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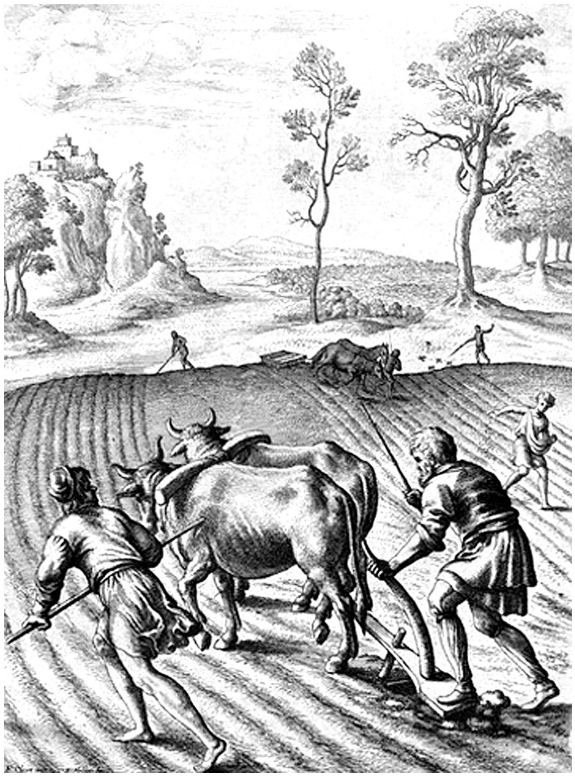

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Combination of Azolla and Urea Nitrogen for Satisfactory Production of Irrigated Boro Rice (BRRI Dhan 29)

Shamima Akhter, M.H. Mian, M.A. Kader and S.A. Begum
Department of Soil Science, Bangladesh Agricultural University, Mymensingh, Bangladesh

Abstract: A suitable combination of Azolla and urea-N was investigated for cultivation of rice (cv. BRRI Dhan 29). For this purpose an attempt was made to apply 30, 40, 50, 60 and 70% of the recommended doses of urea in 2 or 3 splits along with incorporation of one layer of Azolla grown from 0.2 kg m^{-2} inoculum. Two separate treatments of applying 100% of the recommended dose of urea in 3-splits (without Azolla) and no application of N at all from either urea or Azolla were included. Azolla covered the surface area in 13 days after inoculation at 7 DAT and reached the maximum growth in 19 days producing 14.0 to 18.3 t ha^{-1} fresh biomass containing 20.7 to 26.4 kg N ha^{-1}. Incorporation of one layer of Azolla at 30 DAT plus application of 50, 60 and 70 kg N ha^{-1} in 3 splits constituted the application of 72.7, 81.6 and 91.1 kg N ha^{-1} that produced 5.58, 6.00 and 6.02 t ha^{-1} grain yields which were statistically similar to each other but the latter two were statistically superior to the grain yield of 5.40 t ha^{-1} produced by conventional recommended practice. Total uptake of N, P and S by the rice plants (grain + straw) were increased significantly due to incorporation of Azolla. Incorporation of one layer of Azolla plus application of 60% of urea-N appeared as the best combination in this study.

Key words: Azolla, rice, urea, yield, biomass, N, P and S uptake

Introduction

Almost all soils of Bangladesh are very deficient in nitrogen. This situation has arisen mainly due to low level of organic matter caused by rapid decomposition due to warm climate, continuous intensive cropping, introduction of high yielding crop varieties and rare addition of organic manure. The soils of Bangladesh are low in organic matter and hence of nitrogen (Portch and Islam, 1984). Therefore, the use of nitrogenous fertilizers like urea has now become essential for rice production. To attain higher yield of food grain, modern high yielding varieties of rice are being cultivated which remove larger quantities of nutrients from soils. This should be replenished in order to maintain soil fertility and long term soil productivity. Besides, the continuous use of chemical fertilizer deteriorates soil properties in addition to causing micro-nutrients deficiencies. Long term experiments at BRRI farm showed that yield potentialities of rice declined due to use of chemical fertilizers but use of green manures maintained good soil fertility level. So, it is important that green manuring or use of organic fertilizers have now become essential to maintain the soil fertility. The Azolla-Anabaena symbiosis has already become a potential biofertilizer for rice production due to its high N_2-fixing abilities, rapid growth and easily decomposable characteristics (Khan, 1988). *Azolla pinnata* is natural inhabitant of fresh waters of canals, ponds, hills, rivers and water-logged rice fields of Bangladesh. Vigorously growing Azolla contains 3 to 5% nitrogen on a dry weight basis (Anonymous, 1978). Thus Azolla is a potential source of aquatic green manure for rice due to its high nitrogen fixation capacity, ability to supply nitrogen to the growing rice and the compatibility to grow with rice. Azolla also acts as a recycling source of P, S and other essential nutrients to the rice plants (Mian and Azmal, 1989). Therefore, Azolla has a bright future as a source of both nitrogen and organic matter for lowland rice soils and the soils that are irrigated for rice cultivation in Bangladesh since it can grow simultaneously with standing rice crop saving the need of an extra land and time (Mian, 2002). The present study was, therefore, undertaken to test the extent of Azolla biomass production and nitrogen fixation by growing Azolla simultaneously with BRRI Dhan 29 in boro season and the extent of reduction of urea-N by incorporating the produced Azolla biomass for cultivation of BRRI Dhan 29.

Materials and Methods

The experiment was conducted in the Soil Science Field Laboratory of Bangladesh Agricultural University, Mymensingh during boro season of 2000. Combinations of one layer of Azolla were made with urea nitrogen for obtaining a satisfactory yield of irrigated BRRI Dhan 29 rice in boro season. Characteristically, the soil was silt loam having pH 6.6, organic matter 3.183%, total N 0.16%, available phosphorus 8.5 ppm, available S 10.0 ppm. Mechanical analysis of soil was done by hydrometer method (Buoyoucos, 1926) and the textural class was determined following "Marshall's Triangular Coordinates" using USDA system. The pH of the soil was determined with the help of a glass electrode pH meter using the soil:water ratio of 1:2.5 (Jackson, 1962). Wet oxidation method was followed to determine the percentage of organic carbon according to Page *et al.* (1989) and then the organic matter content was calculated by multiplying the percent organic carbon with the Van Bemmelen factor, 1.73 (Piper, 1950). The N, P and S contents of the soil samples were determined by the following of standard procedure (Page *et al.*, 1989). The twelve treatments used in this experiment were as follows; T_0: control (no N as Azolla or urea); T_1: inoculation of 0.2 kg m^{-2} Azolla at 7 DAT and incorporation of the formed layer at 30 DAT + 30% of urea-N in 2 splits (15 and 60 DAT); T_2: as in T_1 but 40% of urea-N; T_3: as in T_1 but 50% of urea-N; T_4: as in T_1 but 60% of urea-N; T_5: as in T_1 but 70% of urea-N; T_6: as in T_1 but 30% of urea-N in 3 splits (15, 45 and 60 DAT); T_7: as in T_6 but 40% of urea-N; T_8: as in T_6 but 50% of urea-N; T_9: as in T_6 but 60% of urea-N; T_{10}: as in T_6 but 70% of urea-N and T_{11}: 100% of urea-N (recommended dose) applied in 3 equal splits. Triple super phosphate, muriate of potash, gypsum and zinc sulphate were applied to all the experimental plots as basal dose @ 20 kg P, 40 kg K, 10 kg S and 1 kg Zn ha^{-1}, respectively. The treatments were laid out in randomized complete block (RCBD) design with 3 replications. The unit plot size was 4 × 2.5 m^2 and the total number of plots were 36. Forty days old seedlings of BRRI Dhan 29 rice were transplanted on 2 February, 2000 at 25 × 15 cm^2 spacing. Irrigation was done in the experimental plots to maintain 5-10 cm water up to 30 DAT to grow Azolla. The weights of fresh biomass of Azolla were recorded as per design. The crops were harvested at maturity on 25 May, 2000. Ten hills were randomly selected from each plot at maturity to record the yield contributing characters. Grain and straw yields were recorded plot wise. The grain and straw samples were dried in an oven at 60°C for about 48 h and then ground. The samples were digested and then analyzed for the determination of N, P and S contents following standard procedure (Page *et al.*, 1989). The data were statistically analyzed by F-test and the means were compared by Duncan's multiple range test (DMRT).

Results

Biomass production and N yield of Azolla: Azolla inoculum (0.2 kg m^{-2}) started growing with the rice plants and covered the water surface in 13 days (i.e. layer formation) producing 6.40 to 8.17 t ha^{-1} fresh biomass (Fig. 1). The fronds continued growing until reaching the peak of maximum production of 14.0 to 18.3 t ha^{-1} fresh biomass containing 20.7 to 26.4 kg N ha^{-1} (Table 1) in 19 days after inoculation. Thereafter, the biomass started declining due to the degeneration of older fronds and came down to 7.4–8.83 t ha^{-1} before incorporation into soil at 30 DAT (25 days

Table 1: Pattern of growth of Azolla simultaneously with BRRI Dhan 29 from 5 to 30 DAT in boro season of 2000

	Azolla biomass (t ha^{-1})					Total N in Azolla biomass (kg ha^{-1})				
Treatments	18 DAT*	21 DAT	24 DAT	27 DAT	30 DAT	18 DAT	21 DAT	24 DAT	27 DAT	30 DAT
Urea-N applied in 2 splits (15 & 45 DAT)										
T_1 = 1 L AZ plus 30% urea-N	8.17	14.7	18.3	17.0	7.73	11.3	20.3	25.3	23.5	10.7
T_2 = 1 L AZ plus 40% urea-N	7.50	14.5	16.7	16.2	6.83	11.4	22.1	25.5	24.7	10.4
T_3 = 1 L AZ plus 50% urea-N	7.68	13.5	16.2	15.0	7.40	10.9	19.2	23.1	21.2	10.5
T_4 = 1 L AZ plus 60% urea-N	6.75	13.1	15.0	14.5	7.50	9.32	18.1	20.7	17.3	10.4
T_5 = 1 L AZ plus 70% urea-N	6.33	9.67	14.6	11.5	8.00	10.0	15.3	22.0	18.2	12.6
Urea-N applied in 3 splits (15, 45 & 60 DAT)										
T_6 = 1 L AZ plus 30% urea-N	7.33	10.5	17.0	15.2	8.83	9.42	13.5	21.9	19.5	11.4
T_7 = 1 L AZ plus 40% urea-N	7.92	14.4	16.6	14.5	8.00	12.6	22.9	26.4	23.1	12.7
T_8 = 1 L AZ plus 50% urea-N	7.75	10.9	14.1	14.3	7.67	12.5	17.5	22.7	20.4	12.3
T_9 = 1 L AZ plus 60% urea-N	7.42	9.40	14.3	10.8	8.23	11.9	15.2	21.6	17.4	13.2
T_{10} = 1 L AZ plus 70% urea-N	6.4	11.3	14.0	12.5	8.23	9.66	17.1	21.1	18.9	12.4
Average	7.3	12.2	15.6	14.2	7.84	10.9	18.1	23.0	20.4	11.7

Table 2: Effect of simultaneously grown Azolla (from 0.2 kg m^{-2} inoculation) with different dose of urea-N on different growth parameters of rice (cv. BRRI Dhan 29) grown in boro season of 2000

Treatments	Plant height (cm)	Effective tillers hill^{-1}	Panicle length (cm)	No. of filled grain panicle^{-1} (No.)	Thousand grain weight (g)
T_0 = No N as Azolla or urea	80.04d	8.76d	23.10	88.6e	18.59
Urea-N applied in 2 splits (15 & 45 DAT)					
T_1 = 1 L AZ* plus 30% urea-N	84.38cd	9.76cd	21.50	89.5e	19.37
T_2 = 1 L AZ plus 40% urea-N	85.28c	10.49a-d	21.77	102.7c-e	19.20
T_3 = 1 L AZ plus 50% urea-N	90.25ab	10.69a-d	23.43	109.4b-d	19.79
T_4 = 1 L AZ plus 60% urea-N	93.06a	10.69a-d	22.53	119.4ab	20.67
T_5 = 1 L AZ plus 70% urea-N	92.77a	11.80a-c	23.43	131.0a	21.20
Urea-N applied in 3 splits (15, 45 & 60 DAT)					
T_6 = 1 L AZ plus 30% urea-N	83.10cd	9.95b-d	23.00	96.5de	19.80
T_7 = 1 L AZ plus 40% urea-N	87.18bc	10.97a-c	22.60	105.2b-d	20.42
T_8 = 1 L AZ plus 50% urea-N	86.93bc	11.16a-c	23.90	117.3a-c	21.47
T_9 = 1 L AZ plus 60% urea-N	92.30a	12.03ab	23.43	126.8a	21.09
T_{10} = 1 L AZ plus 70% urea-N	93.83a	12.25a	23.40	130.3a	21.28
T_{11} = 100% of urea-N	90.96ab	11.50a-c	23.37	107.8b-d	20.03
Sx	7.27	0.663	1.428	4.770	0.919
CV (%)	3.05	10.61	10.78	7.49	7.83

Table 3: Effect of incorporated one layer of Azolla in combination with different rates of urea-N on the grain and straw yields of BRRI Dhan 29 in boro season of 2000

	Grain yield (at 14% moisture)		Straw yield (sun dry) (grain + straw)		Total yield (grain + straw)	
Treatments	t ha^{-1}	% increases over control	t ha^{-1}	% increases over control	t ha^{-1}	% increases over control
T_0= No N as Azolla or Urea	3.07e	-	3.59 e	-	6.66 f	-
Urea-N applied in 2 splits (15 & 45 DAT)						
T_1= 1 L AZ* plus 30% urea-N	4.00d	30.3	4.77d	32.7	8.77e	31.7
T_2= 1 L AZ plus 40% urea-N	4.60c	49.8	5.35cd	49.0	9.95de	49.4
T_3= 1 L AZ plus 50% urea-N	5.18bc	68.7	5.63b-d	56.8	10.8b-d	62.2
T_4= 1 L AZ plus 60% urea-N	5.38b	75.2	6.16a-c	71.6	11.5a-c	72.7
T_5= 1 L AZ plus 70% urea-N	5.73ab	86.6	6.53ab	81.9	12.3a	84.7
Urea-N applied in 3 splits (15, 45 & 60 DAT)						
T_6= 1 L AZ plus 30% urea-N	4.63c	50.8	5.61bcd	56.3	10.3cd	54.7
T_7= 1 L AZ plus 40% urea-N	5.17bc	68.4	5.59b-d	55.7	10.8cd	62.2
T_8= 1 L AZ plus 50% urea-N	5.58ab	81.8	6.40ab	78.3	12.0a-c	80.2
T_9= 1 L AZ plus 60% urea-N	6.00a	95.4	6.60ab	83.84	12.6a	89.2
T_{10}= 1 L AZ plus 70% urea-N	6.02a	96.1	6.83a	90.3	12.9a	93.6
T_{11}= 100% of urea-N	5.40b	75.9	6.21a-c	73.0	11.6ab	74.2
Sx	0.184		0.305		0.415	
CV (%)	6.30		9.15		6.63	

The figures in a column having the similar letter do not differ significantly at 5% DMRT

1 L AZ* : One layer of Azolla (1L) formed from 0.2 kg m^{-2} initial inoculum within 7 to 30 DAT incorporated (DAT = Days after transplanting).

100% of N as urea = 100 kg N ha^{-1} as urea

Azolla N in 1 L AZ = 19.9 to 25.5 kg ha^{-1} for the treatments T_1 to T_5 and 198 to 26.4 kg ha^{-1} for the treatments T_6 to T_{10}

Table 4: Effect of incorporated one layer of Azolla in combination with different rates of urea-N on N, P and S uptake by rice (cv. BRRI Dhan 29) in boro season of 2000

	N uptake (Kg ha^{-1})			P uptake (Kg ha^{-1})			S uptake (Kg ha^{-1})		
Treatments	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total
T_0 = No Azolla, no urea	29.9d	18.6d	48.5e	9.75e	6.27f	16.0h	3.01g	3.08e	6.09f
Urea-N applied in 2 splits (15 & 45 DAT)									
T_1 = 1 L AZ* plus 30% urea-N	41.0c	20.2cd	61.1f	10.4e	7.1ef	17.5gh	4.09f	3.71de	7.80ef
T_2 = 1 L AZ plus 40% urea-N	40.3c	22.7c	63.0cd	13.0cde	7.4d-f	20.4fg	4.94e	4.48b	9.42b-e
T_3 = 1 L AZ plus 50% urea-N	47.8a-c	23.4bc	71.1bc	14.6bc	8.6cd	23.2d-f	5.70c-e	4.38bc	7.08ef
T_4 = 1 L AZ plus 60% urea-N	51.6ab	26.4ab	78.1ab	15.3bc	8.1de	23.4d-f	6.19bc	5.19a	11.4a-d
T_5 = 1 L AZ plus 70% urea-N	53.9ab	30.0a	83.9a	16.6ab	9.68bc	26.3b-d	6.85ab	5.75a	12.6a
Urea-N applied in 3 splits (15, 45 & 60 DAT)									
T_6 = 1 L AZ plus 30% urea-N	46.2bc	21.3cd	67.5cd	11.2de	7.84de	19.0gh	5.09de	3.79cd	8.88d-f
T_7 = 1 L AZ plus 40% urea-N	46.6bc	21.9cd	68.5cd	14.0b-d	8.08de	22.0ef	5.96b-d	3.08e	9.01c-f
T_8 = 1 L AZ plus 50% urea-N	52.5ab	27.2a	79.7ab	16.1a-c	10.7ab	27.1bc	6.53a-c	5.54a	12.1a-c
T_9 = 1 L AZ plus 60% urea-N	54.1ab	28.3a	82.4a	17.2ab	10.5b	28.7ab	7.34a	5.15a	12.5ab
T_{10} = 1 L AZ plus 70% urea-N	56.8a	31.6a	88.4a	19.3a	11.9a	31.2a	7.39a	5.66a	12.9a
T_{11} = 100% of urea-N	50.5ab	27.8a	78.3ab	16.0bc	8.36c-e	24.4c-e	7.24a	4.10b-d	11.3a-d
Sx	2.89	1.13	2.94	1.08	0.43	0.99	0.29	0.21	0.97
CV (%)	10.52	7.87	7.03	12.9	8.63	7.3	8.49	8.42	16.58

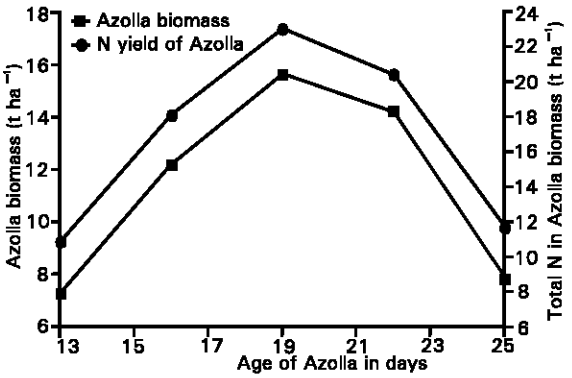

Fig. 1: Biomass production and N yield of Azolla growing simultaneously with BRRI Dhan 29 from 7 to 30 DAT in Boro season of 2000

old Azolla) (Fig. 1, Table 1). The growth pattern in terms of the amounts of biomass and nitrogen yields are also presented (Table 1). This study demonstrated that it is possible to grow sufficient amount of Azolla biomass (up to 18.3 t ha^{-1}) simultaneously with BRRI Dhan 29 in 19 days from 0.2 kg m^{-2} inoculum making up to 26.4 kg N ha^{-1} available for adding into soil which will help reducing the conventional use of urea fertilizers.

Yield contributing characters of BRRI Dhan 29: The combined application of Azolla biomass plus urea-N significantly increased the plant height, number of effective tillers hill^{-1}, panicle length and filled grains panicle^{-1} (Table 2). The tallest plant (93.8 cm) was recorded for T_{10} which was statistically similar to that of T_3, T_4, T_5, T_9 and T_{11}. The highest number of effective tillers hill^{-1} (11.0) was also observed in the treatment T_{10} which was statistically different from T_1 and T_2 only. Panicle length ranged form 21.5 to 23.9 cm. The maximum panicle length was recorded for T_{10} i.e. incorporation of one layer Azolla plus 70% urea-N applied in three splits which was, non significant. The number of filled grains panicle^{-1} ranged from 85.6 to 115.6. Among the treatments, T_{10} produced the highest number of grains panicle^{-1} which was statistically similar to that of T_3, T_4, T_5, T_8 and T_9. The weight of 1000 grains was not influenced significantly by the combined application of Azolla and urea. The weights varied from 17.6 to 19.7 g (Table 2). The treatments T_{10} produced the highest 1000-grains weight of 19.7 g.

Grain and straw yields of BRRI Dhan 29: The grain yield of BRRI Dhan 29 was influenced significantly by the combined use of Azolla and urea-N. The highest grain yield (6.02 t ha^{-1}) was obtained with T_{10} which was statistically similar to that of T_5, T_8 and T_9. The lowest yield (3.07 t ha^{-1}) was recorded in the control treatment. Gain yield was found increased by 30.3 to 96.1% over the control. The straw yield was also influenced significantly by the combined use of Azolla and urea-N (Table 3). The straw yields ranged from 3.59 to 6.83 t ha^{-1}. The highest straw yield was found with the treatment T_{10} which was statistically similar to that of T_4, T_5, T_8, T_9 and T_{11}. Total biological yield was the reflection of grain and straw yields. Total yield responded significantly to incorporation of Azolla plus application of urea-N. The total yield ranged from 6.66 to 12.9 t ha^{-1}. The highest total yield (12.9 t ha^{-1}) was obtained due to the treatment T_{10} which was statistically similar to the yields of T_4, T_5, T_8, T_9 and T_{11}.

Nutrient uptake by BRRI Dhan 29: The N uptake in both grains and straw increased significantly by the incorporation of one layer of Azolla containing 20.7 to 26.4 kg N ha^{-1} and application of 30-70% of urea-N (Table 4). The highest amount of nitrogen uptake (56.8 kg ha^{-1}) was recorded for T_{10} which did not differ significantly with nitrogen uptake by T_3, T_4, T_5, T_8, T_9 and T_{11}. Almost similar was the case in N uptake by straw. The total N uptake in grain and straw ranged from 48.5 to 88.4 kg ha^{-1}.

The P uptake in both grains and straw of rice was significantly influenced by different combinations of Azolla and urea (Table 4). The highest amount of P uptake in grain (19.3 kg ha^{-1}) and straw (11.9 kg ha^{-1}) were recorded in treatment T_{10}. While the lowest amounts were observed in the control (9.75 and 6.27 kg ha^{-1}, respectively). The highest total P uptake in grain and straw (31.2 kg ha^{-1}) was recorded in T_{10} which was found statistically different from all other treatments except T_9.

The S uptake in grain and straw was also increased significantly with different combinations of Azolla and urea-N (Table 4). The highest S uptake in grains (7.39 kg ha^{-1}) was recorded in T_{10} which was statistically identical to that of T_5, T_8 and T_9. The highest S uptake in straw (5.66 t ha^{-1}) was also recorded in T_{10} identical to that of T_4, T_5, T_8 and T_9 treatments. Total S uptake (grain plus straw) ranged from 6.09 to 12.9 kg ha^{-1} due to different treatments.

Discussion

It has already been established that Azolla can grow simultaneously with irrigated rice plants during their early period of growth, preferably within 30-40 days after transplanting (Mian, 2002). Present study revealed that Azolla (0.2 kg m^{-2} inoculum) grew well with rice plants, covered the surface in 13 days and

produced the maximum amount of biomass as 14.0 to 18.3 t ha^{-1} containing 20.7 to 26.4 kg N ha^{-1} in 19 days. Another treatment was 100% of the recommended dose of urea-N to compare with the effect of Azolla-urea combination treatments. Incorporation of one layer of Azolla having 14.0 t ha^{-1} biomass containing 21.1 kg N ha^{-1} and the application of 70% of N as urea in 3 splits (Treatment T_{10}) equaled the total application of 91.1 kg N ha^{-1} (70 kg N as urea + 21.1 kg N as Azolla). Similarly, the total N applied in T_9 was 81.6 kg ha^{-1} (21.6 kg Azolla + 60 kg N ha^{-1} as urea) and that in T_8 was 72.7 kg ha^{-1} (22.7 kg ha^{-1} as Azolla + 50 kg ha^{-1} as urea). The treatments T_{10}, T_9, T_8 and T_5 produced 6.02, 6.00, 5.58 and 5.73 t ha^{-1} grain yields, respectively, which were non significantly different from each other particularly, the performance of T_9 and T_{10} were practically equal and they both differed significantly from the grain yield of 5.40 t ha^{-1} obtained by using 100% of the recommended dose of urea i.e. 100 kg N ha^{-1}. These grain yield results indicated that growing and incorporating one layer of Azolla may save 40% of the use of urea i.e. the recommended dose may stand as incorporation of one layer of Azolla plus 60% of urea-N application in 3-splits. So, it is quiet possible to grow Azolla along with the standing boro rice crop for subsequent incorporation to supply substantial amount of N and some amounts of nutrients (e.g. P, S) to the rice plants of Azolla for their better growth. Simultaneous growth of Azolla with rice plants have been reported elsewhere. For example Mamun *et al.* (2000) obtained 15.8 –17.1 t ha^{-1} Azolla biomass from 0.2 kg m^{-2} inoculum in 19 days containing 25.1 to 27.8 kg N ha^{-1}. These authors also reported a significant increases in rice yield due to incorporation of Azolla biomass. Total N, P and S uptake by the rice plants has been increased due to incorporation of Azolla biomass which helped in increasing the yields. Availability of N from Azolla in substantial amounts has already been proved by Mian (2000). The availability of P and S from Azolla has been reported by many others (Singh, 1998; Mian and Azmal, 1989). Now it is beyond hesitations that Azolla-urea combination can increase the rice yields significantly in some instances, Azolla-urea combinations are better than using the recommended dose of urea alone. Kolhe and Mittra (1990) reported the highest grain yield of 5.07 t ha^{-1} due to the incorporation of 10 t ha^{-1} Azolla plus 30 kg N ha^{-1} as urea. Mian (2000) has clearly demonstrated that incorporation of one layer of Azolla may reduce the use of urea by 30-50% for cultivation of irrigated boro rice. The present study, however, stated the use of 60% of urea-N for this purpose.

In conclusion inoculation of 0.2 kg m^{-2} Azolla may grow simultaneously with BRRI Dhan 29 in boro season to yield about 14.2 to18.3 t ha^{-1} of biomass within 30 DAT containing 20.7-26.4 kg N ha^{-1} for incorporation in order to reduce the use of urea fertilizer by about 40%. However, this needs further study for confirmation.

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