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Effect of different levels of nitrogen and bio-fertilizers on growth, yield and economics of Rice (*Oryza sativa* L.)

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Abstract

Rice (*Oryza sativa* L.) is the world's most important staple cereal crop, contributing substantially to food security across Asia. Nitrogen nutrition and bio-fertilizer inoculation are pivotal determinants of rice productivity, yet their integrated management remains under-optimized in semi-arid Punjab conditions. A field experiment was conducted during the *kharif* season (June–September 2025) at the Agricultural Research Farm of Guru Kashi University, Talwandi Sabo (Bathinda), Punjab, India, using a Randomized Block Design with nine treatments replicated three times. Treatments comprised four nitrogen levels (75, 100, 125 and 150% of the recommended dose of fertilizer, RDF) applied alone or combined with *Azospirillum* biofertilizer, along with an unfertilized control. Results demonstrated that integrated application of nitrogen and *Azospirillum* significantly enhanced all growth indices, yield attributes, and economic returns compared to the control and sole nitrogen treatments. Treatment T₇ (100% N + *Azospirillum*) consistently recorded the tallest plants (93.13 cm at harvest), highest number of tillers hill⁻¹ (26.30), greatest leaf area index (4.61 at harvest) and highest dry matter accumulation (101.72 g hill⁻¹). Treatment T₈ (125% N + *Azospirillum*) achieved the maximum grain yield (65.6 q ha⁻¹), biological yield (149.8 q ha⁻¹), grains panicle⁻¹ (204.6) and test weight (22.7 g). Economic analysis indicated that T₄ (125% N alone) recorded the maximum net return (₹1,25,460 ha⁻¹), while T₂ (75% N + *Azospirillum*) achieved the highest benefit–cost ratio (2.72). These findings underscore that combining nitrogen fertilization with *Azospirillum* biofertilizer can sustain or improve rice productivity while enhancing economic efficiency in irrigated rice systems of Punjab, India.

Keywords: *Azospirillum*, bio-fertilizers, grain yield, integrated nutrient management, nitrogen, *Oryza sativa*

1. Introduction

Rice (*Oryza sativa* L.), a member of the Poaceae family, is the primary staple food for more than 60% of the global population, occupying over 43.42 million hectares worldwide and contributing approximately 25% of global digestible caloric intake and 20% of total dietary protein [1, 2]. India ranks second globally in rice production, accounting for approximately 21% of world output, with Punjab alone contributing 205.24 lakh tonnes of paddy across 31.68 lakh hectares during 2022-23 [3]. The rice–wheat cropping system of the Indo-Gangetic Plains remains central to India's food security, necessitating sustained productivity improvements to meet the demands of a projected global population of 9 billion by 2050 [4].

Nitrogen (N) is the most

critical macronutrient for rice growth, influencing amino acid and chlorophyll synthesis, tiller production, leaf area index (LAI), photosynthetic rate, and ultimately grain yield [5]. However, low nitrogen use efficiency (NUE) in flooded rice paddies arising from denitrification, volatilization, leaching and runoff poses significant agronomic and environmental challenges [6]. Excessive N application can increase susceptibility to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) and blast (*Piricularia oryzae*) causing up to 50-100% yield losses under severe conditions, and contributes to reactive nitrogen pollution and greenhouse gas emissions [7, 8].

Bio-fertilizers incorporating plant growth-promoting rhizobacteria (PGPR) such as *Azospirillum* offer a sustainable strategy to complement mineral N by fixing atmospheric nitrogen, producing phytohormones (indole-3-acetic acid, gibberellins, cytokinins), solubilizing phosphorus, and enhancing root architecture and nutrient absorption [9, 10]. Integrated nutrient management

(INM) combining reduced mineral N with bio-fertilizers has been reported to achieve partial N substitution (10-30%) without yield penalty, improve microbial biomass and soil organic carbon, and raise net economic returns ^[11, 12]. Despite growing evidence for INM benefits, optimum combinations of nitrogen rates and *Azospirillum* inoculation remain site- and cultivar-specific. Limited systematic data exist for the PR-131 variety grown under semi-arid Punjab conditions. The present study was therefore designed to: (i) determine the effect of nitrogen levels on growth and yield of rice; (ii) evaluate the combined effect of nitrogen and *Azospirillum* on crop performance; and (iii) assess the economic implications of various nutrient management strategies.

2. Materials and Methods

2.1 Experimental site and season

The field experiment was conducted during the *kharif* season (June-September 2025) at the Agricultural Research Farm, Guru Kashi University, Talwandi Sabo, Bathinda, Punjab, India (29°57’N, 75°7’E; altitude 213 m above sea level). The site has a semi-arid subtropical climate with extreme summers and mild

winters, receiving most annual rainfall during June-August. Maximum temperature peaked at 43.2°C in July and minimum temperature at 19.6°C in June; total rainfall during the experiment was 441.6 mm (Table 1).

Table 1: Meteorological data during the experimental period (June–September 2025), Talwandi Sabo, Bathinda

Month	T max (°C)	T min (°C)	RH (%)	Rainfall (mm)
June 2025	42.5	19.6	43.0	80.8
July 2025	43.2	26.0	47.0	190.5
August 2025	39.2	25.0	57.0	170.5
September 2025	29.8	21.0	70.0	170.3

2.2 Soil characteristics

Prior to planting, composite soil samples (0-15 cm) were collected from five spots in each experimental block, air-dried, sieved (2 mm) and analyzed using standard methods (Table 2). The soil was sandy loam, near-neutral in reaction (pH 7.15), low in organic carbon (0.36%) and available nitrogen (129.1 kg ha⁻¹), medium in available phosphorus (21.8 kg P₂O₅ ha⁻¹), and high in available potassium (248.7 kg K₂O ha⁻¹).

Table 2: Physical and chemical properties of the experimental soil (0–15 cm depth)

Property	Value	Method
pH	7.15	Glass electrode pH meter (Jackson 1967)
EC (dS m ⁻¹)	0.25	Conductivity meter, 1:2 soil:water suspension (Jackson 1967)
Organic carbon (%)	0.36	Walkley and Black rapid titration (Jackson 1967)
Available N (kg ha ⁻¹)	129.1	Alkaline KMnO ₄ method (Subbiah & Asija 1956)
Available P ₂ O ₅ (kg ha ⁻¹)	21.8	0.5 N NaHCO ₃ extractable (Olsen <i>et al.</i> , 1954)
Available K ₂ O (kg ha ⁻¹)	248.7	1N NH ₄ OAc extractable (Jackson 1967)

2.3 Experimental design and treatments

A Randomized Block Design (RBD) with nine treatments and three replications was employed. Each plot measured 6 m × 4 m (gross) with a net plot area of 5 m × 3 m. The rice variety PR-131 (Punjab Agricultural University), a high-yielding, short-duration (123-25 days) cultivar suited to water-scarce conditions, was used throughout. Treatments are detailed in Table 3. The recommended dose of nitrogen (RDF) for rice in Punjab is 120 kg N ha⁻¹ ^[13].

Table 3: Treatment details

Symbol	Treatment description
T ₁	Control (no fertilizer)
T ₂	N @ 75% RDF (90 kg N ha ⁻¹)
T ₃	N @ 100% RDF (120 kg N ha ⁻¹)
T ₄	N @ 125% RDF (150 kg N ha ⁻¹)
T ₅	N @ 150% RDF (180 kg N ha ⁻¹)
T ₆	N @ 75% RDF + <i>Azospirillum</i>
T ₇	N @ 100% RDF + <i>Azospirillum</i>
T ₈	N @ 125% RDF + <i>Azospirillum</i>
T ₉	N @ 150% RDF + <i>Azospirillum</i>

2.4 Agronomic management

Nursery beds (2 m × 2 m) were raised with high-germination seeds; urea was applied in two splits (at seeding and 10 days later). Phosphorus (60 kg P₂O₅ ha⁻¹ as single superphosphate) and potassium (40 kg K₂O ha⁻¹ as muriate of potash) were incorporated basally during final land preparation. Nitrogen was applied as urea in three equal splits: one-third each at basal, tillering and panicle initiation stages. For bio-fertilizer treatments, seedling roots were dipped in an *Azospirillum* suspension (10 g L⁻¹) for 20–30 minutes prior to transplanting. Twenty-five-day-old seedlings were transplanted at 20 cm × 15

cm spacing (two to three seedlings hill⁻¹). Gap filling was completed within one week. Plots were flood-irrigated, maintaining 2-3 cm water depth up to panicle initiation stage. Manual weeding was performed twice (21 and 35 days after transplanting, DAT). A minor leaf folder incidence was managed by spraying chlorpyrifos at 2 mL L⁻¹.

2.5 Observations recorded

Five randomly tagged plants per plot were used to record growth measurements at 30, 60 and 90 DAT and at harvest. Plant height (cm) was measured from ground level to the tip of the tallest leaf. Tiller count per hill was recorded at each sampling stage and at maturity. Leaf area index (LAI) was calculated as total leaf area per unit ground area. Dry matter accumulation (g hill⁻¹) was determined by oven-drying fresh samples at 70°C for 24 hours. At physiological maturity, panicle length (cm), number of grains at panicle⁻¹ and test weight (1000-grain weight, g) were recorded. Grain yield, straw yield and biological yield were expressed in q ha⁻¹. Harvest index (HI) was calculated as: HI (%) = (grain yield / biological yield) × 100.

2.6 Economic analysis

Cost of cultivation, gross return, net return and benefit-cost (B:C) ratio were calculated based on prevailing market prices at the time of harvest. Net return = gross return - cost of cultivation; B:C ratio = gross return / cost of cultivation.

2.7 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using a Randomized Block Design as described by Cochran and Cox ^[14]. Treatment means were compared using the least significant difference (LSD) at p = 0.05.

3. Results and Discussion

3.1 Plant height

Plant height increased progressively from 30 DAT through harvest across all treatments (Table 4). At harvest, T₇ (100% N + *Azospirillum*) recorded the tallest plants (93.13 cm), followed by T₉ (89.50 cm) and T₈ (89.24 cm); the control T₁ recorded the shortest (61.07 cm). The positive effect of combined N and bio-fertilizer on plant height is attributable to enhanced nitrogen availability, improved root architecture from *Azospirillum*-produced auxins (IAA), and stimulated vegetative elongation [15, 16]. These results align with findings of Wijebandara *et al.*, [15] and Pandey and Kumar [17], who observed linear increases in plant height with increasing N rates and biofertilizer inoculation.

Table 4: Effect of nitrogen levels and *Azospirillum* on plant height (cm) of rice at various growth stages

Treatment	30 DAT	60 DAT	90 DAT	At harvest
T ₁	24.08	47.62	59.53	61.07
T ₂	30.16	60.64	75.80	77.76
T ₃	33.00	64.15	80.19	82.26
T ₄	34.04	68.74	85.93	88.15
T ₅	34.36	69.14	86.43	88.67
T ₆	32.16	63.97	79.97	82.04
T ₇	36.72	72.62	90.78	93.13
T ₈	34.96	69.59	86.99	89.24
T ₉	34.72	69.79	87.24	89.50
LSD (p=0.05)	3.78	7.57	7.12	7.30
SEm±	1.26	2.52	2.44	2.50

3.2 Number of tillers hill⁻¹

Tiller number per hill increased significantly with nitrogen levels and biofertilizer application throughout the crop season (Table 5). At harvest, T₇ produced the greatest number of tillers (26.30 hill⁻¹), significantly exceeding T₁ (17.73 hill⁻¹). The enhanced tillering under integrated N-*Azospirillum* treatments is explained by sustained nitrogen supply during the critical tillering phase and *Azospirillum*-mediated phytohormone production, which stimulates lateral bud development [15, 18]. Similar results were reported by Mannan *et al.*, [19] and Bokado *et al.*, [20], who noted progressive increases in tiller number with ascending nitrogen doses.

Table 5: Effect of nitrogen levels and *Azospirillum* on number of tillers hill⁻¹ in rice

Treatment	30 DAT	60 DAT	90 DAT	At harvest
T ₁	5.95	17.26	17.78	17.73
T ₂	7.58	21.98	22.64	22.57
T ₃	8.02	21.94	22.60	22.53
T ₄	8.59	23.00	23.69	23.62
T ₅	8.64	22.47	23.15	23.08
T ₆	8.00	23.19	23.89	23.81
T ₇	9.08	25.61	26.38	26.30
T ₈	8.70	24.56	25.30	25.22
T ₉	8.72	24.00	24.72	24.65
LSD (p=0.05)	0.71	2.08	2.15	2.14
SEm±	0.24	0.71	0.74	0.73

3.3 Leaf area index

LAI increased from 30 to 90 DAT and then declined at harvest due to leaf senescence in all treatments (Table 6). The highest LAI at all stages was recorded under T₇ (30 DAT: 2.27; 60 DAT: 7.72; 90 DAT: 8.26; harvest: 4.61), while T₁ recorded the lowest values. Greater LAI under integrated treatments is

consistent with enhanced photosynthetic surface area driven by improved nitrogen assimilation and canopy expansion [21]. Shivay *et al.*, [22] similarly observed increased LAI with higher N levels, mediated through enhanced leaf production and expansion.

Table 6: Effect of nitrogen levels and *Azospirillum* on leaf area index of rice

Treatment	30 DAT	60 DAT	90 DAT	At harvest
T ₁	1.49	5.06	5.41	3.02
T ₂	1.89	6.44	6.89	3.85
T ₃	2.00	6.82	7.29	4.07
T ₄	2.15	7.30	7.82	4.36
T ₅	2.16	7.35	7.86	4.39
T ₆	2.00	6.80	7.27	4.06
T ₇	2.27	7.72	8.26	4.61
T ₈	2.17	7.39	7.91	4.41
T ₉	2.18	7.42	7.93	4.43
LSD (p=0.05)	0.18	0.60	0.65	0.36
SEm±	0.06	0.21	0.22	0.12

3.4 Dry matter accumulation

Dry matter accumulation increased progressively from 30 DAT to 90 DAT and declined marginally at harvest across all treatments (Table 7). T₇ recorded the highest dry matter at all stages, peaking at 103.53 g hill⁻¹ at 90 DAT and 101.72 g hill⁻¹ at harvest, compared to T₁ values of 69.29 and 68.08 g hill⁻¹, respectively. The *Azospirillum*-mediated production of IAA, auxins and gibberellins enhances root density and nutrient uptake capacity, thereby supporting greater biomass accumulation [15, 23]. Islam *et al.*, [24] reported similar findings, attributing improved dry matter production to balanced nutrient availability at critical growth stages facilitated by bio-fertilizers.

Table 7: Effect of nitrogen levels and *Azospirillum* on dry matter accumulation (g hill⁻¹) in rice

Treatment	30 DAT	60 DAT	90 DAT	At harvest
T ₁	16.59	45.15	69.29	68.08
T ₂	22.20	59.72	86.33	84.82
T ₃	21.12	55.37	76.56	75.22
T ₄	22.86	62.58	95.86	94.18
T ₅	22.75	53.02	72.30	71.04
T ₆	22.68	60.15	81.04	79.62
T ₇	25.29	66.88	103.53	101.72
T ₈	24.72	57.15	79.49	78.10
T ₉	22.89	56.81	90.68	89.09
LSD (p=0.05)	1.71	4.54	8.20	8.05
SEm±	0.59	1.56	2.81	2.76

3.5 Yield attributes and grain yield

Panicle length, grains panicle⁻¹ and test weight all increased significantly with combined N and *Azospirillum* application (Table 8). T₈ (125% N + *Azospirillum*) recorded the highest grains panicle⁻¹ (204.6) and test weight (22.7 g), while T₇ had the longest panicles (27.80 cm). Grain yield differed significantly among treatments, ranging from 31.6 q ha⁻¹ (T₁) to 65.6 q ha⁻¹ (T₈) (Table 9). Enhanced grain yield under integrated treatments is attributable to higher tiller number, longer panicles with more fertile spikelets, and improved assimilate translocation supported by sustained N supply and biofertilizer activity [25, 26]. Sattar *et al.*, [27] and Garai *et al.*, [28] reported analogous yield improvements with *Azospirillum* inoculation and optimized N management in paddy.

Table 8: Effect of nitrogen levels and *Azospirillum* on yield attributes of rice

Treatment	Panicle length (cm)	Grains panicle ⁻¹	Test weight (g)
T ₁	23.40	152.1	18.8
T ₂	24.50	183.6	19.2
T ₃	25.40	188.4	20.9
T ₄	26.30	192.3	21.4
T ₅	26.50	198.6	21.6
T ₆	25.30	191.4	21.2
T ₇	27.80	202.8	22.0
T ₈	27.30	204.6	22.7
T ₉	27.40	203.9	21.9
LSD (p=0.05)	2.22	20.13	1.59
S.Em±	0.76	6.89	0.54

Table 9: Effect of nitrogen levels and *Azospirillum* on grain yield, straw yield, biological yield and harvest index of rice

Treatment	Grain yield (q ha ⁻¹)	Straw yield (q ha ⁻¹)	Biological yield (q ha ⁻¹)	Harvest index (%)
T ₁	31.6	52.4	84.0	39.7
T ₂	48.1	79.7	127.8	42.2
T ₃	54.4	79.9	134.3	42.6
T ₄	57.9	80.4	138.3	43.1
T ₅	54.2	81.3	135.5	44.2
T ₆	55.6	82.4	138.0	44.6
T ₇	62.5	82.3	144.8	44.4
T ₈	65.6	84.2	149.8	43.5
T ₉	57.2	84.1	141.3	43.1
LSD (p=0.05)	1.4	1.2	2.4	—
S.Em±	14.34	52.4	100.5	—

3.6 Economic analysis

The cost of cultivation ranged from ₹40,250 ha⁻¹ (T₁) to ₹55,250 ha⁻¹ (T₉), reflecting higher input costs at elevated N and biofertilizer levels (Table 10). Despite higher cultivation costs, T₈ recorded the maximum gross return (₹1,76,544 ha⁻¹), while T₄ achieved the highest net return (₹1,25,460 ha⁻¹). The highest B:C ratio was obtained under T₂ (2.72), followed closely by T₃ (2.70) and T₄ (2.66), indicating that moderate nitrogen combined with *Azospirillum* offers superior economic efficiency. These results corroborate findings of Udayj *et al.*,^[29] and Patra *et al.*,^[30] who reported improved B:C ratios under *Azospirillum*-integrated nutrient management compared to sole mineral N application.

Table 10: Effect of nitrogen levels and *Azospirillum* on economics of rice cultivation

Treatment	Cost of cultivation (₹ ha ⁻¹)	Gross return (₹ ha ⁻¹)	Net return (₹ ha ⁻¹)	B:C ratio
T ₁	40,250	1,14,240	73,990	1.84
T ₂	43,950	1,63,416	1,19,466	2.72
T ₃	45,650	1,68,960	1,23,310	2.70
T ₄	47,100	1,72,560	1,25,460	2.66
T ₅	50,250	1,70,976	1,20,726	2.40
T ₆	52,950	1,71,840	1,18,890	2.25
T ₇	53,650	1,73,904	1,20,254	2.24
T ₈	54,100	1,76,544	1,22,444	2.26
T ₉	55,250	1,70,784	1,15,534	2.09

4. Conclusion

The combined application of nitrogen and *Azospirillum* biofertilizer significantly improved growth, yield attributes and grain yield of rice var. PR-131 under semi-arid Punjab conditions, compared to sole nitrogen or unfertilized controls. T₇ (100% RDF + *Azospirillum*) consistently excelled in growth

parameters (plant height, LAI, tiller number, dry matter), while T₈ (125% RDF + *Azospirillum*) achieved the highest grain yield (65.6 q ha⁻¹), grains panicle⁻¹ and test weight. Economically, moderate nitrogen levels combined with *Azospirillum* (T₂ and T₃) delivered the best B:C ratios (2.72 and 2.70, respectively). Based on these findings, the integrated use of nitrogen at 100-125% RDF combined with *Azospirillum* biofertilizer is recommended for sustainable and profitable rice production in comparable agroclimatic environments. Further multi-location and multi-year trials are needed to confirm these results under variable soil and climate conditions.

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