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Effect of time of *Azolla* incorporation and inorganic fertilizer application on growth and yield of Basmati rice

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Azolla tissue contains 5% N, which is slowly released into the soil upon decomposition. Timing of incorporation is therefore important for maximum benefit to a crop. The effect of time to incorporate Azolla biomass on growth and yield of rice was investigated in Mwea-Kenya. Treatments consisted of 7.5 t ha⁻¹ Azolla biomass applied at transplanting, 7.5 t ha⁻¹ Azolla applied at 21 days after transplanting (DAT) and 30 kg N ha⁻¹ inorganic N applied in splits at O, 21 and at 55 DAT. There were control treatments without Azolla and without inorganic N application. The treatments were laid out in a Randomized Complete Block Design (RCBD) with three replications. Phosphorus and potassium were applied at 50 Kg ha⁻¹ each as P_2O_5 and K_2O . Plant height and tiller numbers were recorded at 21 (rooting/tillering), 32 (tillering), 42 (maximum tillering), 60 (flowering) and 75 DAT (heading) while yield parameters were determined at physiological maturity (120 DAT). Data were analysed using SAS software and means separated using the least significant difference test (p≤0.05). Azolla incorporation at transplanting significantly enhanced panicle m⁻², grain weight and grain yield while incorporating it at 21 DAT only significantly enhanced panicle m⁻². Higher environmental temperatures enhanced *Azolla* effect. The effect of Inorganic N significantly increased plant height, tiller number, grain weight and spikelets panicle⁻¹. However, percentage grain filling was reduced. The effect of interaction between Azolla application and inorganic N was significant on spikelets panicle⁻¹ and grain weight. Observations therefore indicate that the effect of Azolla on yield and yield components was more when incorporated at transplanting.

Key words: Azolla, incorporation time, inorganic fertilizer, rice yields.

INTRODUCTION

Azolla is a fern found in still or slow-moving water bodies (Campbell, 2011). It has a symbiotic association with nitrogen fixing blue-green algae, *Anabaena azollae* (Bocchi and Maglioglio, 2010). The association enables it

to fix nitrogen, which it releases upon decomposition thus making *Azolla* an important source of bio-fertilizer (Wager, 1997). Nitrogen, Phosphorus and potassium constitute 4-5, 1.5 and 3% of *Azolla* respectively on a dry

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> weight basis. In addition, *Azolla* can provide 1.8–3 tons ha⁻¹ dry matter per crop, (IRRI, 1990). According to Kannaiyan (1993) about 20 t/ha *Azolla* is capable of providing 40 kg N ha⁻¹ inorganic nitrogen requirement upon incorporation in the soil. According to IRRI (1990) about 50% of the nitrogen is released within the first 6-8 weeks of incorporation into the soil.

Due to its nitrogen fixing capability, Azolla has been used as a bio-fertilizer in rice paddies for increased productivity (Kamalasanana et al., 2002). For centuries, the potential of Azolla and its nitrogen-fixing partner Anabaena azollae has been exploited to increase rice yields in China and Asian countries (Armstrong, 1979; Carrapiço et al., 2000). However, the advent of the industrial revolution resulted in increased use of inorganic fertilizers leading to reduction in the traditional use of Azolla as a green manure (Carrapiço et al., 2000). In China, at least 3.2 million acres of rice paddies were planted with Azolla by 1980. In Northern Italy at Po Valley, Azolla incorpored in paddies produced equivalent of 30-40 Kg N/ha (Bocchi and Maglioglio, 2010). In India, Singh and Singh (1987) reported significant increase in rice yields from Azolla application at transplanting and Azolla dual incorporation. Research in Guinea Bissau on the comparative effects of Azolla on rice yields showed that incorporating 7 tons ha⁻¹ Azolla biomass gave an equivalent effect of 43.5 kg N ha⁻¹ (Carrapiço et al., 2000). Findings by Malyan et al. (2019) showed that the use of Azolla reduces the need for application of urea fertilizer by 25% in rice production with no effects on yields. According to Razavipour et al. (2018), Azolla use increases tillers, grain weight, yield of rice and is a desirable management practice in rice production Field studies at Ahero Irrigation Research Station in Kenya (1980) confirmed the positive benefit of 4.8 tons ha⁻¹ Azolla when used with inorganic nitrogen (AIR Report, 57). However, the need for extra fields for mass multiplication of Azolla proved uneconomical. In West Kano Irrigation Scheme, incorporation of Azolla + urea gave significantly higher grain yields and plant height (Serrem et al., 2013).

Azolla is a major source of nitrogen when grown in paddies and incorporated into the soil as green manure. The process of incorporating Azolla in the soil can be done either at transplanting or during active tillering, thus making it a dual crop with the paddy rice (Bocchi and Maglioglio, 2010). According to IIRR (Low input Rice Production-LIRP Technology Kit), three methods are commonly used; (i) Azolla is grown with the rice crop in paddies and incorporated as green manure; (ii) Azolla is incorporated once at 20 days after transplanting; (iii) Azolla is incorporated during subsequent cropping. Azolla in the soil provides organic matter, which improves soil quality and provides nutrients for the current and subsequent crops (Ferentinos et al., 2002). However, the timing of Azolla application and the benefit to the crop is affected by the environmental conditions (Wagner, 1997).

Although Azolla is beneficial to rice production, its use has not been widely accepted due to several constraints including labour for its incorporation (Carrapiço et al., 2000). Consequently, farmers continue to use inorganic fertilizers. Inorganic fertilizers have been shown to improve yields initially but their impacts are however not sustainable over a long period of time (Patro et al., 2011). This is because of creation of nutrients imbalance which consequently leads to a reduction in soil fertility and crop yields (Singh et al., 2001). Application of Azolla combined with inorganic nitrogen gives optimum grain yields (Kannaiyan, 1993). Ito and Watanabe (1985) showed that early incorporation of Azolla in the soil increases nitrogen availability. Farmers in Mwea incorporate Azolla in the soil during weeding as a management strategy (Oyange et al, 2019). Considering the abundance of Azolla in Mwea paddies, its integration with inorganic fertilizers and timely incorporation in the soil can help reduce the cost of inorganic fertilizers and consequently the cost of paddy rice production. The objective of the study was to determine the effect of timing of Azolla incorporation on paddy rice growth and yield.

MATERIALS AND METHODS

Site description

The study was done at Mwea Irrigation Scheme during the year 2015 and 2016. Mwea lies within agro-ecological zones LM3 and LM 4 (Marginal cotton zones). Rainfall pattern is bimodal; long rainy season begins from March to May and the short rainy season from October to November. Annual mean rainfall is about 930 mm, out of which 510 mm is received during long rainy season, with 66% reliability. The mean temperature is 22°C with a minimum of 17°C and a maximum of 28°C. During the experimental period, the average temperature was 23°C with relative humidity of 78% (Appendix Table 1). The average and maximum temperatures were higher during growth stage but lower during heading and maturity stages for second than first season. Relative humidity was lower for the first season than for the second season. The experimental plots had black cotton soils, imperfectly drained, with ideal pH (Table 1). The N and P levels were near threshold while the K levels were low. Azolla tissue N, P and K levels were 4.0, 0.45 and 1.1%, respectively for Mwea (Table 2).

Experimental design

Treatments consisted of 7.5 t ha⁻¹ *Azolla* incorporated at transplanting, 7.5 t ha⁻¹ *Azolla* applied at 21 DAT and no *Azolla* application combined with inorganic N application of 0 and 30 kg N ha⁻¹ as Sulphate of Ammonia. The treatments were laid out in a RCBD with a split plot arrangement. Inorganic N was applied in three equal splits each of 10 kg N ha⁻¹ at transplanting, 21 DAT and 50 DAT respectively. Nutrient P and K were applied at Mwea Irrigation Agricultural Development Centre (MIAD) standard rates of 50 kg ha⁻¹ P₂O₅ and 50 kg ha⁻¹ K₂O as triple super phosphate and muriate of potash, respectively. Irrigation was carried out to maintain a water depth of 2-5 cm above the ground. Basmati 370 rice variety sourced from MIAD was grown at a spacing of 30 cm x 15 cm. One seedling per hill was transplanted 21 days after sowing and the field was kept weed free by manual weeding at 21, 32 and 45 DAT.

| | S1 | S2 | S3 | S4 | S5 | S 7 | Average | Classification |
|-----------|-------|-------|-------|-------|-------|------------|---------|----------------|
| рН | 6.26 | 6.84 | 6.06 | 5.96 | 5.97 | 5.74 | 6.14 | Ideal |
| N% | 0.105 | 0.119 | 0.144 | 0.130 | 0.133 | 0.151 | 0.130 | * |
| P(ppm) | 15.0 | 13.0 | 13.0 | 12.0 | 14.0 | 14.0 | 13.5 | * |
| K (me %) | 0.085 | 0.17 | 0.17 | 0.127 | 0.127 | 0.085 | 0.127 | Low |
| E.C µS/cm | 663 | 475 | 451 | 323 | 172 | 235 | 387 | Ideal |

Table 1. Soil nutrient status at Mwea paddy fields.

S= Sample, * = the level is near the threshold of that particular element.

Table 2. Tissue nutrient content of Azolla accessions (dry weight basis) in Kenya.

| Accession | N (%) | P (%) | K (%) |
|------------|--------|--------|--------|
| Mwea | 4.0 | 0.45 | 1.1 |
| Ahero | 5.1 | 0.21 | 2.2 |
| West Kano | 4.8 | 0.18 | 1.6 |
| Bunyala | 3.4 | 0.23 | 1.5 |
| Taveta 1 | 3.2 | 0.20 | 1.3 |
| Taveta 2 | 3.4 | 0.22 | 2.0 |
| TARDA 2 | 3.4 | 0.40 | 1.9 |
| Mean | 3.9 | 0.27 | 1.6 |
| P-value | <0.001 | <0.001 | <0.001 |
| LSD (0.05) | 0.2 | 0.14 | 0.2 |
| CV (%) | 1.9 | 2.1 | 3.8 |

Data collection

Data collected included plant height, tiller numbers, grain yield and grain yield components (panicle number, spikelets per panicle, 1000 grain weight, % filled grains and % ripened grains). Ten hills per plot were sampled to determine plant height and tiller numbers at 21, 35, 42, 60 and 75 DAT, corresponding to rooting, tillering, maximum tillering, flowering and heading stages respectively. Soil samples from the experimental site were analyzed for N, P, K and pH, prior to crop establishment. *Azolla* biomass (100 g) each was collected from the canal drains within the six major irrigation schemes in Kenya namely: Mwea, Ahero West Kano Bunyala, Taveta and TARDA for N, P and K analysis.

Data analysis

Data collected were subjected to analysis of variance using SAS statistical package and means separated using the least significant difference (LSD) test at p≤0.05. Linear regression analysis was done to determine the linear regression relationship between yield and yield components.

RESULTS

Soil nutrient status in Mwea

The soil N, P and K averaged 0.13%, 13.5 ppm and 0.13%, respectively. The pH was on average 6.1. The N and P levels were within threshold limits while the pH was

ideal.

Azolla plant tissue nutrient levels

The total *Azolla* plant tissue N% on a dry weight basis ranged between 3.14 and 5.06% (Table 2). Mwea Azolla accession had tissue N levels of 4.0%

Effect of time of incorporation on tilers and plant height

Time of *Azolla* incorporation in paddy rice plots had no significant effect on tiller numbers and plant height during both seasons. However, application of 30 kg N ha⁻¹ significantly increased panicles/m², grain weight, and % grain filling during the first season, while spikelets/panicle significantly increased during the second season. The effect of interaction between time of *Azolla* application and inorganic N on tiller numbers and plant height was not significant.

Time of *Azolla* incorporation significantly affected the number of spikelets/panicle, neck node, panicle/m², grain weight and grain yield. Application of 7.5 t ha⁻¹ *Azolla* at transplanting gave significantly more spikelets/panicle higher grain weight and grain yield than when 7.5 t ha⁻¹ *Azolla* was incorporated at 21 DAT (Table 3). However,

Table 3. Effect of time of *Azolla* biomass application on number of tillers and plant height in Mwea Irrigation Scheme during 2015/2016.

| Veriable | | | Season 1 | | | | | | | |
|--|--------|--------------------------------|-------------------|--------|--------|--------|--|--|--|--|
| variable | | Tiller numbers m ⁻² | Plant height (cm) | | | | | | | |
| Treatment | 30 DAT | 60 DAT | 75 DAT | 30 DAT | 60 DAT | 75 DAT | | | | |
| No Azolla (control) | 302.9 | 367.4 | 351.5 | 53.6 | 93.9 | 117.1 | | | | |
| 7.5 t ha ⁻¹ Azolla at transplanting | 278.1 | 365.9 | 345.2 | 49.3 | 87.4 | 115.3 | | | | |
| 7.5 t ha ⁻¹ <i>Azolla</i> at 21 DAT | 258.9 | 382.9 | 357.7 | 50 | 91.2 | 119.2 | | | | |
| Mean | 280 | 372.1 | 351.4 | 51 | 90.9 | 117.2 | | | | |
| P-value | 0.25 | 0.86 | 0.94 | 0.07 | 0.37 | 0.46 | | | | |
| LSD (0.05) | NS | NS | NS | NS | NS | NS | | | | |
| CV (%) | 15.4 | 15.6 | 18 | 6 | 8.3 | 4.5 | | | | |
| 0 kg N ha ⁻¹ (control) | 296.8 | 345.4 | 329.4 | 51.4 | 88.9 | 117 | | | | |
| 30 kg N ha⁻¹ | 263.2 | 398.7 | 373.5 | 50.6 | 92.8 | 117.4 | | | | |
| Mean | 280 | 372.1 | 351.4 | 51 | 90.9 | 117.2 | | | | |
| P-value | 0.13 | 0.08 | 0.17 | 0.59 | 0.304 | 0.89 | | | | |
| LSD (0.05) | NS | NS | NS | NS | NS | NS | | | | |
| CV (%) | 15.4 | 15.6 | 18 | 6 | 8.3 | 4.5 | | | | |
| N x Azolla - P-value | 0.16 | 0.71 | 0.93 | 0.6 | 0.4 | 0.73 | | | | |
| | | | Season 2 | | | | | | | |
| No Azolla (control) | 124.4 | 395.2 | 435.1 | 34.8 | 48.4 | 118.7 | | | | |
| 7.5 t ha-1 Azolla at transplanting | 125.2 | 378.5 | 442.6 | 35.6 | 51 | 122 | | | | |
| 7.5 t ha-1 Azolla at 21 DAT | 127.4 | 382.9 | 402.2 | 35.9 | 52.6 | 124.4 | | | | |
| Mean | 125.7 | 385.5 | 426.6 | 35.4 | 50.7 | 121.7 | | | | |
| P-value | 0.98 | 0.82 | 0.83 | 0.87 | 0.21 | 0.11 | | | | |
| LSD (0.05) | 39.1 | 60 | 154.3 | 4.4 | 4.9 | 5.4 | | | | |
| CV (%) | NS | NS | NS | NS | NS | NS | | | | |
| 0 kg N ha ⁻¹ (control) | 109.9 | 373.1 | 420 | 34 | 48.5 | 119 | | | | |
| 30 kg N ha ⁻¹ | 141.5 | 398 | 433.3 | 36.8 | 52.8 | 123.9 | | | | |
| Mean | 125.7 | 385.5 | 426.6 | 35.4 | 50.7 | 121.7 | | | | |
| P-value | 0.05 | 0.28 | 0.82 | 0.12 | 0.04 | 0.05 | | | | |
| LSD (0.05) | 31.5 | 49 | 126 | 3.6 | 4 | 4.4 | | | | |
| CV (%) | 24.2 | NS | NS | NS | 7.6 | 3.5 | | | | |
| N x Azolla - P-value | 0.93 | 0.82 | 0.8 | 0.98 | 0.62 | 0.6 | | | | |

incorporation of 7.5 t ha ⁻¹ *Azolla* at 21 DAT during second season, resulted in significantly longer neck node, more panicle m⁻² than basal application of the *Azolla*. The treatment significantly increased the number of spikelets panicle⁻¹ during the first season. The effect of interaction between time of *Azolla* incorporation and inorganic nitrogen application was significant on spikelets panicle⁻¹ and grain weight during the second season (Table 4). Linear regression relationship between spikelets panicle⁻¹ and yield (Figures 1 and 2) showed a positive significant relationship (r² = 0.149). There was also a strong positive linear relationship (r²=0.42) between panicle m⁻² and yield (Figure 1).

DISCUSSION

The soils in Mwea had N, P and K levels within threshold

limits (Table 1). The pH was ideal (6.1), and within acceptable levels for maximum P availability; Fe and Al ions had no detrimental effects on other nutrients (Miller, 2016). The total *Azolla* plant tissue N% on a dry weight basis ranged from 3.14 to 5.06% (Table 2). This is consistent with the findings of Watanabe and Berja (1983), which showed 4-5% tissue N and 0.7-1.85 ppm P. The tissue P level was in conformity with the reported amounts of 0.1- 0.5% (Better Crops, 1999). The soil N levels were near the threshold limits thus necessitating external application

Azolla incorporation in the soil at transplanting significantly increased spikelets panicle⁻¹, grain weight and yield while incorporation in the soil at 21 DAT significantly enhanced number of panicles, neck node length, and grain weight (Table 5). Time of *Azolla* incorporation had no significant effect on plant height and tiller numbers both seasons (Table 4). The significant

Table 4. Effect of time of incorporation of *Azolla* biomass on neck node, spikelets, panicles and panicle length in Mwea Irrigation Scheme during 2015/2017.

| | Season 1 | | | | | | | | | | |
|--|----------------|---------------------------------|------------------------------------|---------------------|--|--|--|--|--|--|--|
| Treatment | Neck node (cm) | Number of Spikelets/ panicle | Number of Panicles m ⁻² | Panicle length (cm) | | | | | | | |
| No Azolla (control) | 3.6 | 75.2 | 465.1 | 25.7 | | | | | | | |
| 7.5 t ha ⁻¹ Azolla at transplanting | 3.7 | 84.9 | 428.8 | 26.4 | | | | | | | |
| 7.5 t ha ⁻¹ <i>Azolla</i> at 21 DAT | 3.5 | 80.1 | 470.3 | 25.7 | | | | | | | |
| Mean | 3.6 | 80 | 454.7 | 26.1 | | | | | | | |
| P-value | 0.205 | 0.016 | 0.83 | 0.11 | | | | | | | |
| LSD (0.05) | NS | 6.1 | NS | NS | | | | | | | |
| CV (%) | 3.7 | 5.9 | 28.4 | 2 | | | | | | | |
| 0 kg N ha ⁻¹ (control) | 3.6 | 76.1 | 425.6 | 26.1 | | | | | | | |
| 30 kg N ha⁻¹ | 3.6 | 83.9 | 483.9 | 26 | | | | | | | |
| Mean | 3.6 | 80 | 454.7 | 26.1 | | | | | | | |
| P-value | 0.68 | 0.01 | 0.36 | 0.58 | | | | | | | |
| LSD (0.05) | NS | 5 | NS | NS | | | | | | | |
| CV (%) | 3.7 | 5.9 | 28.4 | 2 | | | | | | | |
| N x Azolla - P-value | 0.37 | 0.55 | 0.81 | 0.46 | | | | | | | |
| | | Seaso | on 2 | | | | | | | | |
| No Azolla (control) | 3.72 | 75.7 | 344.5 | 24.7 | | | | | | | |
| 7.5 t ha-1 Azolla at transplanting | 3.81 | 74.4 | 362.1 | 25.2 | | | | | | | |
| 7.5 t ha-1 Azolla at 21 DAT | 3.94 | 75.5 | 409.3 | 25.8 | | | | | | | |
| Mean | 3.8 | 75.2 | 372 | 25.2 | | | | | | | |
| P-value | 0.05 | 0.91 | 0.03 | 0.15 | | | | | | | |
| LSD (0.05) | 0.17 | NS | 47.1 | NS | | | | | | | |
| CV (%) | 3.5 | 7.1 | 9.8 | 3.5 | | | | | | | |
| 0 kg N ha-1 (control) | 3.8 | 74.5 | 345.7 | 25 | | | | | | | |
| 30 kg N ha-1 | 3.8 | 75.9 | 398.2 | 25.4 | | | | | | | |
| Mean | 3.8 | 75.2 | 372 | 25.2 | | | | | | | |
| P-value | 0.44 | 0.58 | 0.01 | 0.4 | | | | | | | |
| LSD (0.05) | NS | NS | 38.4 | NS | | | | | | | |
| CV (%) | 3.5 | 7.1 | 9.8 | 3.5 | | | | | | | |
| N x Azolla - P-value | 0.65 | 0.03 | 0.73 | 0.57 | | | | | | | |

DAT= days after transplanting.



Figure 1. Linear regression relationship between spikelets panicle⁻¹ and grain yield at Mwea Irrigation Scheme.



Figure 2. Linear regression relationship between number of panicle (m^{-2}) and grain yield at Mwea Irrigation Scheme.

| Table | 5. Effect | of time | of Azolla | a incorporation | on | grain | weight, | % | grain | filling | and | yield | of | paddy | rice | in |
|-------|-----------|---------|------------|-----------------|----|-------|---------|---|-------|---------|-----|-------|----|-------|------|----|
| Mwea | Irrgation | Scheme | e during 2 | 2015/2016. | | | | | | | | | | | | |

| Tractment | Season 1 | | | | | | | | | |
|--|------------------|-----------------|--------------|--|--|--|--|--|--|--|
| Treatment | Grain weight (g) | % filled grains | Yield (t/ha) | | | | | | | |
| No Azolla (control) | 0.0213 | 0.68 | 5.18 | | | | | | | |
| 7.5 t ha ⁻¹ Azolla at transplanting | 0.0216 | 0.77 | 6.21 | | | | | | | |
| 7.5 t ha ⁻¹ Azolla at 21 DAT | 0.0217 | 0.71 | 5.7 | | | | | | | |
| Mean | 0.022 | 0.72 | 5.7 | | | | | | | |
| P-value | 0.712 | 0.430 | 0.440 | | | | | | | |
| LSD (0.05) | NS | NS | NS | | | | | | | |
| CV (%) | 4.08 | 14.6 | 23.6 | | | | | | | |
| 0 kg N ha ⁻¹ (control) | 0.0216 | 0.73 | 0.51 | | | | | | | |
| 30 kg N ha ⁻¹ | 0.0215 | 0.71 | 6.3 | | | | | | | |
| Mean | 0.022 | 0.72 | 5.7 | | | | | | | |
| P-value | 0.770 | 0.610 | 0.080 | | | | | | | |
| LSD (0.05) | NS | NS | NS | | | | | | | |
| CV (%) | 4.08 | 14.6 | 23.6 | | | | | | | |
| N x Azolla - P-value | 0.77 | 0.4 | 0.27 | | | | | | | |
| | Season 2 | | | | | | | | | |
| No Azolla (control) | 0.0226 | 0.86 | 5.2 | | | | | | | |
| 7.5 t ha ⁻¹ Azolla at transplanting | 0.0237 | 0.86 | 6.4 | | | | | | | |
| 7.5 t ha ⁻¹ Azolla at 21 DAT | 0.0229 | 0.88 | 5.69 | | | | | | | |
| Mean | 23 | 0.87 | 5.8 | | | | | | | |
| P-value | 0.002 | 0.660 | 0.030 | | | | | | | |
| LSD (0.05) | 0.01 | NS | 0.83 | | | | | | | |
| CV (%) | 1.6 | 3.7 | 11.2 | | | | | | | |
| 0 kg N ha ⁻¹ (control) | 0.0228 | 0.89 | 5.5 | | | | | | | |
| 30 kg N ha ⁻¹ | 0.0233 | 0.85 | 6.1 | | | | | | | |
| Mean | 0.023 | 0.87 | 5.80 | | | | | | | |
| P-value | 0.010 | 0.050 | 0.080 | | | | | | | |
| LSD (0.05) | 0.004 | 0.034 | NS | | | | | | | |
| CV (%) | 1.6 | 3.7 | 11.2 | | | | | | | |
| N x Azolla - P-value | 0.01 | 0.63 | 0.25 | | | | | | | |

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effect of *Azolla* incorporation on yield and yield components but not on growth stages of rice crop can be attributed to a comparatively slow rate of nutrients release by *Azolla*. Watanabe et al. (1991) reported that the rate of mineralization in *Azolla* is gradual. The slow rate of mineralization is due to existence of lignified tissues, which make decomposition to be slow, leading to gradual availability of tissue nutrients (Watanabe et al., 1991). According to Ito and Watanabe (1985), 60% of the tissue N is released within the first four weeks.

Inorganic nitrogen application significantly affected both vegetative and reproductive components of rice plant. Plant height, numbers of tillers, grain weight and spikelets/panicle were significantly increased while percentage grain filling was reduced. Inorganic nitrogen application increased spikelets/panicle in the first season and number of panicles, grain weight and % filled grains in the second season. Inorganic N application has been reported to enhance growth, tillers and yield of paddy rice (Yesuf and Balcha, 2014; Chaturvedi, 2005). The enhancement of growth and yield components can be attributed to supply of readily available nitrogen source throughout the growing period. In this study, N was applied in equal splits at 0, 21 and 53 DAT respectively. This consequently benefitted both vegetative and reproductive phases of rice crop and led to the significant increase realized. Enhanced vegetative growth increases solar radiation reception by the plant canopy (Marshall and Roberts, 2000) and this had a positive effect on plant height, tiller numbers and yield components. Yoshida (1972) reported that increased reproductive tillers concurrently increased rice yields.

A positive correlation between the number of spikelets/panicle, number of panicles m⁻² and yield suggests the beneficial effect of effective timing of *Azolla* incorporation. It also suggests that *Azolla* should be incorporated at transplanting for maximum benefit to farmers, especially where temperatures are relatively low. The effect of interaction between time of incorporation and inorganic N application was not significant for all parameters except for spikelets panicle⁻¹.

The significant effects of Azolla incorporation were more pronounced during the second season. This can be attributed to relatively higher temperature and relative humidity, which may have enhanced mineralization of Azolla during vegetative stage of the second season. During the first season, average temperatures were lower (22°C) at growth stage and higher at reproductive (23.5°C) stage while in the second season, temperatures were higher at growth stage (22.9°C) and lower at reproductive stage (22.1°C). Relative humidity was also higher in second season (79%) than in the first (69%). Consequently, Azolla mineralization could have been faster in the second season leading to the response observed. These results are in concurrence with the findings of Subedi and Shrestha (2015) who reported that the rate of nutrient release upon decomposing Azolla

increases with increasing environmental temperatures and relative humidity.

Conclusion

Time of *Azolla* application affects growth and yield of paddy rice. Application of *Azolla* at planting is more beneficial to paddy rice as it increases both yield and yield components of paddy rice

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDIX

Appendix Table 1. Average temperatures, relative humidity and rainfall during 2015/2016.

| Season 1 | | | | | | | | | | | | | | | | |
|----------------|-----------------|------|------|------|------|-------|------|------|------|------|------|------|------|------|------|------|
| Month | Nov Dec Jan Feb | | | | | March | | | | | | | | | | |
| Week | W3 | W4 | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 | W1 | W2 |
| Max Temp. (°C) | 28.5 | 29.8 | 28.1 | 28.4 | 29.7 | 26.6 | 31.0 | 31.4 | 31.1 | 31.1 | 31.7 | 32.1 | 32.3 | 33.1 | 33.2 | 32.7 |
| Min Temp.(°C) | 17.2 | 16.0 | 15.3 | 14.9 | 14.9 | 15.3 | 13.7 | 13.6 | 14.6 | 14.1 | 14.3 | 15.0 | 16.5 | 15.1 | 13.8 | 14.6 |
| Av Temp.(°C) | 22.9 | 22.9 | 21.7 | 21.6 | 22.3 | 20.9 | 22.4 | 22.5 | 22.8 | 22.6 | 23.0 | 23.5 | 24.4 | 24.1 | 23.5 | 23.6 |
| RH (%) | 77.0 | 79.0 | 72.0 | 63.0 | 64 | 60.0 | 59.0 | 34.0 | 55.0 | 52.0 | 53.0 | 65.0 | 64.0 | 34.0 | 53.0 | 64.0 |
| Rainfall (mm) | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.8 | 3.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Season 2 | | | | | | | | | | | | | | | | |
| Month | Sep | | | | Oct | | | | Nov | | | | Dec | | | |
| Week | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 | W1 | W2 | W3 | W4 |
| Max Temp. (°C) | 27.2 | 28.8 | 29.0 | 29.4 | 29.9 | 30.6 | 30.7 | 31.0 | 30.3 | 29.7 | 26.9 | 27.4 | 28.0 | 28.1 | 29.1 | 28.9 |
| Min Temp.(°C) | 16.2 | 16.1 | 14.4 | 17.7 | 18.0 | 17.3 | 16.3 | 16.9 | 18.0 | 17.5 | 16.8 | 17.2 | 14.9 | 15.9 | 14.1 | 15.0 |
| Av Temp.(°C) | 21.7 | 22.4 | 21.7 | 23.6 | 23.9 | 23.9 | 23.5 | 23.9 | 24.2 | 23.6 | 21.8 | 22.3 | 21.5 | 22.0 | 21.6 | 22.0 |
| RH (%) | 82.0 | 78.4 | 80.0 | 78.0 | 80.9 | 75.3 | 71.9 | 74.1 | 79.3 | 85.3 | 88.7 | 85.4 | 80.3 | 79.8 | 76.4 | 77.8 |
| Rainfall (mm) | 0.0 | 0.0 | 0.0 | 6.3 | 0.6 | 0.1 | 0.0 | 0.8 | 2.1 | 0.8 | 13.5 | 4.2 | 0.2 | 2.8 | 1.0 | 0.5 |

W= week, temp=temperature, Max= maximum, Min=Minimum, Av= average, RH relative humidity.