GLOBAL JOURNAL OF AGRICULTURAL AND ALLIED SCIENCES (2026) VOL 7, ISSUE 1, PAGES 24-28 SHORT COMMUNICATION

Effects of Azotobacter as a Source of Nitrogen on Wheat Yield and Grain Protein

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MANUSCRIPT INFO

Article history:
Received: January 31, 2026
Revised: April 27, 2026
Accepted: May 11, 2026

Keywords:
Azotobacter
Biofertilizer
Grain quality
Nutrient management
Wheat

ABSTRACT

Nitrogen (N) is a key nutrient that governs wheat productivity and grain quality; however, excessive reliance on synthetic N fertilizers poses economic and environmental challenges. Consequently, nitrogen-fixing biofertilizers such as *Azotobacter* have gained attention as environmentally friendly alternatives to urea. For this research, the hypothesis was that *Azotobacter* inoculation could substitute a portion of chemical N fertilizer without reducing wheat yield and quality. The objective of this study was to evaluate the potential of *Azotobacter* in substituting nitrogenous fertilizers for improving wheat yield and grain protein content. A field experiment was conducted at IAAS, Lamjung Campus, from November 2024 to April 2025 using a Randomized Complete Block Design with three replications and seven treatments. The treatments included an untreated control, *Azotobacter* only; *Azotobacter* + farmyard manure (FYM); FYM + 100% recommended N; *Azotobacter* + FYM + 25% N; 50% and 75% N supplied via urea. Phosphorus and potassium were applied uniformly in all treatments. Results showed significant differences ($p \leq 0.05$) among the treatments for most yield and yield-contributing parameters, except harvest index and sterility percentage. The treatment *Azotobacter* + FYM + 75% N recorded the highest number of effective tillers (301 m^{-2}), thousand-grain weight (50.3 g), grain yield (4393 kg ha^{-1}), straw yield (5048 kg ha^{-1}), and grain protein content (12.7%). Although this treatment produced 13.7% higher yield than FYM + 100% N, the yields were statistically comparable ($p > 0.05$), indicating that *Azotobacter* application can effectively replace up to 25% of chemical N fertilizer. Thus, the combined use of *Azotobacter*, FYM, and 75% N can be recommended for sustainable wheat production in the mid-hills of Nepal.

Citation:
Shrestha, S., Dhital, G., & Shrestha, S. (2026). Effects of Azotobacter as a Source of Nitrogen on Wheat Yield and Grain Protein
Global Journal of Agricultural and Allied Sciences, 7(1), 24-28.

1. Introduction

Wheat (*Triticum aestivum*) ranks as the third most important cereal crop in Nepal, after rice and maize, grown widely across diverse agro-climatic regions. Wheat, a heavy nitrogen (N) feeder, requires substantial N inputs (up to 100 kg ha^{-1}) to ensure ideal growth and optimum yield in the mid-hills of Nepal, with nitrogen primarily supplied as chemical fertilizer (urea). However, excessive and haphazard use of such fertilizer leads to soil degradation, environmental and health concerns, and increases production costs (Patel et al., 2013). Furthermore, limited access to agricultural inputs and their high prices, particularly for chemical fertilizers, exacerbated by the absence of domestic production in Nepal, have made these fertilizers unaffordable for marginal farmers (Mishra, 2023). While the annual demand for fertilizers exceeds 8×10^8 kg, only about 4.5×10^8 kg are legally imported to Nepal (Bastakoti et al., 2025). With urea use per hectare increasing by approximately 62% from 2011 to 2023 (MoALD, 2024), maintaining soil fertility has become increasingly difficult. Therefore, efficient and sustainable management of N fertilizer is crucial.

Biofertilizers like *Azotobacter,* an alternative to chemical N fertilizers, are eco-friendly, non-toxic, non-hazardous, and cost-effective (Bastakoti et al., 2025). As a free-living aerobic bacterium, *Azotobacter* can fix 60 to 90 kg N ha^{-1} annually and increase crop yield by 5-28% (Milosevic et al., 2012). In addition to N fixation, *Azotobacter* promotes plant growth through the production of phytohormones, improvement of nutrient uptake, suppression of soil-borne pathogens, and enhancement of microbial activity (Bargaz et al., 2021). Although *Azotobacter* alone cannot fully meet crop N demands, combining it with other fertilizers can increase yield and reduce N application by up to 25% (El-Sorady et al., 2022).

Evaluating the impact of *Azotobacter* alone or in combination with other fertilizers is essential to identify efficient nutrient management practices. Integrated use of *Azotobacter* with other fertilizers can boost N use efficiency and support sustainable wheat production in Nepal (Paudel et al., 2024). Farmyard manure (FYM) improves soil structure, increases organic matter (OM) content, and ensures a slow and sustained release of nutrients in soil. Several studies have reported that combining *Azotobacter* with FYM and chemical fertilizers can reduce mineral N requirements by up to 25% without a yield penalty (Anshuman et al., 2023; Mahato & Kafle,

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https://doi.org/10.35251/gjaas.2026.004

2018). However, the effectiveness of *Azotobacter* under varying N levels remains underexplored in the mid-hills of Nepal.

An experiment was conducted to determine the effects of *Azotobacter* in substituting nitrogenous fertilizer on wheat yield and grain protein content under mid-hill conditions of Nepal. It was hypothesized that the application of *Azotobacter* with FYM could replace a portion of the recommended chemical N fertilizer without reducing wheat yield and quality. The specific objective of this study was to identify the appropriate combination of nitrogen inputs for maximizing wheat yield and grain protein content.

2. Methods

2.1. Experimental Site

The research was conducted at the Agronomy Farm of IAAS, Lamjung Campus, Nepal, in 2024. The farm was located in the sub-tropical climatic zone at 28°30' N latitude and 84°38' E longitude at an elevation of 625 m above sea level.

2.2. Soil Information

Soil samples were collected at the beginning of the experiment from a depth of 15 cm across at least 10 different locations within the experimental plot. The soil samples were analyzed at the Soil and Fertilizer Testing Laboratory, Pokhara. Soil texture was a clay loam with a pH of 5.8 and OM content of 3.88%. The soil contained 0.16% total N, 5.37 kg ha^{-1} P_2O_5, 108 kg ha^{-1} K_2O, and 0.315 mg kg^{-1} boron.

2.3. Experimental Details

The experiment was conducted in a Randomized Complete Block Design (RCBD) with 7 treatments and 3 replications. Each plot was of 6 m^2 (3 m × 2 m) area. The buffer distance between the blocks and between experimental units within a block was 1 m and 0.5 m, respectively.

Borlaug-2020 wheat variety was planted at 120 kg ha^{-1}. The seeds were collected from Lumbini Seed Company Pvt. Ltd. The treatments included a control; *Azotobacter* only; *Azotobacter* + FYM, FYM + 100% recommended N; *Azotobacter* + FYM + 75% recommended N; *Azotobacter* + FYM + 50% recommended N; and *Azotobacter* + FYM + 25% recommended N, with constant rates of phosphorus and potassium application. A detailed treatment description is presented in Table 1. The 100%, 75%, 50%, and 25% of the recommended N rates corresponded to 100, 75, 50, and 25 kg N ha^{-1}, respectively. Farmyard manure was incorporated at 8 t ha^{-1} in the soil as the main source of OM. Its nutrient composition was not analyzed and was assumed based on typical values (0.5–1.0% N, 0.15–0.20% P_2O_5, 0.5–0.6% K_2O) (Food and Agriculture Organization of the United Nations, 2006). Urea (46% N), diammonium phosphate (18% N and 46% P_2O_5), and muriate of potash (60% K_2O) were used as sources of inorganic fertilizers and were applied at the rate of 100:50:50 kg NPK ha^{-1} (MoALD, 2024). Half of N and full rates of K_2O and P_2O_5 were applied as a basal dose, while the remaining doses of N were top-dressed in two equal splits at 22 and 52 days after sowing (DAS).

100 mL of liquid *Azotobacter,* with a colony-forming unit count of 3 × 10^8 mL^{-1}, diluted with 1 L of sugar solution (prepared by adding 100 g of sugar to 1 L of water), was applied to seeds and left overnight before sowing (P. Manandhar, personal communication, November 20, 2024). Seeds were sown with 20 cm spacing between rows at a depth of 2-3 cm. Plots were irrigated weekly during the initial four weeks and again at the critical growth stages of the crop, such as Zadoks (Z)-21 (crown root initiation), Z-22 (tillering), Z-37 (jointing), Z-61 (flowering), Z-75 (milking), and Z-85 (dough stage). Harvesting was done manually on 142 DAS from the plots.

Table 1. *Details of treatments for the experiment, 2024-2025, IAAS Agronomy Farm, Lamjung campus, Nepal.*

Treatment	Details
T1	Control (No manure and fertilizers)
T2	*Azotobacter* (Seed inoculated)
T3	*Azotobacter* + FYM (8 t ha^{-1})
T4	100% NPK (100:50:50 kg ha^{-1}) + FYM (8 t ha^{-1})
T5	*Azotobacter* + 75% N (75 kg ha^{-1}) + P (50 kg ha^{-1}) + K (50 kg ha^{-1}) + FYM (8 t ha^{-1})
T6	*Azotobacter* + 50% N (50 kg ha^{-1}) + P (50 kg ha^{-1}) + K (50 kg ha^{-1}) + FYM (8 t ha^{-1})
T7	*Azotobacter* + 25% N (25 kg ha^{-1}) + P (50 kg ha^{-1}) + K (50 kg ha^{-1}) FYM (8 t ha^{-1})

2.4. Assessment

To evaluate the effects of *Azotobacter* in substituting N fertilizers, different yield and yield attributes were assessed along with the grain protein content of wheat. The number of effective tillers per m^2 was counted at harvest from each plot. A ruler was used to measure spike length from the base to the tip of the spike. Ten spikes were randomly selected from the central rows of each plot, excluding two border rows, and the number of spikelets, florets, and grains per spike was counted. Sterility percentage was calculated using the following formula as described by Bhatta et al. (2005).

$$\text{Sterility \%} = \frac{Numberof\ florets\ per\ spike - Number\ of\ grains\ per\ spike}{Total\ number\ of\ florets\ per\ spike} \times 100$$

1000-grain weight was measured by weighing 1000 grains at 14% moisture content obtained from each plot. Grain moisture content was determined using a grain moisture meter. Grain and straw yields were calculated from the net plot harvest and converted into kg ha^{-1}. Grain N content was determined from oven-dried grain samples collected at harvest using the Kjeldahl method. Grain protein content was derived as % N content × 5.7. Harvest index was calculated by using the formula given by Reddy (2013).

$$\text{Harvest index} = \frac{Economic\ yield\ (Grain\ yield)}{Biological\ yield\ (Grain+Straw)}$$

2.5. Statistical Analysis

The data were compiled and arranged in MS-Excel 2021, while R software (version 4.4.3) was used for analysis. Analysis of variance (ANOVA) was performed to test treatment effects, and treatment means were compared using the Least Significant Difference (LSD) test at a probability threshold of 0.05 (Gomez & Gomez, 1984).

3. Results

3.1. Effect on Yield Attributes of Wheat

The yield attributes differed significantly ($p \leq 0.05$) among treatments, except for sterility (Table 2). The maximum number of effective tillers per m^{2} (301) was observed in *Azotobacter* + FYM + 75% N, followed by FYM + 100% N (285). The longest spike length (9.1 cm) and highest 1000-grain weight (50.3 g) were observed in the treatment *Azotobacter* + FYM + 75% N, and were statistically similar to FYM + 100% N, *Azotobacter* + FYM + 50% N, and *Azotobacter* + FYM + 25% N. *Azotobacter* + FYM + 75% N, also produced the highest number of spikelets, florets, and grains per spike. The control treatment consistently recorded the lowest values for all yield attributes. Although sterility percentage did not differ significantly ($p > 0.05$), the highest numerical value (49.6%) was observed in the control, whereas the lowest value (12.9%) was in the treatment FYM + 100% N.

Table 2. *Yield attributes of wheat as influenced by Azotobacter and other fertilizer treatments, 2024-2025, IAAS Agronomy Farm, Lamjung campus, Nepal.*

Treatments	No. of effective tillers (m^{-2})	Spike length (cm)	No. of spikelet per spike	No. of florets per spike	No. of grains per spike	1000-grain weight (g)	Sterility (%)
Control	171.3^{d}	5.1^{b}	10.7^{d}	28.6^{d}	19.5^{e}	42.1^{d}	49.6
Azotobacter seed inoculated	198.2^{cd}	5.5^{b}	12.7^{c}	35.1^{cd}	24.0^{de}	43.5^{cd}	47.6
Azotobacter + FYM	238.6^{b}	6.1^{b}	13.7^{c}	39.7^{c}	30.1^{c}	45.1^{bcd}	34.7
FYM + 100% N	285.3^{a}	8.3^{a}	16.9^{ab}	53.5^{b}	47.8^{a}	47.6^{abc}	12.9
Azotobacter + FYM + 75% N	300.9^{a}	9.1^{a}	18.2^{a}	64.9^{a}	48.9^{a}	50.3^{a}	32.9
Azotobacter + FYM + 50% N	246.9^{b}	8.1^{a}	16.4^{ab}	56.8^{ab}	46.2^{ab}	49.1^{ab}	22.9
Azotobacter + FYM + 25% N	215.7^{bc}	7.7^{a}	15.8^{b}	53.3^{b}	37.3^{bc}	46.7^{abc}	47.6
LSD (0.05)	36.6	1.4	1.8	9.5	9.3	4.2	29.8
SEM (±)	11.9	0.5	0.6	3.1	3.0	1.4	9.7
F- Probability	***	***	***	***	***	*	NS

Note: Means followed by different lowercase letters indicate significant differences among treatments (*$p \leq 0.05$, *** $p \leq 0.001$); LSD = Least Significant Difference, SEM = Standard Error of Mean, and NS = Not significant.

Table 3: *Wheat yield and harvest index as influenced by Azotobacter and other fertilizers, 2024-2025, IAAS Agronomy Farm, Lamjung campus, Nepal.*

Treatments	Grain Yield (kg ha^{-1})	Straw Yield (kg ha^{-1})	Harvest index
Control	706.9^{d}	1065.7^{d}	0.40
Azotobacter seed inoculated	1084.4^{dMO}	1161.8^{d}	0.48
Azotobacter + FYM	1880.9^{c}	1642.6^{d}	0.52
FYM + 100% N	3865.1^{ab}	3958.3^{b}	0.49
Azotobacter + FYM + 75% N	4392.9^{a}	5048.1^{a}	0.47
Azotobacter + FYM + 50% N	3296.0^{b}	3060.9^{c}	0.51
Azotobacter + FYM + 25% N	2373.9^{c}	2580.1^{c}	0.46
LSD (0.05)	734.3	697.5	0.09
SEM (±)	238.3	226.4	0.03
F- Probability	***	***	NS

Note: Means followed by different lowercase letters indicate significant differences among treatments (*$p \leq 0.05$, *** $p \leq 0.001$); LSD = Least Significant Difference, SEM= Standard Error of Mean, and NS = Not significant

3.2. Effect on the Yield of Wheat

Grain and straw yields varied significantly ($p \leq 0.001$) among the treatments (Table 3). The highest grain yield (4393 kg ha^{-1}) and straw yield (5048 kg ha^{-1}) were recorded in the treatment *Azotobacter* + FYM + 75% N, which was statistically at par with FYM + 100% N. The lowest yields were observed in the control, significantly lower than all other treatments. However, harvest index did not differ significantly among the treatments. The highest harvest index (0.52) was recorded in *Azotobacter* + FYM, while the lowest harvest index (0.40) was in the control.

3.3. Grain Protein Content

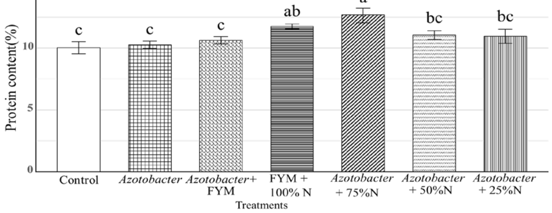

Figure 1. *Grain protein content (Means ± SEM) of wheat 2024-2025, IAAS Agronomy Farm, Lamjung campus, Nepal (^{abc} $p \leq 0.05$).*

Significant variation ($p \leq 0.05$) was observed in grain protein content among different treatments (Fig. 1). The highest grain protein content (12.7 %) was recorded in *Azotobacter* + FYM + 75% N, followed by FYM + 100% N, whereas the lowest grain protein content was observed in the control.

4. Discussion

The combined application of *Azotobacter*, FYM, and reduced N fertilizer improved yield attributes, yield, and grain quality of wheat. The increased number of effective tillers per m^2 observed under *Azotobacter*-inoculated treatments can be primarily attributed to improved soil fertility and N availability, which enhanced root growth and nutrient uptake. *Azotobacter* is known to fix atmospheric N and produce phytohormones like indole acetic acid, gibberellins, and cytokinins, which stimulate cell division and axillary bud outgrowth, resulting in more tillers. Similar benefits of integrated nutrient management on wheat tillering have been reported previously (Mahato & Kafle, 2018; Spaepen & Vanderleyden, 2011).

Improved N availability plays a vital role during spike initiation and development. Optimal N fertilization supports cell division and elongation in spike primordia, leading to longer spikes (Hawkesford, 2014). Phytohormones such as gibberellins, whose levels may be influenced by *Azotobacter* through plant-microbe interactions, may enhance shoot apical meristem activity and spike differentiation (Plackett & Wilson, 2018; Spaepen & Vanderleyden, 2011). The incorporation of FYM further improves soil OM, increases microbial biomass, and ensures a sustained release of nutrients. This creates favorable conditions for tiller survival and spike development, resulting in longer spikes (Mahato & Kafle, 2018; Kumari et al., 2024).

Increase in the number of spikelets, florets, and grains per spike under integrated nutrient management can also be explained by the combined hormonal and nutritional effects. *Azotobacter* produces cytokinins that promote meristematic activity, delay senescence, and enhance floret survival, leading to improved spikelet initiation and a higher number of fertile florets (Jameson & Song, 2016; Spaepen & Vanderleyden, 2011). Adequate N supply also increases N availability in leaves with continuous transport of assimilates to spikes, reducing floret abortion and improving grain set (Zhang et al., 2024). Improved photosynthesis efficiency under optimal N fertilization further supports grain filling, resulting in increased grain number and weight (Kumawat et al., 2018; Namvar & Khandan, 2013).

The increase in grain and straw yield with *Azotobacter*-inoculated treatments resulted from cumulative improvements in yield-attributing traits. These effects arise not only from atmospheric N fixation but also from the production of growth-regulating substances that improve balanced nutrient availability throughout crop growth (Jaga et al., 2017). Comparable yield performance between *Azotobacter* + FYM + 75% N and the full N dose suggested that the biofertilizers can replace 25% synthetic N (urea) without reducing photosynthetic activity during the grain development stage. Despite increased biomass production, harvest index did not differ significantly among treatments. This may be because harvest index is considered a genetically stable trait and generally remains unaffected unless stress conditions alter assimilate partitioning between grain and straw (Dai et al., 2016).

The synergistic effects of biological N fixation, FYM mineralization, and reduced chemical N supplementation also contributed to increasing grain protein content in wheat. Sustained N supply may have delayed leaf senescence, prolonged the functional photosynthetic period, and improved post-anthesis N remobilization into developing grains (Hawkesford, 2014). In contrast, the lower protein content observed in the control reflects inadequate N availability for amino acid synthesis and protein accumulation (Sinclair & Rufty, 2012). Despite the commonly reported yield–protein trade-off in cereals (Simmonds, 1995), the *Azotobacter* + FYM + 75% N treatment improved both grain yield and protein content, suggesting improved N uptake efficiency and reduced dilution effects, in agreement with earlier findings (Gupta et al., 2023).

5. Conclusions

The present study demonstrated that the combined application of *Azotobacter* with Farmyard Manure (FYM) and reduced synthetic N significantly improved wheat yield, yield attributes, and grain protein content. Among the treatments, *Azotobacter* + FYM + 75% N produced the highest grain yield (4393 kg ha^{-1}) and protein content (12.7%). While this treatment produced 13.7% higher numerical yield than the full recommended dose of N, the difference was not significant. This indicates that *Azotobacter* inoculation, in combination with FYM, can effectively replace 25% of mineral N without compromising wheat yield and quality. Therefore, the integrated use of biofertilizers, FYM, and reduced inorganic N offers a sustainable, cost-effective, and eco-friendly nutrient management strategy for wheat production in the mid-hills of Nepal. However, further location-specific studies along with economic analysis are needed to assess the comparative performance of *Azotobacter* under diverse agro-ecological conditions and develop practical recommendations for farmers.

Acknowledgments

Authors are thankful to the Institute of Agriculture and Animal Science, Lamjung Campus, for facilitating the research, and to everyone who contributed to the study. Sincere gratitude is extended to Lumbini Seed Company Pvt. Ltd., Bhairahawa, Rupandehi, for providing planting material; Nepal Bio-Science Research Laboratory (NBRL), Kathmandu, for supplying *Azotobacter* inoculants; and Soil and Fertilizer Testing Laboratory, Pokhara, for soil testing.

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