

ENHANCING WHEAT (*TRITICUM AESTIVUM* L.) PERFORMANCE THROUGH COMBINED USE OF BIOFERTILIZERS AND ZINC SULPHATE

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Abstract

A field experiment was conducted during the Rabi season of 2024 at Govt. Post College, Timergara, District Dir Lower, Khyber Pakhtunkhwa, Pakistan, to investigate the effects of biofertilizers and zinc sulphate on the growth and yield of wheat (*Triticum aestivum* L.). The study included treatments with phosphate-solubilizing bacteria (PSB), *Azotobacter*, their combination (PSB + *Azotobacter*), and zinc sulphate applied at 20, 25, and 30 kg/ha. The experimental soil was sandy loam, nearly neutral in pH (7.8), and low in organic carbon (0.35%). Results revealed that the integrated application of PSB, *Azotobacter*, and zinc sulphate at 30 kg/ha significantly improved growth and yield attributes. The treatment achieved maximum plant height (159.03 cm), plant dry weight (162.70 g/plant), crop growth rate (26.25 g/m²/day), cobs per plant (1.8), rows per cob (16.8), seeds per cob (553.4), 100-seed weight (29.3 g), grain yield (6.5 t/ha), straw yield (12.9 t/ha), and harvest index (33.8%). These improvements can be attributed to the synergistic role of biofertilizers in enhancing nutrient availability, particularly phosphorus and nitrogen, through increased microbial activity in the rhizosphere, alongside the physiological benefits of zinc in enzymatic and metabolic functions. The findings highlight integrated nutrient management, combining biofertilizers with zinc supplementation, as a sustainable approach to improving wheat productivity while reducing reliance on chemical fertilizers.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a globally cultivated cereal crop and serves as one of the cornerstones of global food security, providing nearly 20% of the total dietary calories and protein intake for the human population (Irshad et al., 2025). In India, wheat occupies a prominent place in the agricultural landscape, ranking second after rice in both area and production (Ullah et al., 2025a). However, the continuous intensification of wheat cultivation, coupled with declining soil fertility, has led to stagnation in yield levels in many regions (Ullah et al., 2025b). This necessitates the adoption of improved and sustainable agronomic practices to

ensure optimal productivity without compromising environmental health (Divakar Reddy et al., 2023). One of the key challenges in wheat production is the imbalance in soil nutrient availability, particularly the widespread deficiency of micronutrients such as zinc (Zn) Ullah et al., 2025c). Zinc plays a pivotal role in various physiological and biochemical functions in plants, including enzyme activity, protein and nucleic acid synthesis, photosynthesis, and membrane integrity (Khan et al., 2018a). Zinc deficiency is especially prevalent in sandy loam and alkaline soils, common in wheat-growing regions of northern India. This micronutrient deficiency is often linked with

stunted plant growth, chlorosis, poor grain filling, and ultimately, yield losses (Reddy et al., 2023).

Zinc sulphate (ZnSO_4) is widely recommended to correct zinc deficiency due to its high solubility and effectiveness in supplying bioavailable zinc to crops (Manan et al., 2025). However, reliance solely on inorganic fertilizers is often economically unsustainable for smallholder farmers and may lead to environmental degradation through nutrient leaching and accumulation. This has led to increased interest in integrated nutrient management (INM) strategies that combine chemical fertilizers with biofertilizers, which are natural, living microorganisms that improve soil fertility and plant nutrition through biological processes (Kumar et al., 2021; Shakir et al., 2023a and 2023b).

Biofertilizers such as Azotobacter (a free-living nitrogen-fixing bacterium) and Phosphate Solubilizing Bacteria (PSB) play significant roles in improving nutrient uptake efficiency in non-leguminous crops like wheat. Azotobacter not only fixes atmospheric nitrogen but also synthesizes growth-promoting substances like indole acetic acid (IAA), gibberellins, and Cytokinins, enhancing root development and plant vigor (Shakir et al., 2023b). PSB enhances the availability of phosphorus by converting insoluble phosphate forms into soluble ones that are readily absorbed by plants. When used in combination, these biofertilizers promote a more robust and efficient root system, which further facilitates the uptake of macro and micronutrients, including zinc (Ssemugenze et al., 2025).

The integrated application of biofertilizers and zinc sulphate has shown promising results in improving not only the growth parameters such as plant height, dry matter accumulation, and tillering ability but also important yield attributes including spike length, number of grains per spike, 1000-grain weight, grain yield, and harvest index (Khan et al., 2028b; Lubna et al., 2025). The synergistic interaction between beneficial microbes and micronutrients creates a favorable rhizosphere environment, enhances nutrient use efficiency, and supports long-term soil health (Samantaray et al., 2024).

Despite growing evidence on the individual roles of biofertilizers and zinc in crop production, studies focusing on their combined influence on wheat performance are relatively scarce, especially under field conditions specific to the Indo-Gangetic plains

(Khan et al., 2018). Therefore, the present study was undertaken to evaluate the integrated use of biofertilizers (PSB and Azotobacter) along with zinc sulphate at graded levels (20, 25, and 30 kg/ha) for their effects on the growth, yield, and overall productivity of wheat (*Triticum aestivum* L.) (Khosravi et al., 2024). The study aims to contribute to the development of sustainable and cost-effective nutrient management practices for improving wheat cultivation in zinc-deficient soils.

Materials and Methods

The field experiment was conducted in 2024 at the Govt Post College, Timergara District, Dir Lower Khyber Pakhtunkhwa, Pakistan. The experimental site is geographically located at 25°24'42" N latitude, 81°50'56" E longitude, and at an altitude of 98 meters above mean sea level (Asif et al., 2025).

The experiment was laid out in a Randomized Block Design (RBD) with ten treatments, each replicated three times. The individual plot size was 3 m × 3 m. The study aimed to investigate the integrated effect of biofertilizers and zinc sulphate on the growth and yield of wheat (*Triticum aestivum* L.) (Kumar, 2022).

Experimental Factors

• Biofertilizer Treatments:

- Phosphate Solubilizing Bacteria (PSB) @ 10 ml/kg seed
- Azotobacter @ 10 ml/kg seed
- PSB + Azotobacter @ 10 ml each/kg seed

• Zinc Sulphate (ZnSO_4) Levels:

- 20 kg/ha
- 25 kg/ha
- 30 kg/ha

Treatment Combinations

- T₁ – PSB + ZnSO_4 @ 20 kg/ha
- T₂ – PSB + ZnSO_4 @ 25 kg/ha
- T₃ – PSB + ZnSO_4 @ 30 kg/ha
- T₄ – Azotobacter + ZnSO_4 @ 20 kg/ha
- T₅ – Azotobacter + ZnSO_4 @ 25 kg/ha
- T₆ – Azotobacter + ZnSO_4 @ 30 kg/ha
- T₇ – PSB + Azotobacter + ZnSO_4 @ 20 kg/ha
- T₈ – PSB + Azotobacter + ZnSO_4 @ 25 kg/ha
- T₉ – PSB + Azotobacter + ZnSO_4 @ 30 kg/ha
- T₁₀ – Control (no biofertilizer or ZnSO_4)

Crop Management

Wheat was sown on 17th November 2022 using recommended agronomic practices. Zinc sulphate was applied as a basal dose and thoroughly mixed in the soil before sowing (Ullah et al., 2024). The biofertilizers were applied as seed inoculants before sowing. At maturity, the crop was harvested by collecting samples from a 1 m² area in the center of each plot to minimize border effects. From each sample plot, five representative plants were randomly selected for recording biometric and yield-related parameters (Chaudhary, 2022).

Parameters Recorded

The following growth and yield parameters were observed:

- Plant height (cm)
- Dry weight (g/plant)
- Crop growth rate (g/m²/day)
- Number of cobs per plant
- Number of seed rows per cob
- Number of seeds per cob
- Seed index (100-seed weight in grams)
- Grain yield (t/ha)
- Stover yield (t/ha)

Statistical Analysis

The recorded data were subjected to analysis of variance (ANOVA) as per the procedure described by Gomez and Gomez (1976) to test the significance of treatment effects. Where applicable, the critical difference (CD) at a 5% level of significance was used for comparing the means.

Growth Parameters

Plant Height (cm)

At harvest, a significantly higher plant height (159.03 cm) was recorded in Treatment T₉ (PSB + Azotobacter + ZnSO₄ @ 30 kg/ha), followed closely by Treatment T₈ (PSB + Azotobacter + ZnSO₄ @ 25 kg/ha), which was found to be statistically at par with T₉. The results indicate that the integrated application of biofertilizers as seed inoculants, along with zinc sulphate, positively influenced plant height. The increase in plant height may be attributed to the timely and balanced availability of essential nutrients, particularly nitrogen and phosphorus from biofertilizers and zinc from ZnSO₄, which play a pivotal role in cell elongation, division, and internodal growth. Additionally, zinc is known to enhance the synthesis of indole acetic acid (IAA), a plant hormone that promotes shoot elongation. The

bacterization of wheat with Azotobacter likely stimulated root proliferation and nutrient uptake, which in turn supported shoot development. These findings are consistent with the observations reported by Garima Joshi and Aaradhana Chilwal (2018) and Alka Jyoti Sharma et al. (2020), who also found that the integration of biofertilizers and micronutrients significantly increased plant height in cereal crops.

Crop Growth Rate (g/m²/day)

At the 60-80 days after sowing (DAS) interval, the highest crop growth rate (64.12 g/m²/day) was recorded in Treatment T₉ (PSB + Azotobacter + ZnSO₄ @ 30 kg/ha), while Treatment T₈ (PSB + Azotobacter + ZnSO₄ @ 25 kg/ha) was statistically at par with T₉. The results demonstrate that the combined application of biofertilizers and a higher zinc dose significantly enhanced the crop growth rate during this critical growth stage. The improved crop growth rate can be attributed to the enhanced nitrogen availability resulting from the biological nitrogen fixation capacity of Azotobacter, which supports vigorous vegetative development (Ullah et al., 2018g). This finding aligns with the study of Monib et al. (1979), who reported that Azotobacter inoculation increased soil nitrogen content, thereby contributing to higher biomass accumulation and growth rate. Additionally, Fallik et al. (1988) observed a noticeable enhancement in both root and shoot growth of Zea mays under controlled conditions when biofertilizers were applied. Enhanced root systems facilitate greater nutrient and water uptake, directly supporting higher photosynthetic activity and dry matter production. According to Ritchie et al. (1993), although plants require relatively small amounts of nutrients during their early developmental stages, a high concentration of available nutrients in the root zone during these stages is critical for promoting robust early growth, which in turn influences subsequent crop performance (Ullah et al 2023). The combined presence of PSB and Azotobacter, along with adequate zinc from ZnSO₄, likely created a favorable rhizosphere environment, improving nutrient solubilization and uptake. This contributed to greater leaf area expansion, higher chlorophyll content, and longer duration of active photosynthesis, all of which supported higher crop

growth rate during the 60–80 DAS interval (Ullah et al., 2024).

Dry Weight per Plant (g)

At harvest, the highest plant dry weight (162.70 g) was recorded under Treatment T₉ (PSB + Azotobacter + ZnSO₄ @ 30 kg/ha), which was significantly superior over all other treatments. However, Treatment T₈ (PSB + Azotobacter + ZnSO₄ @ 25 kg/ha) was statistically at par with T₉, indicating that the combined application of biofertilizers and zinc sulphate had a substantial impact on dry matter accumulation (Ullah et al., 2018g).

The increase in plant dry weight may be attributed to the inoculation of Azotobacter, which not only fixes atmospheric nitrogen, but also plays a vital role in modifying microbial communities, suppressing soil-borne pathogens, and enhancing the bioavailability of phosphorus (Ullah et al., 2018h). Moreover,

Azotobacter produces growth-promoting substances such as auxins, cytokinins, and gibberellins, which stimulate early plant development and contribute to enhanced vegetative growth. These findings align with the observations reported by Meshram and Shende (1982), who noted that Azotobacter improves plant growth through multiple physiological mechanisms.

The beneficial effects of Azotobacter on plant dry weight are further supported by Jarak et al. (2012), who reported similar results in maize. In addition, Ghodpage et al. (2008) emphasized the importance of adequate zinc supply throughout the crop life cycle, which enhances photosynthetic activity, prolongs leaf area duration, and supports metabolic processes essential for dry matter production. The observed increase in dry biomass under integrated treatments reflects the synergistic role of biofertilizers and zinc in optimizing plant metabolic efficiency and overall growth performance (Ullah et al., 2018).

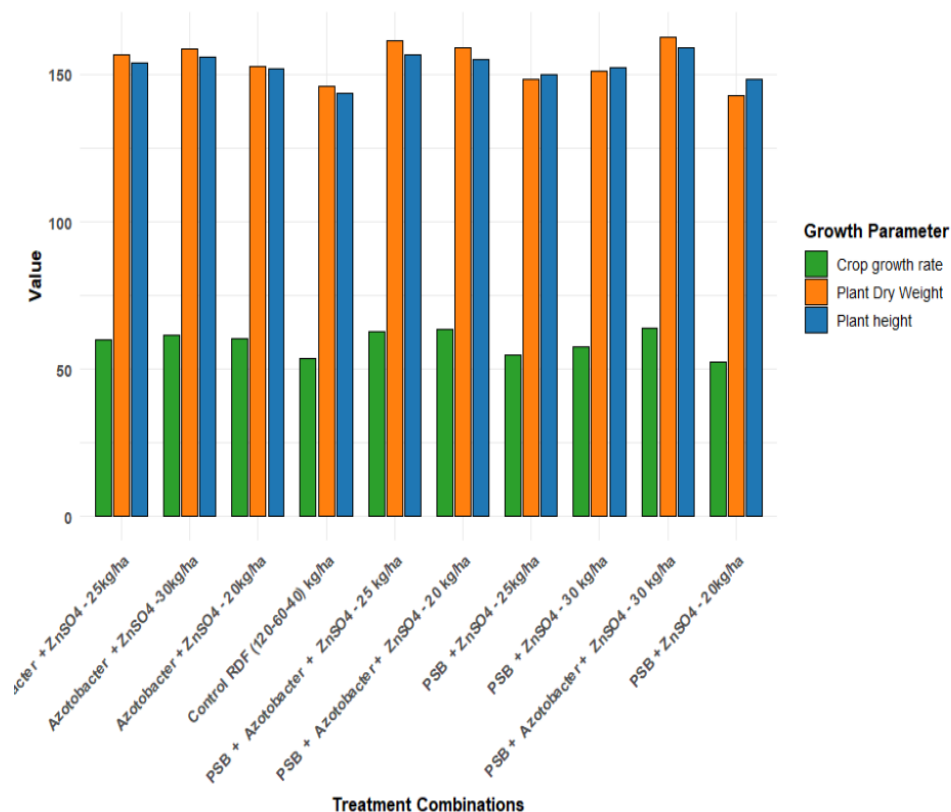


Figure 1 represents the crop growth rate, plant dry weight, and plant height

Yield Attributes

Number of Cobs per Plant

A significantly higher number of cobs per plant (1.8) was recorded in Treatment T₉ (PSB + Azotobacter + ZnSO₄ @ 30 kg/ha), which was significantly superior

over all other treatments. However, Treatment T₈ (PSB + Azotobacter + ZnSO₄ @ 25 kg/ha) was statistically at par with T₉.

The increased number of cobs per plant may be attributed to improved soil nutrient status due to biofertilizer inoculation, which created a favorable rhizosphere environment for root proliferation and nutrient uptake (Ullah et al., 2018c). Azotobacter and PSB are known to produce growth-promoting substances such as gibberellins, Cytokinins, and auxins, while also contributing to nitrogen fixation. These microbial activities collectively enhance plant vigor and reproductive potential. These results are from Singh and Totawat (2002).

Number of Seeds per Cob

The maximum number of seeds per cob (553.4) was also observed in Treatment T₉, followed by T₈, which was statistically similar. The combined application of biofertilizers and zinc enhanced reproductive development and seed setting in wheat (Shakir et al., 2023a).

The improvement in grain number may also be linked to the presence of magnesium, which plays a role in pollen viability and fruit set. As noted by Mahgoub et al. (2010) and Siam et al. (2008), magnesium positively influences pollen formation and viability, contributing to a higher grain count per cob. While the increase in grain number was not drastic, it was significant enough to reflect the effectiveness of integrated nutrient management (Ullah et al., 2018; 2025).

Number of Rows per Cob

A significantly higher number of rows per cob (16.85) was recorded in Treatment T₉, with T₈ again being statistically at par. The improved number of rows may result from enhanced zinc availability, which supports reproductive growth, grain setting, and development of reproductive structures.

The positive response of maize yield components to zinc application may be attributed to its essential role in enzyme activation, protein synthesis, and hormone regulation, all of which are critical during the flowering and grain filling stages. These results are in agreement with findings reported by Gupta et al. (2018).

Seed Index (100-Seed Weight in g)

Treatment T₉ exhibited the highest 100-seed weight (29.35 g), indicating better seed development. Treatment T₈ was statistically equal to T₉. The increased nitrogen availability from Azotobacter inoculation likely contributed to enhanced leaf area and photosynthetic efficiency, leading to greater assimilate accumulation in developing grains. These findings align with Kader et al. (2002), who reported that Azotobacter improved nitrogen use efficiency and increased seed size and weight in cereal crops (Ullah et al., 2019c).

Grain Yield (t/ha)

The highest grain yield (6.5 t/ha) was recorded in Treatment T₉, significantly outperforming all other treatments, with T₈ being statistically at par. The improvement in grain yield can be linked to the positive effects of biofertilizers on nutrient availability and root development, as well as the role of zinc in chlorophyll formation and enzymatic activity (Ullah et al., 2019b).

Biofertilizer-treated plants exhibited enhanced photosynthetic activity, improved metabolic functions, and better synthesis of growth regulators, which together contributed to increased reproductive efficiency and grain filling. These findings are consistent with those of (Shaikh Wasim Chand et al. 2017) and Chandra Naik et al. (2020).

Stover Yield (t/ha)

The maximum stover yield (12.9 t/ha) was also recorded under Treatment T₉, indicating the significant role of integrated nutrient application in promoting total biomass production. Treatment T₈ was again statistically at par (Ullah et al., 2018e). Zinc fertilization is known to promote plant metabolism and vegetative growth, which leads to increased straw yield. Similar results have been reported by Tariq et al. (2014) and Palai et al. (2018), who highlighted the beneficial effects of zinc and biofertilizers on overall plant vigor and fodder production (Ullah et al., 2019a; Ullah et al., 2018f).

Harvest Index (%)

The highest harvest index (33.8%) was observed in Treatment T₉, which was significantly higher than the other treatments, with T₈ again being statistically at par (Ullah et al., 2018b). The increased harvest index indicates improved partitioning of assimilates

toward grain production relative to total biomass (Ullah et al., 2021). This improvement may be attributed to the enhanced availability of phosphorus and other nutrients facilitated by biofertilizers, especially in calcareous soils, where nutrient

availability is typically constrained. These results are supported by Afzal et al., who demonstrated the beneficial role of biofertilizers in nutrient uptake and efficient biomass utilization (Ullah et al., 2018d).

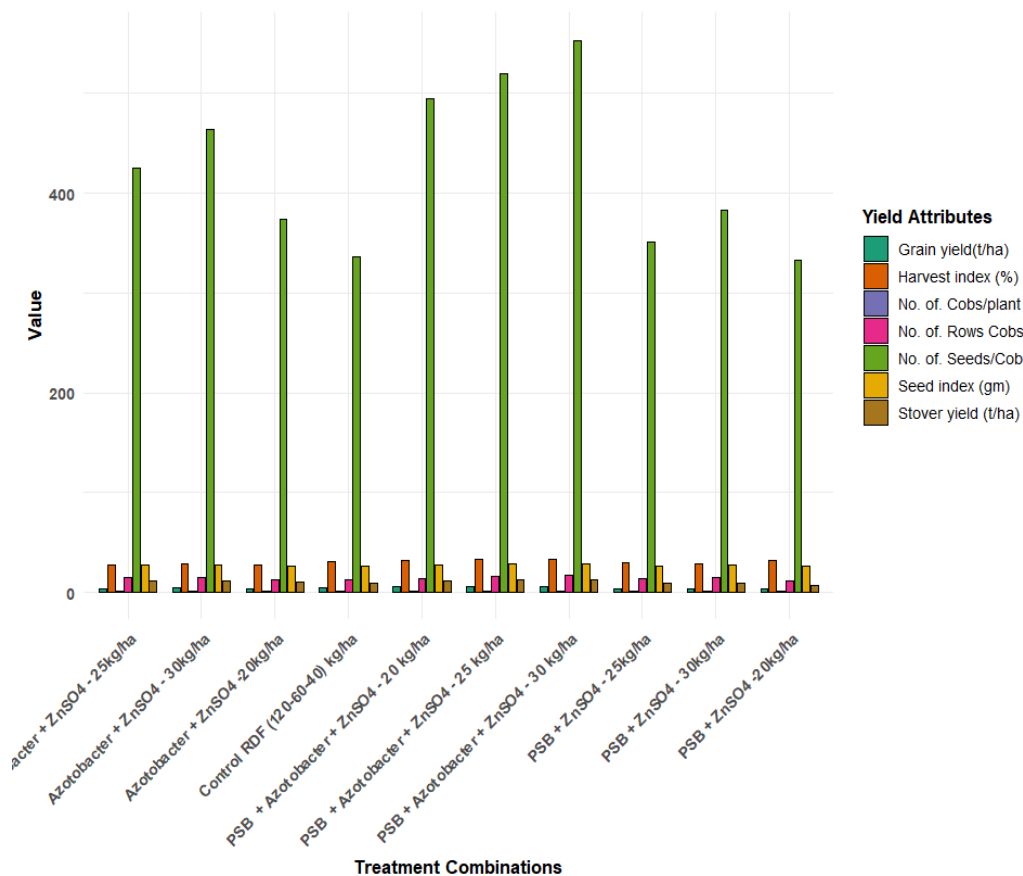


Figure 2. Resented no of cobs/plan, no of rows of cobs, no of seeds/cob, seed index (gm), grain yield(t/ha), stover yield (t/ha), harvest index (%)

Conclusion

The present investigation demonstrated that the integrated application of biofertilizers (PSB + Azotobacter) and zinc sulphate, particularly at 30 kg/ha, significantly improved the growth parameters and yield attributes of wheat (*Triticum aestivum* L.) under field conditions. Among all treatments, Treatment T₉ (PSB + Azotobacter + ZnSO₄ @ 30 kg/ha) consistently recorded the highest values in plant height, dry weight, crop growth rate, number of cobs per plant, rows and seeds per cob, seed index, grain yield (6.5 t/ha), stover yield (12.9 t/ha), and harvest index (33.8%).

The positive effects observed are likely due to the synergistic interaction between beneficial microbes

and zinc, which enhanced nutrient solubilization, nitrogen fixation, root development, and physiological activity, ultimately leading to better growth and higher productivity. Biofertilizers improved nutrient use efficiency and soil health, while zinc played a vital role in hormonal regulation and reproductive development. Therefore, it can be concluded that the integrated use of PSB + Azotobacter + ZnSO₄ at 30 kg/ha is a viable and eco-friendly strategy for enhancing wheat growth, yield, and sustainability in nutrient-deficient soils. Adoption of such integrated nutrient management practices can reduce the dependency on chemical fertilizers and support sustainable agriculture.

Novelty Statement

This study provides novel insights into the synergistic effects of biofertilizers (PSB and Azotobacter) and zinc sulphate on the growth and yield performance of wheat (*Triticum aestivum* L.) under field conditions. Unlike previous research focusing on either biofertilizers or micronutrients alone, this work uniquely demonstrates that their combined application, particularly at 30 kg/ha of ZnSO₄, significantly enhances crop productivity. The findings contribute to the development of sustainable nutrient management strategies, offering a practical alternative to excessive chemical fertilizer use while improving soil health and crop performance in zinc-deficient soils.

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