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Performance of Purple Non-Sulphur Bacterial Isolates on the Growth and Yield Attributes of Rice under Pot and Field Conditions at Graded Levels of Nitrogen Fertiliser

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Abstract

Biological nitrogen fixation by phototrophic purple non-sulphur bacteria (PPNSB) may contribute to rice nutrition and reduce dependence on mineral nitrogen fertiliser. This study evaluated indigenous PPNSB isolates for their effects on rice growth and yield under pot and field conditions at graded levels of the recommended nitrogen dose (RDN). Five isolates were screened for pot evaluation, and the two better-performing isolates, NKPRPPNSB(H10) and NKPRPPNSB(S6), were advanced to field assessment alongside PPNSB and Azospirillum reference strains. The pot experiment comprised 24 treatments in a completely randomised design with three replications, whereas the field experiment comprised 15 treatments in a randomised complete block design with three replications. The pot results showed that the Azospirillum reference strain at 75% RDN (T14) recorded a grain yield of 32.70

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g plant^{-1} and a straw yield of 21.14 g plant^{-1}; several growth and yield attributes were comparable with those of selected treatments receiving 100% RDN. In the field experiment, the PPNSB reference strain at 100% RDN (T11) recorded a grain yield of 88.70 q ha^{-1}, compared with 87.67 q ha^{-1} for the uninoculated 100% RDN treatment (T15). PPNSB and Azospirillum populations were also enumerated during crop growth. The findings indicate that selected PPNSB isolates warrant further assessment as rice bioinoculants. However, inconsistencies between some narrative descriptions and tabulated treatment values should be resolved before the magnitude of any nitrogen-fertiliser savings can be established.

Keywords: Rice; purple non-sulphur bacteria; biological nitrogen fixation; nitrogen fertilizer; bioinoculants; Rhodobacter; Azospirillum; pot experiment; field experiment; growth attributes; yield attributes.

1. Introduction

Rice (*Oryza sativa* L.) is recognised as a major commodity for humankind because it is a staple food, a cultural tradition and a source of livelihood for millions. It is an important staple food, providing 66–70 per cent of consumers' daily calorie intake. The uneven distribution of the benefits of the Green Revolution and the resulting increase in cultivation costs have raised concerns about future food security (International Rice Research Institute [IRRI], 2013). To ensure food security in rice-consuming countries, rice production should increase by 50 per cent by 2025. This additional rice will need to be produced using less land, water, labour and fewer chemicals (Sundar *et al.*, 2024). Similarly, to achieve the projected production targets of 680 and 771 million tonnes by 2015 and 2030, respectively, rice productivity must be increased through the adoption of suitable and improved technologies. Nitrogen is one of the most important nutrients directly influencing rice growth, development, yield and quality. Nitrogenous chemical fertilisers are costly inputs for rice production and are also in limited supply. Reducing nitrogen fertiliser use in rice cultivation while maintaining or increasing crop output is desirable from both economic and environmental perspectives (Roger & Ladha, 1992). Recent field evidence indicates that biofertiliser supplementation can allow reductions in mineral nitrogen application while maintaining rice yields and limiting nitrogen losses from paddy fields (Hu *et al.*, 2024). Increasing biological nitrogen fixation (BNF) associated with rice plants is an attractive approach to increasing crop yield and reducing nitrogen fertiliser requirements. Nitrogen-fixing phototrophic purple non-sulphur bacteria (PPNSB) are increasingly being used in agriculture. The phototrophic purple non-sulphur bacteria are among the most diverse and versatile groups of phototrophic purple bacteria (Kobayashi, 1982). Their role in the nitrogen nutrition of lowland rice has been recognised (Kobayashi & Haque, 1971; Sundar *et al.*, 2024; Anh *et al*., 2025), and their production of auxin has also been reported (Rajasekhar *et al.*, 1999). PPNSB have been reported to benefit rice growth and grain yield under laboratory, pot and lysimeter conditions (Elbadry & Elbanna, 1999; Harada *et al.,* 2003). In addition to N_2 fixation, these bacteria can solubilise phosphorus and produce growth hormones, *viz.,* indole-3-acetic acid (IAA), cytokinins and 5-aminolevulinic acid (ALA) (Koh & Song, 2007). The use of PPNSB as a biofertiliser for rice under field conditions, in combination with chemical nitrogen fertiliser, has also been investigated. In addition to rice, these bacteria have shown beneficial effects in other plants, including tomato (Koh & Song, 2007; Kondo *et al.*, 2010) and komatsuna (*Brassica campestris*) (Kondo *et al.,* 2008).

Recent studies have also reported that the addition of purple non-sulphur bacteria can improve soil nutrient dynamics and rice growth (Khuong *et al.*, 2024), while the contribution of biofertilisers to non-symbiotic nitrogen fixation in paddy soils varies with soil and inoculant characteristics (Xu *et al.*, 2025). Studies of bacterial inoculants in rice have reported effects on plant growth and nutrient content (Khanam *et al.*, 2022), while endophytic isolates from rice have been reported to possess plant-growth-promoting traits (Kavitha *et al.*, 2025).

However, the performance of selected indigenous PPNSB isolates under both pot and field conditions across graded nitrogen levels, including their comparison with reference strains, requires further evaluation.

The objective of this study was to evaluate the effects of efficient PPNSB isolates on rice growth and yield under pot and field conditions at graded levels of nitrogen fertiliser.

2. Material and Methods

The evaluation of selected PPNSB isolates under pot-culture conditions in rice was conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India. PPNSB isolates were obtained from the rhizosphere soils of major rice-growing districts in northern Karnataka. The isolates were characterised morphologically, biochemically and molecularly, confirming that they belonged to the PPNSB group comprising *Rhodobacter* and *Rhodopseudomonas* species. All isolates were coded as NKPRPPNSB (North Karnataka Rice Rhizosphere Purple Non-Sulphur Bacteria). Based on functional activity and hydroponic-culture experiments, the most efficient isolates were selected for the pot experiment to evaluate their performance in rice at graded levels of the recommended dose of nitrogen (RDN) under puddled conditions. Two reference strains were included for comparison: *Rhodobacter capsulatus* and *Azospirillum* ACD15, obtained from the Department of Agricultural Microbiology, UAS, Dharwad, Karnataka, India. The locally popular rice variety Abhilasha was selected, and the local package of cultivation practices was followed. Nitrogen fertiliser was calculated according to the graded level specified for each treatment. The experiment was laid out in a two-factor completely randomised design (CRD), with 24 treatments and three replications. The treatment combinations were as follows: T1- NKPRPPNSB(H10)+ 50 % RDN, T2- NKPRPPNSB(S6) + 50 % RDN, T3- NKPRPPNSB(D7)+ 50 % RDN, T4- NKPRPPNSB(R16)+ 50 % RDN, T5- NKPRPPNSB(H2)+ 50 % RDN, T6- Reference strain of PPNSB +50 % RDN, T7- Reference strain *Azospirillum* ACD15 +50 % RDN, T8- NKPRPPNSB(H10)+ 75% RDN, T9- NKPRPPNSB(S6) + 75% RDN, T10- NKPRPPNSB(D7)+ 75% RDN, T11- NKPRPPNSB(R16)+ 75% RDN, T12- NKPRPPNSB(H2)+ 75% RDN, T13- Reference strain of PPNSB +75%RDN, T14- Reference strain *Azospirillum* ACD15 +75% RDN, T15- NKPRPPNSB(H10)+ 100% RDN, T16- NKPRPPNSB(S6) + 100% RDN, T17- NKPRPPNSB(D7)+ + 100% RDN,

T18- NKPRPPNSB(R16)+ 100% RDN, T19- NKPRPPNSB(H2)+ 100% RDN, T20- Reference strain of PPNSB +100%RDN, T21- Reference strain *Azospirillum* ACD15+100% RDN, T22-50%RDN, T23-75% RDN and T24- 100% RDN.

An inoculum was prepared by inoculating broth with a 72-h-old culture. The flasks were incubated at 28 + 2°C under light for five days to obtain a population of 10^{9} cells mL^{-1} of liquid culture. The culture broth was then mixed with sterilised lignite powder at a rate of 30 mL per 100 g, together with one per cent sterile carboxymethyl cellulose (CMC) and one per cent gum arabic. The *Azospirillum* ACD15 reference-strain inoculant was prepared by inoculating broth with a 24-h-old culture. The flasks were incubated at 28 + 2°C under light for five days to obtain a population of 10^{7} cells mL^{-1} of liquid culture. The culture broth was then mixed with sterilised lignite powder at a rate of 30 mL per 100 g, together with one per cent sterile CMC. Rice seeds obtained from ARS, Mugad, UAS, Dharwad, Karnataka, India, were used. The seeds were surface-sterilised with 4% sodium hypochlorite for 25 minutes and then thoroughly rinsed twice with sterile water. They were then placed in a CMC-based culture suspension and air-dried overnight in a laminar airflow chamber (LFC). Seeds coated only with the CMC suspension served as the control (Elbadry & Elbanna, 1999). The treated seeds were sown in the nursery and used for transplanting after 21 days.

Plastic pots (5 kg capacity) without drainage holes were selected for the pot experiment. Each pot was filled with 5 kg of soil and FYM and left under puddled conditions for 4 days. Before transplanting, all pots were uniformly fertilised according to the local package of practices, except that nitrogen was applied at the graded level specified for each treatment: 75% as a basal application (one day before transplanting) and 25% at the tillering stage (45 days after transplanting). Bacterial inoculation was carried out at transplanting by dipping the rice seedlings for 30 minutes in a cell suspension of the selected efficient isolates prepared using 0.05 M phosphate buffer (pH 7.0), a 10% (w/v) gum arabic solution and CMC. The seedlings were then transplanted at two seedlings per plant according to the treatment combinations. After transplanting, the pots were watered with tap water to maintain a 3-cm water level from day 3 after transplanting until 2 weeks before harvesting. Growth parameters, including plant height (cm), number of tillers per plant, chlorophyll content, total dry matter content (g/plant) and nitrogen content in the plant (%), were recorded. Microbiological parameters, including the enumeration of PPNSB and *Azospirillum* sp., were assessed using the procedures described by Harada *et al.* (2003) and Tyler *et al.* (1979), respectively. Yield parameters, including the number of productive tillers, panicle length (cm), number of spikelets per panicle, and grain and straw yields per plant, were also recorded.

Field Experiment: Based on the pot experiment, the two best-performing isolates of NKPRPPNSB, together with the reference strains, were selected for field assessment under semi-flooded conditions. The efficient isolates used in the experiment were NKPRPPNSB(H10) and NKPRPPNSB(S6). The field experiment was conducted at the Rice Research Station, Mugad, University of Agricultural Sciences (UAS), Dharwad, during the 2013 Kharif season. The experiment was laid out in a two-factor randomised complete block design (RCBD), with nitrogen level and bioinoculant as the two factors and three replications. The treatment combinations were as follows: T1- NKPRPPNSB(H10)+ 50 % RDN, T2- NKPRPPNSB(S6)+ 50 % RDN, T3- Reference strain of PPNSB +50 % RDN, T4- Reference strain *Azospirillum* ACD15 +50 % RDN, T5- NKPRPPNSB(H10)+ 75% RDN, T6- NKPRPPNSB(S6)+ 75% RDN, T7- Reference strain of PPNSB +75%RDN, T8- Reference strain *Azospirillum* ACD15 +75% RDN, T9- NKPRPPNSB(H10)+ 100% RDN, T10- NKPRPPNSB(S6)+ 100% RDN, T11- Reference strain of PPNSB +100%RDN, T12- Reference strain *Azospirillum* ACD15+100% RDN, T13-50%RDN, T14-75% RDN and T15- 100% RDN. The gross plot measured 3.0 m × 2.5 m, and the net plot measured 2.5 m × 2.0 m. Inoculum preparation and inoculation were carried out according to the procedure described by Gamal-Eldin and Elbanna (2011). Based on the recommended nutrient dose for rice (150: 50: 50 N: P_2O_5: K_2O kg/ha), the amount of fertiliser required per plot was calculated according to the treatment and applied to all treatment combinations. The recommended doses of phosphorus and potassium were applied as basal fertilisers at sowing through single superphosphate and muriate of potash, whereas nitrogen was applied in two equal splits, *namely* at the basal stage and at 30 days after sowing (DAS), as urea. Biocoated seeds were hand-dibbled at a rate of two seeds per hill to a depth of approximately four centimetres, at a spacing of 30 cm × 10 cm, and covered with soil. All observations recorded in the pot experiment were also recorded in the field experiment.

3. Results and Discussion

The results of the pot experiment are presented in Table 1. The results revealed that paddy seedlings transplanted after inoculation with NKPRPPNSB isolates and the reference strains at different RDN levels performed better in terms of growth than those transplanted without inoculation. The growth parameters, including plant height (68.17 cm), number of tillers (17.60 per plant), chlorophyll content at 80 DAS (56.50 at the grand growth stage), dry matter production (16.90 g per plant) and nitrogen content (2.35%), and the yield parameters, including the number of productive tillers (9.27 per plant), number of spikelets per panicle (9.67), panicle length (21.80 cm), and straw and grain yields (32.70 and 21.14 g per plant, respectively), were highest in the treatment inoculated with reference strain 2 at 75% RDN. This treatment was on par with the treatments inoculated with reference strain 1 at 100% RDN and the efficient isolate NKPRPPNSB(H10) at 100% RDN.

A similar trend was observed for the measured growth and yield parameters in the field experiment; the harvest data are presented in Table 2. At the UIC 100% RDN level, the growth parameters—plant height (76.33 cm), number of tillers per row length (220.67), chlorophyll content at 80 DAS (66.23), dry weight per row length of tillers (442.00 g) and nitrogen content in the plant (2.33%)—and the yield parameters—productive tillers (164.50 per row length), spikelets per panicle (17.50), panicle length (20.83 cm), straw yield (120 q/ha) and grain yield (88.70 q/ha)—were found to be on par with those in the treatments inoculated with reference strain 1 at 75% RDN, NKPRPPNSB(H10) at 75% RDN and reference strain 2 at 100% RDN. Increases in rice growth parameters following inoculation have been reported by several workers (Sundar *et al.*, 2024; Merugu *et al*., 2012). The increase in plant height may be due to the availability of abundant nitrogen during plant growth. Ammonia excretion might have increased nitrogen availability to the plant through biological nitrogen fixation. The present study reaffirmed that inoculated plants produced higher biomass than the uninoculated control. Dewan and Subba Rao (1979) reported increased root biomass of rice seedlings following inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum*. The root biomass of rice seedlings was also reported to increase following inoculation with *A. brasilense* by Sundar *et al.* (2024).

Table 1. Influence of efficient isolates on growth and yield parameters of rice under graded level of nitrogen levels in pot experiment

Treatments	Plant height (Cm)	Number of tillers	Ch lorophyll content	Dry matter content (g/plant)	% nitrogen content	Productive tillers	Number of spikelet per panicle	Panicle length (cm)	Grain yield (g/plant)	Straw yield (g/plant)
T1	55.1 7^{fg}	10.30^{f}	49.67^{ef}	13.90^{ef}	1.70^{de}	7.46^{e}	6.30^{cde}	14.27^{j}	**25.00^{d}**	**11.80**
T2	54.00^{fg}	10.50^{f}	49.33^{ef}	13.83^{f}	1.70^{de}	7.45^{e}	6.20^{cde}	14.23^{j}	25.00^{d}	11.79
T3	53.50^{gh}	10.40^{f}	48.67^{f}	13.45^{f}	1.65^{ef}	7.50^{e}	6.20^{bcd}	13.97^{j}	24.85^{e}	11.60
T4	53.00^{j}	10.67^{f}	48.50^{f}	13.40^{f}	1.62^{ef}	7.57^{e}	6.10^{fg}	13.50^{j}	24.80^{e}	11.65
T5	52.17^{i}	10.37^{f}	48.33^{i}	12.37^{g}	1.57^{fg}	7.40^{e}	6.10^{fg}	13.27^{j}	24.50^{e}	11.24
T6	57.00^{f}	10.40^{f}	50.50^{de}	12.00^{g}	1.73^{ef}	7.60^{e}	6.10^{fg}	15.67^{ij}	25.20^{ef}	12.06^{e}
T7	59.00^{e}	10.80^{f}	51.17^{d}	13.90^{ef}	1.78^{ef}	7.80^{d}	6.20^{fg}	16.17^{h}	26.00^{ef}	12.81^{e}
T8	61.17^{cd}	15.00^{b}	54.30^{bc}	15.50^{b}	1.90^{bc}	8.27^{b}	9.00^{bc}	20.67^{bcd}	31.00^{b}	17.88^{c}
T9	59.03^{de}	14.67^{cd}	53.00^{c}	15.33^{b}	1.87^{bc}	8.28^{b}	9.00^{bc}	19.89^{de}	30.50^{c}	17.69^{c}
T10	56.50^{f}	12.67^{e}	53.33^{c}	14.33^{c}	1.88^{bc}	8.17^{bc}	8.40^{cd}	18.43^{gh}	30.30^{c}	17.28^{c}
T11	55.50^{fg}	12.40^{e}	52.50^{c}	14.50^{c}	1.78^{d}	8.00^{c}	8.30^{cd}	18.10^{gh}	30.20^{c}	16.64^{c}
T12	56.00^{f}	11.75^{f}	52.93^{c}	14.30^{d}	1.72^{de}	8.00^{c}	8.00^{de}	17.00^{i}	29.20^{d}	16.59^{c}
T13	59.67^{de}	12.67^{de}	54.33^{bc}	14.20^{de}	1.80^{b}	8.27^{b}	8.40^{cd}	20.67^{bcd}	28.75^{c}	18.06^{b}
T14	68.17^{a}	17.60^{a}	56.50^{a}	16.90^{a}	2.35^{a}	9.27^{a}	9.67^{a}	21.80^{a}	32.70^{a}	21.14^{a}
T15	66.67^{a}	18.00a	54.30^{bc}	16.97a	2.27^{a}	9.30^{a}	9.67^{a}	21.67^{a}	32.70^{a}	21.10^{a}
T16	66.17^{a}	17.50a	56.67^{a}	16.60^{a}	1.95^{a}	9.23^{a}	9.50^{a}	21.30^{ab}	32.40^{a}	20.65^{a}
T17	63.33^{b}	15.67b	56.60^{a}	16.40^{a}	1.90^{b}	9.24^{a}	9.00^{bc}	20.43^{bc}	32.20^{a}	20.20^{b}
T18	62.67^{bc}	14.33c	55.03^{bc}	16.10^{a}	1.85^{bc}	9.20^{a}	9.00^{bc}	19.40^{def}	32.10^{a}	20.10^{b}
T19	62.00^{bc}	12.67de	56.00^{bc}	15.50^{b}	1.75^{cd}	9.13^{a}	8.40^{c}	19.00^{de}	32.00^{a}	19.66^{b}
T20	67.50^{a}	17.80a	55.83^{cd}	16.80^{a}	2.35^{a}	9.25^{a}	9.67^{a}	21.83^{a}	32.20^{a}	21.10^{a}
T21	67.33^{a}	16.00b	55.64	15.30^{b}	2.27^{a}	8.29^{b}	9.20^{ab}	20.77^{bc}	31.50^{b}	19.00^{b}
T22	50.00^{j}	9.67^{g}	42.67^{h}	11.17^{h}	1.25^{h}	7.20^{f}	5.70^{g}	13.23^{j}	23.50^{g}	10.83^{f}
T23	55.67^{fg}	10.67^{f}	46.67^{g}	13.50^{g}	1.47^{g}	8.20^{bc}	8.20^{de}	16.50^{ik}	25.40^{d}	13.22^{d}
T2 4	66.40^{a}	15.67^{b}	50.10^{ce}	15.35^{b}	1.88^{bc}	8.28^{b}	9.00^{bc}	20.70^{bc}	31.50^{b}	17.98^{c}

	SeM	CD	Se.M	CD	Se.M	CD	Se.M	CD	Se.M	CD	Se.M	CD	Se.M	CD	Se.M	CD	Se.M	CD	Se.M	CD
Isolates	0.10	0.17	0.08	0.33	0.14	0.42	0.10	0.39	0.03	0.13	0.02	0.06	0.11	0.45	0.08	0.31	0.35	1.03	0.13	0.51
Nitrogen	0.17	0.73	0.14	0.54	0.24	0.92	0.16	0.65	0.05	0.21	0.03	0.11	0.18	0.74	0.13	0.51	0.27	0.79	0.22	0.83
IXN	0.30	1.27	0.24	0.93	0.42	1.60	0.28	0.84	0.09	0.36	0.05	0.19	0.32	1.29	0.20	0.60	1.03	1.78	0.38	1.44

Table 2. Effect of efficient NKPRPPNSB isolates on growth and yield of rice under field condition at graded level of nitrogen levels

Treatment	Plant height (Cm)		Number of tillers		Chlorophyll con tent		Dry matter content		% nitrogen content		Productive tillers		Number of spikelet per panic le		Panicle length (cm)		Straw yield (q/ha)		Grain yield (q/ha)	
1	59.00cd		191.67^{e}		51.33^{h}		430.40^{g}		1.27^{d}		144.00^{g}		15.00^{f}		15.50^{g}		90.50		56.00	
2	58.50^{bc}		191.33e		51.67^{h}		429.00^{g}		1.25^{d}		144.50^{g}		15.00^{f}		15.83^{g}		86.00		55.90	
3	60.80^{bc}		193.00e		53.17^{g}		425.33^{h}		1.28^{d}		146.70^{f}		15.33^{f}		16.00^{ef}		8.52		56.30	
4	59.83^{b}		198.00^{d}		53.50^{g}		420.67^{i}		1.38^{d}		149.00^{e}		16.00^{cd}		16.83^{cde}		89.40		56.00	
5	68.00^{b}		216.00^{b}		58.33^{f}		441.33^{b}		2.10^{b}		164.00^{b}		16.20^{de}		17.67^{de}		115.00		79.00	
6	70.10^{bc}		211.33^{c}		60.33^{e}		440.00^{b}		2.07^{bc}		159.00^{c}		16.27^{de}		17.67^{de}		112.00		72.00	
7	73.00^{bc}		216.67^{b}		62.50^{d}		435.00^{c}		2.27^{a}		158.33^{c}		17.00^{bc}		18.17^{cd}		100.50		70.00	
8	75.50^{a}		222.00^{a}		65.33^{c}		450.33^{a}		2.30^{a}		175.65^{a}		18.00^{a}		20.00^{a}		126.50		86.00	
9	74.33^{a}		221.33^{a}		68.67^{a}		449.33^{a}		2.37^{a}		175.00^{a}		17.87^{ab}		19.50^{a}		126.00		87.82^{ab}	
1 0	74.17^{a}		220.67^{a}		67.50^{ab}		448.33^{a}		2.30^{a}		174.33^{a}		17.50^{abc}		19.23^{b}		124.67		86.80^{ab}	
1 1	75.17^{a}		223.00^{a}		68.67^{a}		449.30^{a}		2.43^{a}		176.00^{a}		18.17^{a}		20.83^{a}		126.00		88.70^{a}	
12	76.33^{a}		220.67^{a}		66.83^{b}		442.00^{b}		2.33^{a}		164.50^{b}		17.50^{abc}		17.80^{bc}		115.00		82.50^{a}	
1 3	54.50^{d}		186.33f		41.00^{j}		419.17^{j}		0.82^{e}		138.50^{h}		13.50^{g}		14.50^{h}		76.60		50.00	
1 4	64.67^{b}		192.33^{e}		47.67^{i}		430.03^{g}		0.97^{e}		155.67^{d}		15.00^{f}		15.93^{h}		124.50		59.00	
15	72.80^{a}		216.00^{b}		59.67^{e}		441.83^{b}		1.93^{c}		163.00^{b}		17.00^{bc}		17.33^{de}		146.00		87.67^{a}	
	SE.m	**CD**	**SE.m**	**CD**	**SE.m**	**CD**	**SE.m**	**CD**	**SE.m**	**CD**	**SE.m**	**CD**	**SE.m**	**CD**	**SE.m**	**CD**	**SE.m**	**CD**	**SE.m**	**CD**
Isolates(I)	0.40	1.14	0.49	1.42	0.29	0.66	0.52	1.52	0.03	0.10	0.368	1.06	0.82	0.38	0.18	0.53	0.28	0.82	0.25	0.72
Nitrogen(N)	0.31	0.69	0.38	1.14	0.17	0.39	0.40	1.17	0.02	0.07	0.476	1.37	2.38	0.29	0.14	0.41	0.36	1.05	0.19	0.56
I X N	0.88	1.98	0.85	2.45	0.51	1.15	0.90	2.63	0.06	0.17	0.82	2.38	0.22	0.66	0.31	091	0.63	1.83	0.43	1.26

Elbadry *and* Elbanna (1999) reported that inoculated mature rice plants showed a more developed root system. They were able to mimic the effects of inoculation on root morphology by supplying a mixture of growth factors to the rooting medium. In the present study, inoculation with *Azospirillum* and PPNSB isolates at graded RDN levels probably contributed to the increased plant growth parameters. This may be due to the production of growth-promoting substances, *viz.,* IAA, GA and cytokinins, by the inoculated organisms. These substances may play an important role in root elongation and shoot growth (Sunayana *et al.,* 2005; Sundar *et al*., 2024; Serdyuk *et al.*, 2000). The possible mechanisms underlying higher nitrogen accumulation may include the transfer of atmospheric nitrogen to the plant through bacterial nitrogen fixation and/or improved nitrogen uptake in inoculated plants (Sundar *et al.*, 2024). In addition, *Azospirillum* inoculation has been reported to influence mineral uptake and rice growth under hydroponic conditions (Murty & Ladha, 1988).

This clearly indicates that inoculation with efficient isolates at 75% RDN can save 25% of the recommended nitrogen dose. Inoculation with the efficient isolate NKPRPPNSB(H10) at 100% RDN was also superior to the UIC at 100% RDN.

The relationship between growth and yield parameters in the present study provides indirect evidence of the increase in yield following inoculation, as yield components are closely associated with crop growth. The production of growth-promoting substances and nitrogen fixation by the inoculated organisms provide further indirect evidence. Similar findings were reported by Elbadry and Elbanna (1999), who observed increased rice yield in a pot trial following inoculation with *R. capsulatus*. Merugu *et al*. (2012) documented increased rice growth and yield following inoculation with *R. capsulatus* KU002, while Harada *et al.* (2005) and Anh *et al*. (2025) reported increased rice yield following PPNSB inoculation under pot-culture conditions. In practical terms, grain yield is the most important factor for determining the effectiveness of an agricultural treatment. In the present study, the maximum increase in grain yield (10.00%) was observed in the treatment inoculated with reference strain 1 at 75% RDN. This treatment was on par with those inoculated with the efficient isolates NKPRPPNSB(H10) and NKPRPPNSB(S6) at 100% RDN, compared with the UIC at 100% RDN. The precise mechanism underlying the increase in grain yield following inoculation is unknown. Generally, yield parameters depend on plant growth characteristics. In the present study, however, the inoculated microorganisms possessed the ability to fix nitrogen and produce growth-promoting substances. These growth-promoting activities may contribute to vigorous plant growth, which in turn is associated with yield attributes. The results for the yield attributes are consistent with earlier findings by Gamal-Eldin and Elbanna (2011) and Anh *et al*. (2025).

In the present investigation, the population-enumeration results for the pot-culture experiment are presented in Table 4. The NKPRPPNSB population was highest in the treatments inoculated with reference strain 1 at 100% RDN and with the efficient isolate NKPRPPNSB(H10) at 100% RDN. These populations were on par with those observed in treatments inoculated with efficient isolates at 75% RDN in both pot and field experiments at all plant-growth stages. Similar results were observed in the field-experiment data presented in Table 3.

Table 3. Population dynamics of PPNSB isolates and *Azospirillum* sp. under field condition at graded level of nitrogen

Treatments	Population of PPNSB (CFU per g of soil)		Population of *Azospirillum* sp. (CFU per g of soil)	
	80 DAS	At harvest	80 DAS	At harvest
T1	$16.x10^9$	$2.4\ x10^4$	$9.0\ x10^4$	$3.0\ x10^2$
T2	$15X10^9$	$2.5X10^4$	$9.2\ x10^4$	$3.2\ x10^2$
T3	$13\ X10^9$	$2.3X10^5$	$9.9\ x10^4$	$3.0\ x10^2$
T4	$13X10^4$	$2.0X10^2$	$8.6x10^9$	$5.0\ x10^2$
T5	$22.4x10^9$	$2.40x10^5$	$8.6\ x10^4$	$4.0\ x10^2$
T6	$21.5X10^9$	$3.0X10^5$	$9.7\ x10^4$	$4.3\ x10^2$
T7	$20\ X10^9$	$3.3X10^7$	$8.9\ x10^4$	$5.4\ x10^2$
T8	$15X10^4$	$1.4X10^2$	$9.8\ x10^9$	$8.0\ x10^2$
T9	$2.24x10^9$	$2.24x10^5$	$9\ .0\ x10^3$	$3.0\ x10^2$
T10	$2.2X10^9$	$2.2X10^6$	$9.5\ x10^3$	$3.5\ x10^2$
T11	$2.3X10^9$	$2.3X10^5$	$9.2\ x10^3$	$4.2\ x10^2$
T12	$14X10^4$	$1.8X10^2$	$8.1\ x10^9$	$4.1\ x10^2$
T13	$4.0.x10^2$	$2.3\ X\ 10^3$	$7.6x10^4$	$3.0\ x10^2$
T14	$3.0X\ 10^2$	$2.0\ X\ 10^2$	$9.9\ x10^4$	$3.3\ x10^2$
T15	$4.5X\ 10^2$	$1.5X\ 10^2$	$9.9\ x10^3$	$2.9\ x10^2$

Similarly, the *Azospirillum* population was highest in the treatment inoculated with reference strain 2 at 75% RDN, which was on par with the population recorded for *Azospirillum* ACD15 at 50% RDN.

In the present study, the population of the inoculated groups was relatively low at the transplanting stage, but increased gradually until the maximum-tillering stage and then declined. However, at maturity, the population was generally slightly higher than at transplanting. The decrease in population may be attributable to (1) rapid depletion of available nitrogen in the soil, (2) an increased supply of plant photosynthates to the roots, (3) increased transport of atmospheric nitrogen to the roots through the aerenchyma at heading, and (4) increased availability of carbon compounds resulting from partial degradation of plant roots at this growth stage. These results are in accordance with the earlier findings of Ladha *et al.* (1987), who reported the highest populations of PPNSB and *Azospirillum* at the tillering stage of rice.

Another important observation in this study was that the population of *Azospirillum* decreased as nitrogen levels increased. This may be due to inhibition of nitrogenase enzyme activity in the presence of a nitrogen source. However, the PPNSB population did

not decrease with increasing RDN levels. This may be because nitrogenase activity in the PPNSB group is less affected by the presence of nitrogen than that in other heterotrophic nitrogen-fixing bacteria. A similar trend was reported by Harada *et al.* (2003) and Anh *et al*. (2025), who observed increased PPNSB populations irrespective of the presence or absence of a nitrogen source. The population of heterotrophic N_2-fixing bacteria was generally higher than that of the PPNSB group. Similar results were reported by Elbadry *et al.* (1999). The field experiment was conducted under semi-flooded conditions; that is, the plots were not puddled at sowing. From 30 DAS onwards, the plots were fully flooded until the final two weeks before harvest.

Table 4. Population dynamics of PPNSB isolates and *Azospirillum* sp. under pot culture at graded level of nitrogen

Treatments	Population of PPNSB (CFU per g of soil)		Population of *Azospirillum* sp. (CFU per g of soil)	
	80 DAS	At harvest	80 DAS	At harvest
T1	3.0×10^9	3.0×10^9	3.0×10^3	3.0×10^3
T2	$3 .0 \times 10^8$	$3 .0 \times 10^8$	$3 .0 \times 10^4$	$3 .0 \times 10^2$
T3	3.0×10^8	3.0×10^9	3.0×10^3	3.0×10^3
T4	3.0×10^8	3.0×10^8	3.0×10^3	3.0×10^2
T5	2.0×10^9	2.0×10^7	2.0×10^3	2.0×10^3
T6	6.2×10^8	3.0×10^9	8.2×10^3	3.2×10^2
T7	10.0×10^4	10.0×10^2	10.0×10^9	10.0×10^4
T8	3.5×10^9	3.5×10^9	3.5×10^3	3.5×10^3
T9	3.0×10^9	3.0×10^9	3.0×10^3	3.0×10^2
T10	2.0×10^9	2.0×10^9	2.0×10^4	2.0×10^3
T11	3.5×10^9	3.5×10^8	3.5×10^4	3.5×10^2
T12	3.0×10^8	3.0×10^8	3.0×10^4	3.0×10^2
T13	8.0×10^9	3.0×10^9	3.0×10^4	3.0×10^2
T14	11.0×10^4	11.0×10^3	11.0×10^9	11.0×10^4
T15	2.0×10^9	2.0×10^2	2.0×10^3	2.0×10^2
T16	2.2×10^9	2.2×10^2	2.2×10^3	2.2×10^2
T17	3.0×10^9	3.0×10^2	3.0×10^3	3.0×10^2
T18	1.0×10^8	1.0×10^2	1.0×10^3	1.0×10^2
T19	3.5×10^9	3.0×10^2	3.0×10^3	3.0×10^2
T20	9.5×10^9	2.5×10^2	2.5×10^3	2.5×10^2
T21	10.0×10^4	10.0×10^3	10.0×10^8	10.0×10^4
T22	3.0×10^3	3.0×10^2	3.0×10^3	3.0×10^2
T23	10.0×10^3	1.0×10^3	10.0×10^3	10.0×10^2
T2 4	2.0×10^3	2.0×10^3	2.0×10^3	2.0×10^2

The PPNSB group of organisms is versatile and exhibits growth under different conditions. The efficient NKPRPPNSB isolates performed similarly under pot and field conditions in terms of their functional characteristics. This was also observed under *In vitro* conditions.

At the molecular level, the nitrogen-fixation gene *nifH* was amplified in the NKPRPPNSB isolates irrespective of the presence of a nitrogen source in the medium. The acetylene-reduction assay indicated that nitrogenase activity was not inhibited in the presence or absence of a nitrogen source in the medium. This property was not observed in the other nitrogen fixer, *Azospirillum*. Accordingly, the activity of *Azospirillum dec*reased at 100% RDN. However, at 75% RDN, it performed better than PPNSB at the same nitrogen level.

4. Conclusions

The present study showed that inoculation with the Azospirillum reference strain at 75% RDN (T14) produced a grain yield of 32.70 g plant^{-1} and a straw yield of 21.14 g plant^{-1}. These values were statistically comparable with those recorded for selected treatments receiving 100% RDN, including NKPRPPNSB(H10) and the PPNSB reference strain. This indicates that some inoculated treatments maintained pot-level yield attributes at a reduced nitrogen dose. In the field experiment, the PPNSB reference strain at 100% RDN (T11) recorded the highest grain yield in Table 2 (88.70 q ha^{-1}), while the uninoculated 100% RDN treatment (T15) recorded 87.67 q ha^{-1}. The study also monitored PPNSB and Azospirillum populations under pot and field conditions, with population patterns varying among treatments and sampling stages. Overall, the results support further evaluation of indigenous PPNSB isolates as potential bioinoculants for rice under graded nitrogen fertilisation. Nevertheless, the differences between some narrative treatment descriptions and the corresponding tabulated values need to be resolved before firm conclusions can be drawn about the extent of nitrogen-fertiliser savings or the relative performance of individual isolates.

5. Limitations of the Study

The study was conducted using a single rice variety (Abhilasha) at one field location during a single cropping season. Therefore, the findings may have limited generalisability across different rice varieties, agroecological conditions and seasonal environments. Further evaluation across diverse locations, varieties and growing seasons is necessary to establish the broader applicability of the selected bacterial isolates under varying nitrogen fertilisation regimes.

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Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. ChatGPT, Claude and Perplexity were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that no competing interests exist.

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