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Effects of Azolla Incorporation Timing on Growth and Yield Performance of Spring Rice (*Oryza sativa* L.)

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A B S T R A C T

Spring rice in Nepal often faces nitrogen shortages during peak crop demand, limiting yield potential. Azolla, a nitrogen-fixing aquatic fern, offers an eco-friendly alternative, but its effectiveness depends on inoculation and incorporation timing. A field experiment was conducted in Sapahi, Bara, Nepal (March–June, 2023) using a Randomized Complete Block Design with seven treatments and three replications. Plots measured 2 × 3 m (6 m²). Treatments comprised Azolla inoculated at 25, 35, 45, 55, and 65 days after transplanting (DAT) with recommended P & K, a recommended NPK dose (RDF), and a control. Data on growth, yield, and yield components were recorded and analyzed using ANOVA in RStudio. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at the 5% significance level. RDF recorded the highest grain yield (4.25 t/ha) and effective tillers (302.67 m^{-2}), significantly higher than all other treatments ($p < 0.001$; LSD = 0.050). The best Azolla treatment, 25 DAT + P & K (4.02 t/ha), outperformed later Azolla applications and the control (1.87 t/ha). T1 also achieved more effective tillers (259.67m^{-2}), longer panicles (23.72 cm), and lower sterility (25.44%) than other Azolla timings ($p < 0.001$). Azolla application at 25 DAT with P & K improves yield and reduces reliance on synthetic nitrogen fertilizers, making it a sustainable option for spring rice production in Nepal.

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Introduction

Rice is an important cereal crop of Nepal that contributes to 13.6% of the country's overall agricultural GDP (MoALD, 2024c). The most common rice-growing technique in the nation is flooded rice. Spring rice is a better choice for cultivation in Nepal because of its shorter growth duration and higher yield potential (Ghimire et al., 2021). Furthermore, spring rice productivity is more on average than regular-season rice (Regmi et al., 2023). The majority of farmers use urea as a nitrogen source fertilizer (Marahatta, 2022). Due to the increasing population and shrinkage in cultivation areas, maintaining production to feed the people while ensuring good soil health is challenging (Devkota et al., 2023; M. Tahat et al., 2020). Approximately 426,007 metric tons of synthetic fertilizer were imported during the fiscal year 2022–2023, and 342,723 metric tons of synthetic fertilizer were sold overall in 2022–2023, but only 227,836 metric tons were sold in 2021–2022. The amount of synthetic fertilizer reduction in utilization was around 36,429 metric tons in the previous year (MoALD, 2024b). This heavy reliance on chemical fertilizers has a deleterious effect on soil health in the long run, posing a substantial threat to national food security (Chen et al., 2014; Cui et al., 2018; McLaughlin & Mineau, 1995). In addition, this dependence has exacerbated the denitrification losses in flooded rice fields, reducing the fertilizer use efficiency and contributing to greenhouse gas emissions (Hassan et al., 2022; Marti-Jerez et al., 2023). Furthermore, the indiscriminate use of chemical fertilizers has adversely affected the environment, leading to soil degradation and water pollution (Pahalvi et al., 2021). Despite large fertilizer imports, there is still a discrepancy between potential demand and actual supply. Moreover, the fertilizer shortage problem is severe at peak cropping periods, which has negatively impacted the yield (Palikhe et al., 2024). The uncertainty of fertilizer availability during peak crop demand has compelled farmers to purchase from informal sources at a comparatively higher price, thereby increasing input costs (Panta, 2018). This situation underscores the need for sustainable, eco-friendly, and cost-efficient alternatives to conventional fertilizer management (Marzouk et al., 2024; Ocloo et al., 2025). Azolla inoculation and integration reduce methane emissions and the amount of nitrogen fertilizer applied in a double rice cropping system (Xu et al., 2017). Recently, Biological approaches to nutrient management have garnered more attention as sustainable methods of minimizing soil deterioration and lowering the overuse of artificial fertilizers (Daisy et al., 2024). Biofertilizers could be a promising alternative as they are eco-friendly, cost-effective, and improve soil health (S. Kumar et al., 2022; Marzouk et al., 2024). The aquatic fern Azolla works well in flooded rice systems because it can fix atmospheric nitrogen through its symbiotic relationship with the cyanobacteria, i.e., *Anabaena azollae*, which has been widely used as a biofertilizer in flooded rice due to its well-thriving nature in flooded fields (Bhuvaneshwari & Singh, 2015; Lellinger, 1983). Due to its growing nature, it is more suitable for predominant rice cultivation practices in Nepal (Kandel et al., 2020). Azolla breaks down over three to eight weeks, releasing most of its stored nitrogen as ammonia (Marzouk et al., 2023). Additionally, azolla inhibits the growth of aquatic weeds, which lowers cultivation expenses. Azolla's addition improves the soil's organic carbon content and water-retention ability, among other qualities ((B. Kumar & Shahi, 2016). Similarly, Azizi & Safriani (2024) in their study found a maximum yield of 10.97 tonnes/ha when Azolla was applied at the rate of 4 tonnes/ha, as compared to the 7.27 tonnes/ha yield in the control treatment. Studies in Nepal's mid-hills conditions have shown that combining Azolla with recommended doses of phosphorus and potassium yields comparable results to full doses of N, P, and K fertilizers. Tuladhar (2004) reported a 17% rice grain yield increase with the combined use of Azolla with a nitrogen dose of 60 kg N/ha than nitrogen at the same dose.

However, the application timing greatly affects the efficiency of Azolla in rice fields (Oyange et al., 2020). The ideal time to apply Azolla as a biofertilizer and how it stacks up against traditional fertilizers in Nepalese field settings are still unknown, despite its great potential. Additionally, foliar application of Azolla Liquid Organic Fertilizer significantly increases rice growth and production in swampy soil (Rohayani et al., 2024). This gap needs to be filled to determine whether Azolla could be a useful, eco-friendly addition to or replacement for traditional nitrogen fertilizers in rice growing. This experiment aims to assess an efficient timing for Azolla inoculation and incorporation in rice fields and compare its effectiveness with traditional nitrogen fertilizers. This comparison helps to explore the potential of Azolla to meet crop nutrient demands while reducing reliance on chemical fertilizers and improving soil health.

Hypothesis

Null Hypothesis (H_o): There is no significant effect of Azolla inoculation and incorporation timing on the growth and yield of spring rice.

Alternative Hypothesis ($H_{1)}$: There is a significant effect of Azolla inoculation and incorporation timing on the growth and yield of spring rice.

Materials and Methods

Location and Research Site

The research was conducted on Kolhavi-4, Sapahi village of the Bara district of Nepal. The site's location is at a Latitude of 27.076 °E and a Longitude of 85.175 ^{0}N and an elevation of 131m above the mean sea level. Figure 1 and Figure 2 shows the study area location and agroclimatology conditions, respectively.

Nursery Establishment

Two to three rounds of ploughing and harrowing were used to prepare a nursery plot that was 4 meters by 1 meter (4 m²). This was followed by appropriate levelling to ensure consistent seedbed conditions. Hardinath-1, a spring rice variety, was planted in the prepared nursery on 22nd Falgun, 2079 (or 6th March, 2023). It is advised to grow the enhanced rice variety Hardinath-1 up to 800 meters above sea level in the Terai, Inner Terai, and river basin regions. With a 120-day crop length, this variety has a potential productivity of 4.6 metric tons per hectare (MoALD, 2024a).

Land Preparation and Field Layout

The field was prepared by using a Spring-tine harrow is a primary tillage equipment. After a week of water stagnation, the last puddling was done with a rotavator. The field area was leveled and arranged into a bund and a small plot. 30-day-old seedlings at the rate of 3 seedlings per hill were transplanted manually.

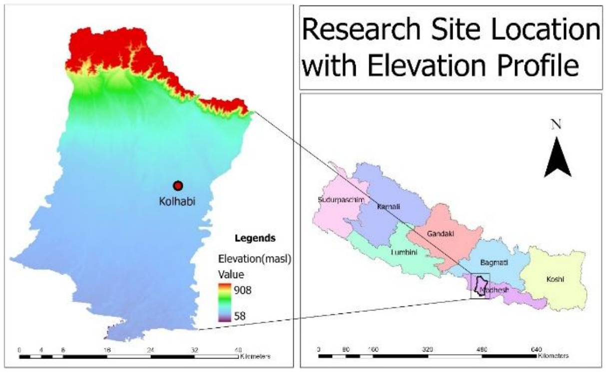

Figure 1. Location of research site
(Image created using ArcGIS Pro Ver 3.1)

Azolla Cultivation

A pond of size 1.5×1.5 m and 0.3 m depth was prepared and leveled. A sturdy 2.75×2.75 m plastic sheet was used to cover the pond. The soil was 0.025–0.05 m (1-2 inches) deep and uniformly spread out throughout the plastic sheet. Next, 0.5 kg of Single Super Phosphate (SSP) in water and 5 kg of cow dung that had been there for 5–10 days were evenly spread over the soil. After that, the pond's water level was kept between three and four inches. Azolla culture (0.5 kg) was spread out in the pond. A shade net that was 0.75 m high and 3 × 3 m was then placed over the pond.

Experimental Details

The experiment was set up using a Randomized. Complete Block Design (RCBD) with 7 treatments and replicated thrice. The dimensions of each plot were 2 m by 3 m (6 m²).

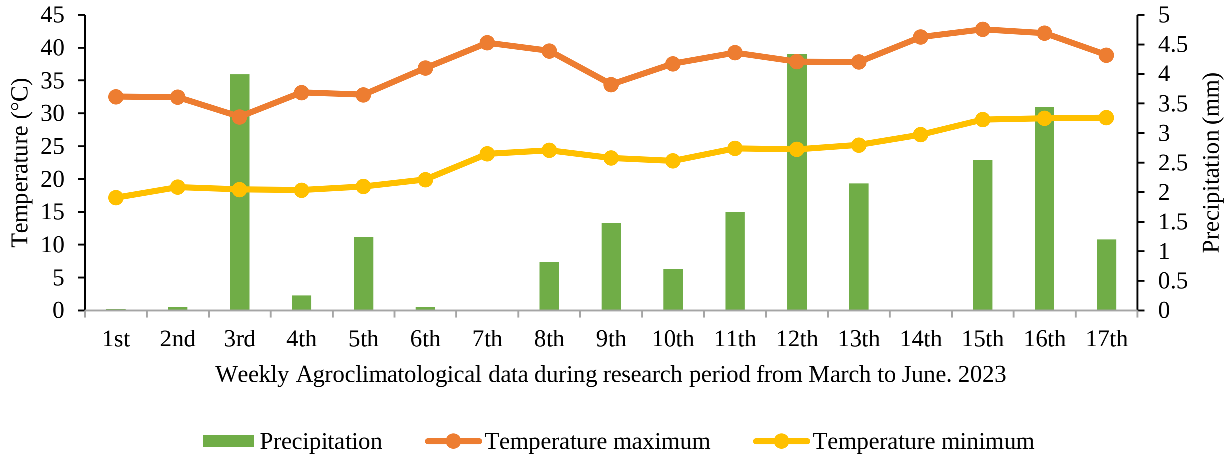

Figure 2. Agroclimatology data of the study site during the four-month research period
(March first week to June last week, 2023, Source: NASA Power Data Viewer)

The crop was planted 20 cm apart for uniformity. To minimize inter-plot interference along with proper field management, a distance of 0.4 m between replications and 0.3 m between treatments was also kept. Treatment details and Sources of Fertilizer in Different Treatments are given in Tables 1 and 2, respectively.

Dose of fertilizers and Azolla (Treatment Application)

The rate of azolla inoculation was 500 kg/ha (300 g/plot). 30 g of MOP and 112.5 g of SSP were applied basally to plots treated with Azolla. Thirty g of MOP, 40 g of DAP, and 130 g of urea were employed in RDF plots; half of the urea and the entire doses of MOP and DAP were applied basally. During the panicle start and tillering phases, two equal splits of the remaining half of the urea were applied.

Observations

Plant Height

From each plot, a random selection of ten plants was made, and height was recorded at a regular interval of 15 days beginning on the 20 DAT and continued up to the physiological maturity stage. Measurement of plant height from the base of the main tillers to the top of the longest leaf. Plant height for each treatment was calculated as the average of 10 plants.

No. of Tillers per Square Meter

The total no. of tillers was counted from 20 DAT until the physiological maturity stage, with 15-day intervals between counts. For the number of tillers, three hills from each plot's second row were used, and the mean was calculated.

Panicle Length

Panicle length was measured from the panicle base node to the end of the panicle and was collected before harvesting.

Number of Effective Tillers per Square Meter

Before harvesting the crop, the total number of effective tillers in a row within the net plot area was recorded in every plot, and then converted into per m^2.

Number of Filled Grains per Panicle

Ten panicles were randomly selected, and the grains were appropriately separated and counted. By counting the number of filled and unfilled grains from 10 randomly selected panicles in each plot, the number of grains per panicle and the sterility % were calculated concurrently.

Sterility percentage

The following formula was used to get the sterility percentage:

$$\text{Sterility percentage} = \frac{\text{Number of unfilled grain}}{\text{Total number of grains}} \times 100\%$$

Thousand Grain Weight (TGW)

From the randomly taken thousand grains, thousand-grain weights (TGW) were taken out and weighed using a portable automatic electronic balance. To calculate TGW, five sample seed lots from each treatment were used. The treatment's grain weight was determined by averaging the five samples' weights.

Table 1. Treatment details used in the experiment

Treatment Code	Treatment Detail
T1	Azolla inoculation (after transplanting) and incorporation at 25 DAT + Recommended dose of P & K
T2	Azolla inoculation at 10 DAT and incorporation at 35 DAT + Recommended dose of P & K
T3	Azolla inoculation at 20 DAT and incorporation at 45 DAT + Recommended dose of P & K
T4	Azolla inoculation at 30 DAT and incorporation at 55 DAT + Recommended dose of P & K
T5	Azolla inoculation at 40 DAT and incorporation at 65 DAT + Recommended dose of P & K
T6	Recommended dose of NPK (100:30:30 kg/ha)
T7	Control (No fertilizer and Azolla)

Table 2. Source of fertilizer used in different treatments

Treatment	N (Urea)	Phosphorus(P)	Potassium (K)
1.	Azolla	SSP	MOP
2.	Azolla	SSP	MOP
3.	Azolla	SSP	MOP
4.	Azolla	SSP	MOP
5.	Azolla	SSP	MOP
6.	Urea	DAP	MOP
7.	-	-	-

Note: SSP, Single Super Phosphate; DAP, Diammonium Phosphate; MOP, Muriate of Potash

Table 3. Effect of Azolla incorporation time on the Plant height of spring rice (Oryza sativa L.)

Treatments	Plant height (cm)				
	20DAT	35DAT	50DAT	65DAT	80DAT
T1-Azolla at 25DAT + P&K	35.05^{b}	44.41	54.52^{b}	75.54	81.21^{b}
T2-Azolla at 35DAT + P&K	33.65^{bc}	44.48	50.95^{c}	74.45	79.66^{bc}
T3-Azolla at 45DAT + P&K	33.93^{bc}	43.62	49.99^{c}	74.80	78.86^{c}
T4-Azolla at 55DAT + P&K	33.25^{c}	44.14	51.84^{bc}	74.03	78.36^{c}
T5-Azolla at 65DAT + P&K	33.96^{bc}	44.30	49.01^{c}	74.31	78.16^{c}
T6- RDF @100:30:30 NPK kg/ha	38.62^{a}	51.98	65.33^{a}	82.81	89.63^{a}
T7-control (no Azolla &Fertilizer)	31.43^{d}	42.23	48.39^{c}	68.23	69.55^{d}
SEm (±)	0.205	0.72	1.14	1.094	0.318
LSD (0.05)	1.397	2.615	3.29	3.223	1.738
CV %	2.29	3.26	3.49	2.42	1.23
F Probability	***	NS	***	NS	***
Grand Mean	34.27	45.02	52.86	74.88	79.35

Note: DAT, Days After Transplanting; SEm, Standard Error of Mean; LSD, Least Significant Difference; CV, Coefficient of Variation; NS, Non-significant; *** represents significant at 1% level and * represents significant at 5% level of Significance. Treatment means followed by the same letter(s) are not significantly different on the Duncan multiple range test at the 0.05 level of significance.

Grain Yield

Each plot was gathered from a 1 m^2 quadrant box. After drying and threshing, the grain was cleaned, sun-dried once more, and its final weight was recorded. Grain yield per hectare was determined for each treatment by using the net plot yields. The grain's percentage of moisture was noted by the Digital Moisture Meter. Finally, the formula proposed by (Shrestha et al., 2021) was used to adjust grain yield at 12% moisture.

$$GY = \frac{(100 - \text{MC})\ \% \times \text{NPY (kg)} \times 10000\ (\text{m2})}{(100 - 12)\ \% \times \text{net plot area (m2)}}$$

Where MC stands for the Moisture content of the fresh grain yield.

GY : Grain yield (kg/ha) at 12% moisture

NPY : net plot yield

Straw Yield

Each net plot area's straw was sun-dried, and the dry weight was calculated and converted to tons per hectare.

Harvest Index

Harvest index (HI) was calculated by using the following formula.

HI% = (grain yield × 100)/ (grain yield + straw yield)

Data Analysis

The data was systematically collected and arranged based on observed parameters. MS-Excel and R Studio were used for the data analysis. The results were then interpreted and compared with existing literature.

Results and Discussion

The research's findings were examined and presented in this section, where tables and figures were used where needed. The collected results were examined together with potential outcomes and supporting evidence from the literature.

Plant Height

The data for plant height were collected at 20 DAT, 35 DAT, 50 DAT, 65 DAT, and 80 DAT. The effect of the time of Azolla inoculation and incorporation in combination with a recommended dose of phosphorus and potassium on plant height, as shown in Table 3

At 20, 50, and 80 DAT, treatments significantly affected plant height; however, at 35 and 65 DAT, the changes were not statistically significant. At 20 DAT, the highest plant height (38.62) was obtained by T6 (RDF @100:30:30 NPK kg/ha), which was noticeably higher than all other treatments. T7 (control) reported the lowest (31.43), followed by T1 (Azolla at 25 DAT + P&K) with 35.05. Similar trends were observed at 50 DAT, with T6 further exceeding other Azolla-based treatments (65.33), T1 surpassing them (54.52), and T7 again showing the lowest height (48.39); these differences were significant (LSD = 3.29, F = ***). T6 was still the tallest plant at 80 DAT (89.63) with an LSD of 1.738, significantly higher than T1 (81.21), which was superior to T7 (69.55). Even though there were no such significant differences in plant height at 35 and 65 DAT, T6 consistently showed numerically larger values at all intervals. When compared to later applications, T1 (Azolla at 25 DAT + P&K) performed better at every stage, indicating that early Azolla with P&K integration and improved plant height.

The experiment shows that the time of Azolla incorporation had a significant effect on plant height. The significant effect of Azolla integration time on rice growth stages can be attributed to the relatively slow rate of nutrient uptake caused by its gradual mineralization rate (Bazihizina et al., 2025). The plants in the Azolla-inoculated field initially did not grow well because N, which was derived from soil and N-fixation, was necessary for Azolla growth and multiplication. The recommended dose of fertilizer (RDF) treatment showed the highest growth as compared to the Azolla treatment because it supplied adequate nitrogen at both the split and basal application, which resulted in higher growth. The fast growth is due to the rice crop's critical stage of nitrogen fulfillment and the plant's simple access to nitrogen. (Ghimire et al., 2021). As compared to Azolla incorporation, which needs time to decompose and release N to rice (Bazihizina et al., 2025).

Number of Tillers Per Hill

The number of tillers per hill was counted at 20, 35, 50, 65, and 90 days after transplanting. together with the recommended potassium and phosphorus dosages. ,(Table 4 illustrates the impact of the timing of Azolla introduction on the number of tillers per hill).

Over time, there was a considerable variation in the number of tillers per hill between treatments. The highest tillers (12.00) were observed at 20 DAT by T6 (RDF @100:30:30 NPK kg/ha), followed by 8.4 by T1 (Azolla at 25 DAT + P&K) and 6.66 by T7 (control); these differences were statistically significant (LSD = 1.30, F = ***). The differences were not significant (NS) at 35 and 50 DAT, despite T6 continuing to display numerically higher tillers (14.2 and 16.26). T6 once more had a great number of tillers (14.96 and 13.86) at 65 and 80 DAT, followed by T1 (11.13 and 10.70), and T7 was still the lowest (6.86 and 6.3). At 65 and 80 DAT, the LSD values were 0.762 and 0.668, respectively, and the F values were very significant (***). The best effect on tillering was seen by the early application of Azolla at 25 DAT (T1), which consistently performed better than later applications. Hence, Azolla applied at 25 DAT together with P & K (T1) was the best combination, continuously outperforming other Azolla treatments in tiller production at every stage.

Yield attributing Character of Rice

The impact of Azolla integration time on yield-attributable characteristics, such as panicle length (PL), total grain weight (TGW), filled grains per panicle (FGp), and effective tiller (ET), Sterility (%) is illustrated in Table 5

Treatments varied significantly in terms of effective tillers/m^2, panicle length, filled grains per panicle, and sterility %, but not in terms of 1000-grain weight. T6 (RDF @ 100:30:30 NPK kg/ha) produced the most productive tillers (302.67 m^{-2}), followed by T1 (Azolla at 25 DAT + P&K) with 259.67 m^{-2} and T7 (control) with the least productive (118.13 m^{-2}) (LSD = 22.851; F = ***). The panicles of T6 and T7 were the longest and shortest, respectively, at 24.40 cm and 20.98 cm (LSD = 0.685; F = ***). The advantages of early Azolla application with P & K were demonstrated by the fact that T1 was the most effective Azolla treatment, while T6 yielded the best overall results. According to LSD = 5.650; F = ***, T6 had the most filled grains per panicle (135.00), followed by T1 (111.30) and T7 (46.60). T6 (22.02%) and T1 (25.44%) had a substantially lower sterility percentage than T7 (53.91%) (LSD = 2.036; F = ***).

Effective Tiller /m^2

The treatments have a major effect on the quantity of effective tillers per square meter. All treatments associated with Azolla had many more tillers per square meter than the control. When the recommended dosage of chemical fertilizer (302.67) was applied, followed by the integration of Azolla at 25 DAT (259.67), the effective tiller per square meter was found to be much greater. Azolla integrated at 25 DAT had a significantly higher effective tiller per square meter than Azolla integrated at 35, 45, 55, and 65 DAT.

Panicle Length

The length of the panicles has been significantly impacted by the treatment. The panicles were noticeably longer than the control in all Azolla-related treatments. However, using the recommended dosage of chemical fertilizer (24.40 cm) and then adding Azolla at 25 DAT (23.72 cm) resulted in a noticeably longer panicle. Among the different dates of incorporation, the panicle length of Azolla incorporated at 25 DAT was significantly longer than that of Azolla incorporated at 35, 45, 55, and 65 DAT.

Table 4. Effect of Azolla incorporation time on tiller number of spring rice (Oryza sativa L.)

Treatments	No of Tillers per hill				
	20DAT	35DAT	50DAT	65DAT	80DAT
T1-Azolla at 25DAT + P&K	8.4^{b}	10.13	12.66	11.13^{b}	10.70^{b}
T2-Azolla at 35DAT + P&K	8.1^{b}	9.33	10.63	10.13^{c}	9.63^{c}
T3-Azolla at 45DAT + P&K	7.96^{bc}	9.40	10.10	9.13^{d}	8.66^{d}
T4-Azolla at 55DAT + P&K	7.46^{bc}	8.56	10.06	9.23^{d}	8.8^{d}
T5-Azolla at 65DAT + P&K	7.86^{bc}	9.70	10.23	9.60^{cd}	9.30^{cd}
T6- RDF @100:30:30 NPK kg/ha	12.00^{a}	14.2	16.26	14.96^{a}	13.86^{a}
T7-control (no Azolla &Fertilizer)	6.66^{c}	7.93	8.30	6.86^{e}	6.3^{e}
SEm (±)	0.178	0.421	0.196	0.061	0.047
LSD (0.05)	1.30	2.00	1.366	0.762	0.668
CV %	8.74	9.90	6.86	4.21	3.91
F Probability	***	NS	NS	***	***
Grand Mean	8.35	9.90	11.18	10.15	9.60

Note: DAT, Days After Transplanting; SEm, Standard Error of Mean; LSD, Least Significant Difference; CV, Coefficient of Variation; NS, Non-significant; *** represents significant at 1% level and * represents significant at 5% level of Significance. Treatment means followed by the same letter(s) are not significantly different on the Duncan multiple range test at the 0.05 level of significance.

Table 5. Effect of Azolla incorporation time on different yield attributing characters of spring rice (Oryza sativa L.)

Treatments	Yield attributing characters				
	Effective tiller/m^2	Panicle length (cm)	Filled grains /panicle	Sterility (%)	1000 grain weight (g)
T1-Azolla at 25DAT + P&K	259.67^{b}	23.72^{ab}	111.30^{b}	25.44^{c}	22.70
T2-Azolla at 35DAT + P&K	234.67^{c}	23.53^{bc}	109.00^{bc}	26.33^{c}	22.73
T3-Azolla at 45DAT + P&K	201.00^{d}	23.01^{bc}	99.83^{d}	29.00^{b}	23.18
T4-Azolla at 55DAT + P&K	208.00^{d}	23.03^{bc}	104.40^{cd}	27.57^{bc}	22.80
T5-Azolla at 65DAT + P&K	220.67^{cd}	22.84^{c}	107.47^{bc}	26.65^{c}	23.83
T6- RDF @100:30:30 NPK kg/ha	302.67^{a}	24.40^{a}	135.00^{a}	22.02^{d}	22.71
T7-control (no Azolla &Fertilizer)	118.13^{e}	20.98^{d}	46.60^{e}	53.91^{a}	22.11
SEm (±)	55	0.049	3.363	0.436	0.032
LSD (0.05)	22.851	0.685	5.650	2.036	0.556
CV %	5.820	1.670	3.115	3.797	1.377
F Probability	***	***	***	***	NS
Grand Mean	165.0	23.07	101.94	30.13	22.726

Note: DAT, Days After Transplantation; SEm, Standard Error of Mean; LSD, Least Significant Difference; CV, Coefficient of Variation; NS represents non-significant, *** represents significant at 1% and * represents significant at 5% level. According to the Duncan multiple range test, treatment means that are followed by the same letter or letters do not differ substantially at the 0.05 level of significance.

Table 6. Different effects of time of Azolla incorporation on spring rice (Oryza sativa L)

Treatments	Yield			
	Grain yield (ton/ha	Straw yield (ton/ha)	Biological yield (ton/ha)	Harvest index (%)
T1-Azolla at 25DAT + P&K	4.02^{b}	5.12^{ab}	9.14^{ab}	45.72^{b}
T2-Azolla at 35DAT + P&K	3.97^{b}	5.16^{a}	9.137^{ab}	44.97^{bc}
T3-Azolla at 45DAT + P&K	3.79^{d}	5.163^{a}	8.95^{c}	43.03^{de}
T4-Azolla at 55DAT + P&K	3.83^{d}	5.17^{a}	9.00^{bc}	42.77^{e}
T5-Azolla at 65DAT + P&K	3.91^{c}	5.19^{a}	9.10^{ab}	44.27^{cd}
T6- RDF @100:30:30 NPK kg/ha	4.25^{a}	4.99^{b}	9.24^{a}	47.34^{a}
T7-control (no Azolla &Fertilizer)	1.87^{e}	3.30^{c}	4.56^{d}	31.47^{f}
SEm (±)	0.0002	0.0018	0.0018	0.185
LSD (0.05)	0.050	0.131	0.131	1.326
CV %	0.79	1.51	0.872	1.74
F Probability	***	***	***	***
Grand Mean	3.57	4.87	8.45	42.80

Note: DAT, Days After Transplantation; Sem, Standard Error of Mean; LSD, Least Significant Difference; CV, Coefficient of Variation; *** represents significant at 1% level and * represents significant at 5% level. Treatment means followed by the same letter(s) are not significantly different on the Duncan multiple range test at the 0.05 level of significance.

Number of Filled Grains per Panicle

Significant effects of the treatments were observed on the number of filled grains per panicle. In every Azolla-related treatment, there were substantially more full grains per panicle than in the control. However, using the recommended dose of chemical fertilizer (135.00) and then incorporating Azolla at 25 DAT (111.30) resulted in a much larger number of filled grains per panicle. The number of filled grains per panicle was substantially larger for Azolla incorporated at 25 DAT than for Azolla incorporated at 35, 45, 55, and 65 DAT, across the various times of incorporation.

The slow rate of mineralization, which makes decomposition to be slow, leading to gradual availability of tissue nutrients (Marzouk et al., 2023). When compared to Azolla treatments, the recommended fertilizer treatment showed noticeably more full grains per panicle because RDF provided enough nitrogen at basal and split application, which was the cause of the higher filled grains per panicle. The increase in filled grains per panicle was due to the fulfillment of N to the rice crop in the critical stage and easily available N (Bhattarai et al., 2024) as compared to Azolla incorporation, which needs time to decompose and release N to rice (Watanabe et al., 1981).

Sterility Percentage

Sterility percentage was significantly impacted by the treatments. The percentage of sterility in all Azolla-associated therapies was much lower than in the control. However, using the prescribed dosage of chemical fertilizer (22.02%) and then incorporating Azolla at 25 DAT (25.44%) resulted in a substantially reduced sterility percentage. In comparison to Azolla incorporated at 35 DAT (26.33%) and 65 DAT (26.65%), Azolla incorporated at 25 DAT exhibited the lowest sterility percentage across the various Azolla incorporation dates.

Thousand Grain Weight (TGW)

Treatments showed no significant effect on TGW. It is a genetic trait that is less influenced by external factors such as nitrogen availability or fertilizer application (Yin et al., 2024). The fact that Azolla does not affect TGW indicates that, although it greatly enhances other yield indices, grain size and quality are unaffected. In comparison to traditional fertilizer-based systems, farmers using Azolla-based systems can attain marketable grain quality and which helps to this consistency in grain weight.

Yield

Significant differences ($p < 0.001$) were seen in all yield parameters across treatments. The best harvest index (47.34%), biological yield (9.24 t/ha), and grain yield (4.25 t/ha) were all found in T6 (RDF @100:30:30 NPK kg/ha). The second-highest harvest index (45.72%) and grain yield (4.02 t/ha) were found in T1 (Azolla at 25 DAT + P&K). The control (T7) produced the least amount of grain (1.87 t/ha) and biological yield (4.56 t/ha), and it had the lowest harvest index (31.47%). For most treatments, the straw yield was statistically comparable, except for T6, which showed a little lower value (4.99 t/ha).

Grain Yield

The recommended chemical fertilizer dose (RDF, 4.25 t/ha) produced a significantly higher grain yield than Azolla incorporation at 25 DAT + P&K (4.02 t/ha), with a mean difference of 0.23 t/ha exceeding the LSD value (0.05 t/ha) at the 5% significance level ($p < 0.001$). The grain yield from Azolla integration at 25 DAT was considerably higher than that from Azolla incorporation at 35 DAT (3.97t/ha), which is statistically comparable.

The timing of Azolla incorporation markedly influenced rice yield, largely because nutrient release from Azolla occurs slowly as its biomass mineralizes over time (Consorti et al., 2024).

According to Marzouk et al. (2023), 60% of the tissue N is released within the first four weeks. Recommended dose of Fertilizer Treatment showed maximum yield as compared to Azolla treatments, as RDF supplied sufficient nitrogen at basal and split application, which was responsible for higher yield. In contrast to Azolla inclusion, which requires time to break down and release nitrogen to the rice (Consorti et al., 2024). The rice crop's key stage of nitrogen fulfillment and readily available nitrogen to plants are the reasons for the yield increase (Sarkar et al., 2023).

Azolla incorporated at 25 DAT demonstrated a noticeably larger yield than Azolla incorporated at 35, 45, 55, and 65 DAT, among other times of inoculation. The earlier introduction of Azolla (25 DAT) ensured a higher N supply to rice than the latter, as the latter may have led to an insufficient supply of nitrogen to the soil and, thus, to the rice plant.

Straw Yield

The effects of the treatments on straw yield were substantial. All Azolla-associated treatments had a significantly higher straw yield than the control. However, the addition of Azolla at 65 DAT (5.19t/ha) and 55 DAT (5.17t/ha) resulted in a noticeably greater straw yield.

Biological Yield

The time of Azolla introduction has a major effect on the biological yield of rice plots. The control plots, devoid of fertilizers or Azolla, produced a minimal biomass of 3.30 tons per hectare. At the same time, the maximum biological yield (9.24 tons/ha) was achieved only by utilizing chemical fertilizer. The highest biological yield throughout this time was 9.14 tons/ha from azolla inclusion at 25 DAT, which was statistically equal to 9.147 tons/ha from azolla incorporation at 35 DAT and 9.10 tons/ha from azolla incorporation at 65 DAT.

Harvest Index

The harvest index was significantly impacted by the timing of Azolla introduction in rice plots. Using solely chemical fertilizer produced the highest harvest index (47.34%), while using neither chemical fertilizer nor Azolla produced the lowest harvest index (31.47%). The maximum harvest index was achieved at 25 DAT (45.72%) and 35 DAT (44.97%) when Azolla was added at different dates.

Implications of the Findings

According to the study, the production and yield attributing traits of spring rice were greatly affected by the timing of fertilization application with Azolla, along with potassium and phosphorus. The 25DAT (T1) treatment has what it takes to beat all successive Azolla treatments when it comes to plant height, number of tillers, effective tillers/m^2, panicle length, filled grains per panicle; however, RDF (T6) would continue to outperform others. In every measure, Treatment T6, which was given the recommended dosage of fertilizer, continuously demonstrated the best results, it seemed like the most effective azolla-based treatment would reduce early growth performance due to large nutrient release retardation from enhanced mineralization of Azolla. However, RDF fared better than others due to its substantial and immediate nitrogen delivery.

Conclusion

This study confirms that the timing of Azolla application plays a crucial role in optimizing rice growth and yield. Early inoculation at 25 DAT, combined with recommended phosphorus and potassium levels (T1), consistently improved plant height, tiller production, panicle length, filled grains per panicle, and reduced sterility, outperforming the control and later Azolla applications. While the recommended dose of fertilizer (RDF–T6) achieved the highest yield due to immediate nitrogen availability, T1 emerged as a sustainable alternative that can reduce dependence on synthetic nitrogen fertilizers. Azolla application beyond 35 DAT is not advisable due to delayed nutrient release and reduced crop performance.

Future studies are encouraged to incorporate plant nutrient analyses to provide a more comprehensive understanding of the effects of Azolla incorporation timing on rice growth and yield.

Declarations

Ethical approval

Since the study featured agronomic field tests on rice, ethical approval was not necessary. Every principle and procedure adhered to accepted standards for agricultural research.

Data availability

All pertinent information is contained in the article and its supporting resources, and it is all kept confidential and safe with the author.

Consent for publication

Since there are no human subjects or personal data in the study, consent for publishing is not applicable.

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