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Research Article

EFFICIENCY OF BIO-FERTILIZER APPLICATION METHODS ON GROWTH, BIOMASS ACCUMULATION, AND YIELD PERFORMANCE OF WHEAT (*TRITICUM AESTIVUM* L)

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Abstract

This study evaluated the influence of different bio-fertilizer application strategies on growth, biomass accumulation, and yield performance of wheat under field conditions using a factorial randomized block design. Treatments included seed inoculation, soil application, broadcast inoculation, and integrated applications of Azotobacter and phosphate-solubilizing bacteria. Key agronomic traits such as plant height, tiller density, dry matter partitioning, and yield components were systematically recorded throughout the crop growth cycle. Results demonstrated that all microbial treatments significantly enhanced vegetative growth and reproductive performance compared to the inoculated control. The integrated treatment (T9) exhibited superior performance, achieving maximum biomass accumulation (239.50 g m^{-1}) and highest grain yield (4607 kg ha^{-1}), representing a 31.6% yield increase. Synergistic microbial interactions improved nitrogen fixation, phosphorus solubilization, Rhizosphere colonization, nutrient uptake efficiency, and photosynthetic productivity. Consequently, enhanced assimilate translocation and sourcesink balance were observed. The study concludes that integrated bio-fertilizer application is an efficient, eco-friendly, and sustainable nutrient management approach for improving wheat productivity and soil fertility while reducing reliance on synthetic fertilizers.

Keywords: Wheat, Azotobacter, Phosphate-solubilizing bacteria, Bio-fertilizers, Grain yield.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated cereal crops in the world and serves as a staple food for a large proportion of the global population.(Shenet al,.2025).It contributes significantly to food security by providing essential carbohydrates, proteins, vitamins, and minerals. (Reshi *et al.,* 2025). With the continuous increase in population and the growing demand for food, enhancing wheat productivity has become a major agricultural priority. Traditionally, crop production has relied heavily on chemical fertilizers to achieve higher yields (Al-Juthery *et al.,* 2025). However, excessive and indiscriminate use of chemical fertilizers has led to soil degradation, nutrient imbalances, environmental pollution, and declining soil fertility, raising concerns about the sustainability of modern agricultural systems (Agarwa *et al.,* 2025). In recent years, bio fertilizers have emerged as an environmentally friendly and sustainable alternative for improving crop productivity (Chaudhary *et al.,* 2022). Bio fertilizers contain beneficial microorganisms that enhance nutrient availability and promote plant growth through natural biological processes (Shahwar *et al.,* 2022). These microorganisms improve soil fertility by fixing atmospheric nitrogen, solubilizing insoluble phosphorus, producing plant growth regulators, and stimulating beneficial microbial activity in the Rhizosphere (Zeng *et al.,* 2022). Among the commonly used bio fertilizers, Azotobacter and phosphate-solubilizing bacteria (PSB) have received considerable attention due to their effectiveness in enhancing crop growth and nutrient uptake (Iftikhar et al).

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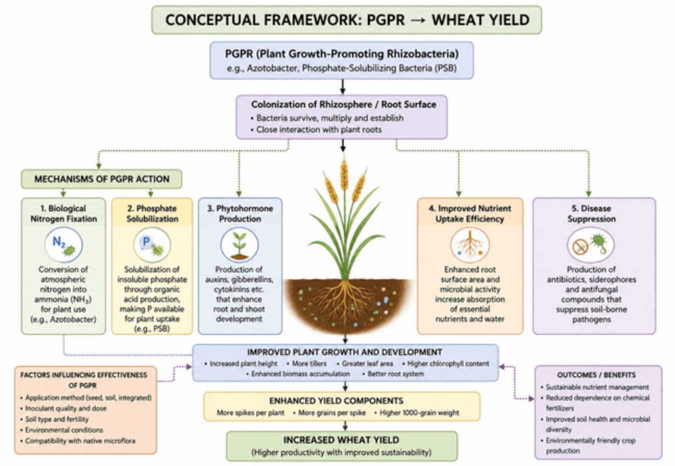
Azotobacter is a free-living nitrogen-fixing bacterium capable of converting atmospheric nitrogen into forms available to plants (Al-Baldawy *et al.,* 2023). In addition, it produces growth-promoting substances such as auxins, gibberellins, cytokinins, vitamins, and antifungal compounds that contribute to improved root development and plant vigor. (Rizvi *et al.,* 2022). Phosphate-solubilizing bacteria play a crucial role in converting insoluble phosphate compounds present in the soil into soluble forms that can be readily absorbed by plants, thereby increasing phosphorus availability and improving crop performance. The combined application of these microorganisms often results in synergistic effects that enhance nutrient-use efficiency, plant growth, and yield. (Jayara *et al.,* 2023). The effectiveness of bio fertilizers largely depends on the method of application. Different techniques such as seed inoculation, soil application, and integrated approaches influence the survival, colonization, and activity of beneficial microorganisms in the soil-plant system. (Khan *et al.,* 2023). Identifying the most efficient application method is essential for maximizing the benefits of bio fertilizers and achieving sustainable crop production. Although numerous studies have reported the positive effects of bio fertilizers on wheat growth and yield, information regarding the comparative effectiveness of different application methods remains limited (Xing *et al.,* 2024). Therefore, the present study was undertaken to evaluate the influence of various bio fertilizer application methods on the growth, biomass accumulation, and yield performance of wheat (Malimbayeva *et al.,* 2026). The findings of this research are expected to contribute to the development of sustainable nutrient management strategies that enhance wheat productivity while reducing dependence on chemical fertilizers and promoting long-term soil health. (Yime *et al.,* 2025).

LITERATURE REVIEW

Wheat (*Triticum aestivum* L.) is a major cereal crop that plays a central role in global food security (Mutanda *et al.,* 2025). Its productivity, however, is increasingly threatened by declining soil fertility, reduced nutrient availability, and the long-term negative impacts of intensive chemical fertilizer use. In response to these challenges, bio fertilizers have gained importance as sustainable agricultural inputs that enhance soil biological activity and improve nutrient cycling in agro-ecosystems (Yasir *et al.,* 2025). Bio fertilizers are microbial inoculants that enhance plant growth by increasing the availability of essential nutrients in the soil. These beneficial microorganisms improve soil fertility through biological nitrogen fixation, phosphorus solubilization, decomposition of organic matter, and production of plant growth-promoting substances. According to Vessey (2003), plant growth-promoting Rhizobacteria (PGPR) significantly improves plant performance through both direct and indirect mechanisms, including nutrient acquisition and disease suppression. Among nitrogen-fixing bacteria, Azotobacter species are widely studied due to their ability to fix atmospheric nitrogen under free living conditions. They not only contribute to nitrogen availability but also produce phytohormones such as insole acetic acid (IAA), gibberellins, and cytokinins, which stimulate root elongation and enhance nutrient uptake efficiency. Similarly, phosphate-solubilizing bacteria (PSB) play a critical role in converting insoluble phosphorus compounds into plant-available forms by secreting organic acids and enzymes, thereby improving phosphorus nutrition in crops (Arif *et al.,* 2017). Several studies have demonstrated the positive effects of bio fertilizers on wheat growth and yield. Increased plant height, higher tiller numbers, improved chlorophyll content, and enhanced grain yield have been reported with the application of Azotobacter and PSB either alone or in combination.(Khandare *et al.,* 2020).The synergistic effect of dual inoculation is often attributed to improved nutrient availability and enhanced microbial activity in the Rhizosphere, which leads to better plant growth and biomass accumulation (Shi *et al.,* 2026). The method of bio fertilizer application is a critical factor influencing its effectiveness. Seed inoculation is considered an efficient method because it ensures early colonization of plant roots by beneficial microorganisms (Kong *et al.,* 2025). Soil application, on the other hand, helps in improving microbial population density in the Rhizosphere. Integrated application methods, combining seed and soil inoculation, have been reported to provide superior results due to better microbial establishment and sustained nutrient availability throughout the crop growth period (Wei *et al.,* 2024). Despite substantial research on bio fertilizers, variations in crop response due to different application methods are still observed across agro-climatic conditions (Roy *et al.,* 2023). This indicates the need for further investigation to identify the most effective application strategy for maximizing wheat productivity under specific environmental conditions.

Theoretical framework

Plant Growth-Promoting Rhizobacteria (PGPR) theory explains that some "good bacteria" live in the soil around plant roots and help plants grow better. These helpful bacteria attach to the roots (called the Rhizosphere) and support plants by improving their nutrition, protecting them from diseases, and stimulating their growth.



Objective of study

1. To evaluate the effect of different bio fertilizer application methods (seed inoculation, soil application, and integrated application) on the growth performance of wheat (*Triticum aestivum* L.).
2. To assess the impact of bio fertilizers, particularly Azotobacter and phosphate-solubilizing bacteria (PSB), on biomass accumulation and yield-related traits of wheat.
3. To identify the most efficient bio fertilizer application method for enhancing wheat productivity under sustainable agricultural practices.

Significance of the Study

This study is significant as it promotes sustainable wheat production by evaluating bio fertilizers as an eco-friendly alternative to chemical fertilizers. It provides valuable insight into how different application methods of bio fertilizers, including seed inoculation, soil application, and integrated application, influence the growth, biomass accumulation, and yield of wheat (*Triticum aestivum* L.). The findings will help identify the most effective use of Azotobacter and phosphate-solubilizing bacteria (PSB) for improving crop performance. This research is beneficial for farmers and researchers in selecting efficient and cost-effective nutrient management practices. It also supports improved soil fertility, enhanced nutrient availability, and reduced environmental pollution. Ultimately, the study contributes to sustainable agriculture, higher wheat productivity, and long-term food security while minimizing dependence on chemical fertilizers.

MATERIALS AND METHODS

A field experiment was conducted to evaluate the efficacy of different bio fertilizer application methods on the growth, biomass accumulation, and yield performance of wheat (*Triticum aestivum* L.). The study was carried out during the Rabi season of (2025–2026) under field conditions. Approximately 70 kg of wheat seed was utilized for experimental sowing across all treatments. The trial was conducted under farmers' field conditions with proper experimental management and supervision. The experimental soil was sandy loam in texture, slightly alkaline in reaction (pH 7.8), low in available nitrogen (152.6 kg ha^{-1}) and adequate in available phosphorus (12.5 kg ha^{-1}) and potassium (346.2 kg ha^{-1}).

The experiment was laid out in a Factorial Randomized Block Design (FRBD) with three replications. Ten nutrient management treatments were evaluated as follows:

- T_0: Recommended Dose of Fertilizer (RDF)
- T_1: Azotobacter seed inoculation (200 g per 10 kg seed)
- T_2: PSB seed inoculation (200 g per 10 kg seed)
- T_3: Azotobacter soil application (3 kg ha^{-1} + 50 kg FYM)
- T_4: PSB soil application (3 kg ha^{-1} + 50 kg FYM)
- T_5: Azotobacter broadcast application (3 kg ha^{-1} + 100 kg FYM at first irrigation)
- T_6: PSB broadcast application (3 kg ha^{-1} + 100 kg FYM at first irrigation)
- T_7: Combination of $T_1 + T_3 + T_5$
- T_8: Combination of $T_2 + T_4 + T_6$
- T_9: Integrated application of all bio-fertilizer treatments ($T_7 + T_8$)

The wheat variety HUW-234 was sown at a row spacing of 20 cm following standard agronomic practices. A uniform fertilizer dose of 120 kg N, 60 kg P_2O_5, 40 kg K_2O, and 25 kg $ZnSO_4$ ha^{-1} was applied to all plots. Half of the nitrogen along with the full doses of phosphorus and potassium was applied at sowing, while the remaining nitrogen was top-dressed after the first irrigation. Farmyard manure (FYM) was incorporated into the soil at the rate of 10 t ha^{-1} one month before sowing. Irrigation was provided at critical crop growth stages, including crown root initiation, tailoring and jointing, flowering and milk stages. For dry matter accumulation studies, plant samples were collected from a 25 cm row length at three randomly selected locations within each plot. Samples were separated into leaves, stems, and spikes, and then sun-dried to constant weight before recording biomass. Spike weight prior to emergence was included in shoot dry matter, and the peduncle was considered part of the shoot biomass, following the methodology described by Bauer et al. (1987). Observations on plant height, tiller density, biomass accumulation, yield attributes, and grain yield were recorded at appropriate growth stages.

The collected data were subjected to analysis of variance (ANOVA) following the procedures applicable to a Factorial Randomized Block Design, and treatment means were compared at the 5% level of significance.

1. Growth and Biomass Accumulation

The results indicated that bio-fertilizer application significantly improved growth and biomass accumulation of wheat (*Triticum aestivum* L.) at all growth stages (Tables 1 and 2). A progressive increase in leaf, shoot, and total dry matter was observed from 30 DAS to harvest in all treatments. Among all treatments, the integrated bio-fertilizer application (T9) recorded the highest biomass accumulation at all stages. At harvest, T9 produced the maximum total dry weight (239.50 g/m row length), followed by T7 (234.00 g/m) and T3 (230.20 g/m), whereas the control (T0) recorded the lowest value (82.20 g/m). A similar trend was observed for leaf, shoot, and panicle dry weight in Table 1, confirming consistent superiority of integrated microbial application. The enhanced biomass production under T9 may be attributed to synergistic interactions between Azotobacter and phosphate-solubilizing bacteria (PSB), which improve nitrogen fixation, phosphorus solubilization, and overall nutrient availability in the Rhizosphere.

2. Dry Matter Partitioning and Growth Dynamics

Dry matter accumulation increased steadily with crop age. A relatively slow growth phase was observed during early stages (30–60 DAS), followed by rapid accumulation from 60 DAS onward, indicating active vegetative growth and canopy development. At maturity, a higher proportion of assimilates was partitioned toward reproductive organs (panicles), indicating efficient source–sink relationships. This suggests that bio-fertilizer application enhanced photosynthetic efficiency and nutrient translocation during grain filling. The reduction in leaf biomass at later stages reflects natural senescence and remobilization of nutrients toward developing grains, which is a typical physiological process in wheat.

Table 1. Effect of bio-fertilizers on leaf, shoot and panicle weight (g/m row length) of wheat under field conditions (experimental period: February–May 2026; seed used 70 kg

Treatment	30 DAS	30 DAS	60 DAS	60 DAS	90 DAS	90 DAS	120 DAS	120 DAS	At Harvest	At Harvest	At Harvest
	Leaf	Shoot	Leaf	Shoot	Leaf	Shoot	Leaf	Shoot	Leaf	Shoot	Panicle
T0	4.90	0.97	18.25	5.85	21.90	38.00	13.25	42.05	4.65	27.10	51.00
T1	6.38	1.22	35.06	9.70	50.00	81.10	40.50	99.76	19.10	70.10	123.98
T2	5.50	1.10	25.50	7.75	32.08	52.65	22.00	59.00	7.62	39.34	73.45
T3	6.46	1.25	37.00	10.25	55.32	86.50	45.25	99.98	20.25	72.78	130.45
T4	6.35	1.21	35.00	10.10	49.30	80.20	40.00	98.75	18.25	68.10	122.00
T5	5.82	1.12	27.10	7.98	35.20	57.98	24.50	66.67	11.20	44.35	84.20
T6	5.10	1.02	21.10	7.34	25.67	43.50	17.20	49.10	8.90	33.25	65.50
T7	6.50	1.25	36.50	11.25	54.24	86.10	45.25	108.06	21.25	75.10	136.56
T8	6.20	1.20	35.50	9.75	51.10	82.25	42.34	101.20	20.21	71.25	128.25
T9	6.51	1.25	38.10	11.50	55.56	88.20	46.15	111.50	21.75	73.45	142.25

Table 2. Effect of bio-fertilizers on total dry weight (g/m row length) of wheat under field conditions

Treatment	30 DAS	60 DAS	90 DAS	120 DAS	At Harvest
T0 – RDF (120:60:40 N:P:K)	5.85	24.10	59.81	79.75	82.20
T1 – Azotobacter seed inoculation	7.60	44.74	132.00	197.60	213.00
T2 – PSB seed inoculation	6.55	32.32	83.32	112.54	119.05
T3 – Azotobacter soil application	7.67	47.06	140.56	215.85	230.20
T4 – PSB soil application	7.56	44.18	128.82	193.30	208.11
T5 – Azotobacter broadcast	7.67	46.70	139.61	213.50	217.75
T6 – PSB broadcast	6.93	32.12	93.10	129.12	137.50
T7 – T1 + T3 + T5	7.70	47.10	140.85	216.00	234.00
T8 – T2 + T4 + T6	7.67	46.67	139.65	214.50	230.40
T9 – Integrated (T7 + T8)	7.76	48.10	143.00	220.95	239.50

Table 3. Effect of bio-fertilizers on yield and yield attributing characters

Treatment	Plant height (cm)	Tillers m^{-2}	Ears m^{-2}	Grains ear^{-1}	1000-grain weight (g)	Grain yield (kg ha^{-1})	% Increase
T0 (RDF NPK)	97.7	290	245	24	34.5	3150	-
T1 (Azotobacter seed inoculation)	99.9	325	268	27	35.8	3433	8.2
T2 (PSB seed inoculation)	99.5	333	268	28	36.1	3415	7.7
T3 (Azotobacter soil application)	108.0	378	320	31	38.0	3883	18.8
T4 (PSB soil application)	100.3	363	295	29	36.9	3633	13.3
T5 (Azotobacter broadcast)	100.0	345	293	30	37.0	3583	12.1
T6 (PSB broadcast)	100.3	355	290	30	36.7	3567	11.7
T7 (Integrated Azotobacter system)	112.2	417	332	34	40.4	4547	30.7
T8 (Integrated PSB system)	116.0	408	325	34	40.5	4497	30.0
T9 (Combined integrated treatment)	114.0	410	330	35	40.8	4607	31.6

3. Effect on Yield and Yield Components

The yield and yield-contributing traits were significantly influenced by bio-fertilizer treatments (Table 3). Plant height, tillers m^{-2}, ears m^{-2}, grains ear^{-1}, 1000-grain weight, and grain yield all increased with bio-fertilizer application compared to control. The highest grain yield (4607 kg ha^{-1}) was recorded in T9, followed by T7 (4547 kg ha^{-1}) and T8 (4497 kg ha^{-1}), whereas the lowest yield was observed in T0 (3150 kg ha^{-1}). The percent increase over control was also maximum in T9 (31.6%). Among individual treatments, soil application of Azotobacter (T3) performed better than seed inoculation (T1) and broadcast application (T5), indicating better microbial establishment in the soil environment.

4. Mechanism of Response

The superior performance of integrated treatments can be attributed to the combined action of Azotobacter and PSB. Azotobacter enhances atmospheric nitrogen fixation and produces growth-promoting hormones such as auxins, gibberellins, and cytokinins, which stimulate root growth and nutrient uptake. PSB increases phosphorus availability by solubilizing insoluble phosphate compounds through organic acid secretion. The combined application improves root proliferation, nutrient absorption efficiency, chlorophyll content, and photosynthetic activity, ultimately leading to higher biomass and grain yield. These findings are consistent with earlier reports that suggest synergistic microbial inoculation enhances crop productivity through improved nutrient cycling and Rhizosphere activity.

Conclusion

The findings of the present investigation unequivocally demonstrated that bio-fertilizer application methods exerted a significant influence on growth dynamics, biomass accumulation, dry matter partitioning, and yield performance of wheat (*Triticum aestivum* L.) under field conditions. The consistent enhancement of growth and productivity parameters across all bio-fertilizer treatments highlights the pivotal role of beneficial microbial inoculants in improving crop performance and nutrient-use efficiency. Among the evaluated treatments, the integrated application of Azotobacter and phosphate-solubilizing bacteria (PSB) (T_9) emerged as the most effective nutrient management strategy. This treatment recorded the highest total biomass accumulation (239.50 g m^{-1} row length), superior yield attributes, and maximum grain yield (4607 kg ha^{-1}), indicating a substantial improvement in crop productivity over the control treatment. The enhanced performance of T_9 can be attributed to synergistic microbial interactions that stimulated biological nitrogen fixation, phosphorus mobilization, Rhizosphere microbial activity, root proliferation, and nutrient acquisition efficiency.

The progressive increase in dry matter accumulation throughout the crop growth cycle further suggests that integrated microbial inoculation enhanced photosynthetic capacity; assimilate production, and carbon allocation efficiency. Moreover, greater translocation of assimilates from vegetative tissues to reproductive sinks during grain filling contributed to improved grain development and yield formation. These responses indicate a strengthened source sink relationship and enhanced physiological efficiency in inoculated plants. The superior performance of soil-applied and integrated treatments compared with individual seed inoculation or broadcast application emphasizes the importance of effective microbial establishment and sustained Rhizosphere colonization. Such enhanced microbial persistence likely facilitated continuous nutrient transformation processes, resulting in improved nutrient availability throughout critical growth stages. Overall, the study provides compelling evidence that the combined application of Azotobacter and phosphate-solubilizing bacteria constitutes a sustainable and environmentally sound approach for improving wheat productivity. The integration of these beneficial microorganisms has the potential to reduce dependence on synthetic fertilizers, enhance soil biological fertility, improve nutrient cycling, and promote long-term agro ecosystem sustainability. Therefore, integrated bio-fertilizer application may be recommended as an effective component of climate-smart and sustainable nutrient management strategies for wheat cultivation under comparable agro ecological conditions. Future research should focus on multi-location validation trials, long-term soil health assessment, and the economic feasibility of large-scale adoption of integrated microbial inoculation technologies.

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