil organic matter status.

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