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Influence of *Bacillus subtilis* Bioinoculant Application Methods on Growth, Yield Traits, and Biochemical Contents of Wheat (*Triticum aestivum* L.) Cultivars

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ABSTRACT

Food security and environmental sustainability are significant challenges in modern agriculture. The overuse of chemical fertilizers has resulted in environmental and soil issues, highlighting the need for eco-friendly alternatives like bio-fertilizers. Among these, phosphate-solubilizing bacteria (PSB), such as *Bacillus subtilis*, are promising, but their effectiveness across different application methods is still being studied. This research compares three application methods - seed dipping, sand-soil granules, and foliar spray - on the growth of two wheat cultivars, Gulzar-19 and Zarghon-21, in a greenhouse setting. The positive control received chemical fertilizers, while the negative control received no supplements. Results showed that using the seed-dipping method, Gulzar-19 had the highest total chlorophyll (approximately 60.88 mg/g) and protein content (approximately 7.87 mg/g). Zarghon-21 produced the most grains per spike (approximately 41) and had higher total chlorophyll (approximately 65.15 mg/g). In the granule method, both cultivars displayed notable root lengths, with Zarghon-21 showing the highest grain weight per spike (approximately 1.53 g). The foliar spray method yielded greater plant height (approximately 82.12 cm) for Zarghon-21 and higher sugar content (approximately 165.69 mg/g) for Gulzar-19. Overall, PSB-inoculated plants consistently outperformed the negative control, demonstrating that seed dipping is the most effective application method, followed by granular and spray methods. This approach can enhance wheat production while reducing chemical fertilizer use.

Keywords: *Bacillus subtilis*, bioinoculant, chemical fertilizers, methods of application, wheat.

INTRODUCTION

The steadily increasing global population, projected to reach nearly 10 billion by 2050, poses a significant challenge to global food security (Muhammad et al., 2025). To meet rising food demand, intensive use of chemical fertilizers and pesticides has become a widespread agricultural practice (Jha et al., 2025). However, the excessive and indiscriminate application of these agrochemicals has resulted in soil and water contamination, environmental degradation, and increased production costs (Mishra et al., 2013; Shahwar et al., 2023). Addressing these issues requires the adoption of sustainable and eco-friendly approaches that can enhance agricultural productivity while maintaining environmental health (Jha et al., 2025).

Wheat is one of the most prevalent grains in the human diet, along with corn and rice. According to global production, wheat makes up 53% of human nutrition in wealthy nations and 85% in developing nations (Cvijanović et al., 2022). In areas where population growth is occurring quickly, it is an essential food source (Donn et al., 2015). Gregory and George (2011) predict that by 2030, worldwide wheat production will need to rise by 67% in order to keep up with population growth. 12% of this increase will

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come from increasing production in already-existing areas, while 21% will come from farming new areas. However, the production and excessive use of nitrogen fertilizers contribute to various environmental problems (Xia et al., 2017). To meet the demands of sustainable production while preserving the environment, the use of microbiological preparations is becoming increasingly important (Cvijanović et al. 2022).

Bioinoculants, also known as biofertilizers, are microbial formulations containing beneficial microorganisms that improve nutrient availability and promote plant growth through mechanisms such as nitrogen fixation, phosphate solubilization, and phytohormone production (Soumare et al., 2020). These microorganisms are commonly found in the rhizosphere or within plant tissues as endophytes (Ibáñez et al., 2023). Bioinoculants can be applied to plant surfaces, seeds, or soil, depending on the target crop and intended function (Allouzi et al., 2022; Petcu et al., 2023). The type of microorganism used is the primary basis for classification, with bacteria and fungi being the most widely utilized groups (Chaudhary et al., 2022). However, the use of microalgae as biofertilizers has also increased in recent years (Kapoore et al., 2021). Among bacterial groups, *Bacillus subtilis* is a well-documented phosphate-solubilizing bacterium known for enhancing phosphorus bioavailability and supporting plant development (Hussain et al., 2023; Ghanaim et al., 2025). Phosphorus is an essential macronutrient involved in photosynthesis, energy transfer, and root growth, yet most soil phosphorus exists in insoluble forms that are unavailable to plants (Fathi and Mehdiniya, 2023).

The effectiveness of bioinoculants depends not only on the microbial strain but also on the method of application. Common techniques such as seed treatment, root dipping, and soil inoculation can result in varying levels of microbial colonization and nutrient uptake, ultimately influencing plant growth and productivity (Cardarelli et al., 2022). *Bacillus subtilis* (*B. subtilis*) as a rhizosphere growth-promoting bacterium. By improving nitrogen fixation and solubilizing phosphate and potassium, *B. subtilis* improves nutrient uptake and boosts overall crop growth (Li et al., 2021). These advantages have led to a rise in their application in agricultural output in recent years. According to Hashem et al. (2019), *B. subtilis* directly promotes plant growth and development by enhancing nitrogen assimilation through biologically active molecules. Furthermore, *B. subtilis* modifies the form of phosphorus in the soil, increasing its availability and boosting crop output and nutrient absorption (Ghanaim et al., 2025). *B. subtilis* has been demonstrated to improve physiological metabolism and total yield in cucumbers by lowering malondialdehyde (MDA) levels in leaves, boosting the activity of protecting enzymes, and promoting growth (Yang et al., 2026). The aim of this study was to evaluate and compare the effectiveness of different application methods - seed dipping, sand-soil granules, and foliar spray of a *B. subtilis*-based phosphate-solubilizing bacterial bioinoculant on the growth, physiological, and yield-related traits of two wheat cultivars (Gulzar-19 and Zarghon-21) and to identify the most efficient method for enhancing wheat performance under greenhouse conditions as a sustainable alternative to excessive chemical fertilizer use.

MATERIAL AND METHODS

Experimental sites and design

This research was conducted in the greenhouse at the Institute of Biotechnology and Genetic Engineering, University of Agriculture, Peshawar, Pakistan. Two wheat (*Triticum aestivum* L.) cultivars, Gulzar-19 and Zarghon-21, were used as test crops and were obtained from the Cereal Crop Research Institute (CCRI), Pirsabak, Nowshera. The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment.

Confirmation of bioinoculant

To confirm the identity and characteristics of the bacterial bioinoculant, several analyses were conducted. The bioinoculant sample was serially diluted, spread on nutrient agar,

and incubated at 37°C for colony formation. Following Sanders (2012), these colonies were used for enumeration and isolation. Gram staining, as described by Biswas et al. (1970), involved staining smears with crystal violet, treating with Gram's iodine, decolorizing with 95% ethanol, and counterstaining with safranin. The samples were examined under a compound microscope at 100× magnification.

Additionally, a wet mount of the bioinoculant was observed, and LB agar colonies were assessed for cell morphology and Gram reaction, noting cell shape, size, and arrangement (e.g., rods or cocci).

Molecular Identification

Bacterial identification involved the amplification of the 16S rRNA gene via PCR. Genomic DNA was extracted from the bioinoculant using a modified phenol-chloroform method. The process included centrifugation of fresh bacterial cultures, resuspension in lysis buffer, and a series of wash and incubation steps with various reagents, ultimately resulting in the air-drying and resuspension of DNA in TE buffer. DNA quality was assessed using a NanoDrop spectrophotometer, followed by PCR amplification with universal 16S rRNA primers and resolution of the amplicons on a 1% agarose gel.

Cultivation conditions and soil characteristics

The experiment was conducted in a greenhouse under controlled conditions, with a temperature range of 25-28 °C, relative humidity of 60-70%, and natural photoperiod. The plants were grown in plastic pots filled with a mixture of sandy loam soil and sterilized sand in a 2:1 ratio to ensure good drainage and aeration. The soil used for the experiment was analyzed to determine its principal chemical makeup using recommended methodologies described by Pathak et al. (2024). Soil was analyzed in the Department of Soil Sciences at the University of Agriculture, Peshawar.

The experiment was arranged in a completely randomized design (CRD) with three replicates per treatment. Each pot was planted with 15 seeds, and uniform irrigation was provided to maintain soil moisture throughout the experiment. Standard agronomic practices, including periodic weeding and pest control, were applied uniformly to all treatments.

Bioinoculant preparation and application

The phosphate-solubilizing bioinoculant (*B. subtilis* strain) used in this study was provided by the Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories, Peshawar. The culture was maintained in a jaggery (gur) solution at 28°C. The optical density (OD_{600}) of the bacterial suspension was measured using a UV - visible spectrophotometer, with the jaggery solution serving as the blank. To evaluate the phosphate-solubilizing ability of the bioinoculant, Pikovskaya's agar plates were prepared, with calcium phosphate added as an insoluble phosphate source. The *B. subtilis* culture was inoculated onto the plates, which were incubated at 28°C for 14 days. Clear zones surrounding microbial growth indicated phosphate solubilization, with the diameter of the zone directly proportional to the activity of the bioinoculant. To determine the most effective application method for the bioinoculant, three different techniques were evaluated: seed dipping, foliar spray, and sand-soil granules.

Seed dipping

For the seed-dipping method, 15 g of wheat seeds were soaked in 30 mL of the liquid *B. subtilis* bioinoculant suspension for 15 minutes prior to sowing. After soaking, the seeds were sown in the prepared soil medium. In addition to the bioinoculant, chemical fertilizers (urea and potash) were applied to meet the nitrogen and potassium requirements of wheat.

Foliar spray

For the foliar spray method, the bioinoculant culture was diluted at a 1:10 ratio with sterile water. Spray treatment was applied to wheat plants after germination, in

combination with chemical fertilizers as described above. Two spray applications were performed: the first at 30 days after sowing and the second at the booting stage. Approximately 5 mL of the diluted suspension was applied per 1 ft² area.

Sand-soil granules

For the sand-soil granule method, the original bioinoculant culture was mixed with a sterilized sand-soil mixture at a 1:2 ratio (sand:soil). One liter of the original culture was incorporated per 10 kg of sand-soil mixture. The granules were applied in two doses: the first at 10 days and the second at 30 days after sowing, with approximately 8 g of granules applied per 1 ft² area. Chemical fertilizers were also added to meet nitrogen and potassium requirements (1.11 g N/ft² and 2.42 g K/ft²), consistent with the other treatments.

Growth parameters and yield parameters

Plant height (cm) and root length (cm) were measured at the booting stage using a measuring tape. The number of leaves per plant and spike length (cm) were also recorded at maturity. Shoot and root fresh weights (g) were determined immediately after harvesting, and dry weights were obtained after drying samples in an oven at 70°C until a constant weight was achieved. At maturity, the number of grains per spike, spike weight, and grain weight per spike (g) were recorded. The total grain yield per plant and 1000-grain weight were also determined to evaluate the overall productivity. All measurements were performed in three replicates per treatment.

Chlorophyll quantification

For chlorophyll measurement, fresh leaf samples were collected and cleaned with methanol. A 0.2 g portion of fresh leaf tissue was accurately weighed, suspended in acetone, and allowed to dissolve the chlorophyll pigments. The chlorophyll extract was then separated by centrifugation. Absorbance of the supernatant was measured at 645 nm (chlorophyll b) and 663 nm (chlorophyll a). Chlorophyll concentration (mg g^{-1}) was calculated using the following equations (Pérez-Patricio et al., 2018).

Biochemical analysis

Protein estimation

To measure protein content, one gram of grains was ground with a mortar and pestle and placed in an Eppendorf tube. After adding 1 mL of Tris-HCl buffer, the tube was vortexed four times with 15-minute intervals. The sample was then incubated for 24 hours at room temperature, centrifuged at 10,000 rpm for 14 minutes, and the supernatant was transferred to a new tube. Finally, 1900 µL of Bradford reagent was mixed with 100 µL of the sample, and the absorbance was measured at 595 nm using a UV spectrophotometer, using Bovine Serum Albumin (BSA) as the standard.

Phenol content measurement

The Folin-Ciocalteu method (Singleton, 1999) was used to measure the phenol content in wheat grains. A 100 mg portion of grain powder was extracted with 80% methanol and centrifuged at 5000 rpm for 5 minutes. Then, 2.5 mL of 10% Folin-Ciocalteu reagent was added to 0.5 mL of the supernatant, followed by the addition of 2 mL of Na_2CO_3. The mixture was kept at room temperature in the dark for 30 minutes. The absorbance was measured at 670 nm using a UV spectrophotometer. Gallic acid was used as the standard.

Total sugar estimation

The sugar content in wheat grains was measured using the method described by DuBois et al. (1956). A 0.6 g sample of milled wheat grains was homogenized in 6 mL of a methanol/chloroform/water mixture (12:5:1). After centrifugation, the clear supernatant was collected. A 1 mL aliquot of the supernatant was mixed with 1 mL of distilled water, 1 mL of 5% phenol, and 5 mL of concentrated sulfuric acid (H_2SO_4). The mixture was vortexed, incubated in a water bath for 30 minutes, and cooled. Absorbance was measured at 490 nm using a UV spectrophotometer, with glucose as the standard and distilled water as the blank.

Statistical analysis

All experimental data were subjected to one-way analysis of variance (ANOVA), and significant differences between treatment means were determined using Duncan's multiple range test at $p<0.05$. Each value represents the mean ± standard deviation from at least three independent biological replicates.

RESULTS AND DISCUSSION

Confirmation of bioinoculant and molecular identification

The viability and morphological characteristics of the bioinoculant were assessed using streak plate culture on Luria-Bertani (LB) agar plates and Gram staining. After 24 hours of incubation at 37°C, the LB agar plates exhibited clear, uniform, creamy-white bacterial colonies, confirming the viability of the bioinoculant (Figure 1a). Gram staining revealed bacilli-shaped cells that retained the crystal violet stain and appeared purple under 100× magnification, indicating that the bacteria were Gram-positive. The rod-shaped morphology confirmed that the bioinoculant was *Bacillus subtilis* (Figure 1b). These results collectively verified the existence, viability, and identity of the bioinoculant strain used in this study.

Genomic DNA from two bacterial isolates was extracted using the phenol-chloroform method and quantified by NanoDrop, yielding concentrations of 774.7 ng/µl and 112.8 ng/µl, with purities of 1.93 and 1.91, respectively. PCR amplification using universal 16S rRNA primers produced ~1456 bp bands in both samples (Figure 1c), confirming the presence of the 16S rRNA gene and identifying the isolates as *B. subtilis*.

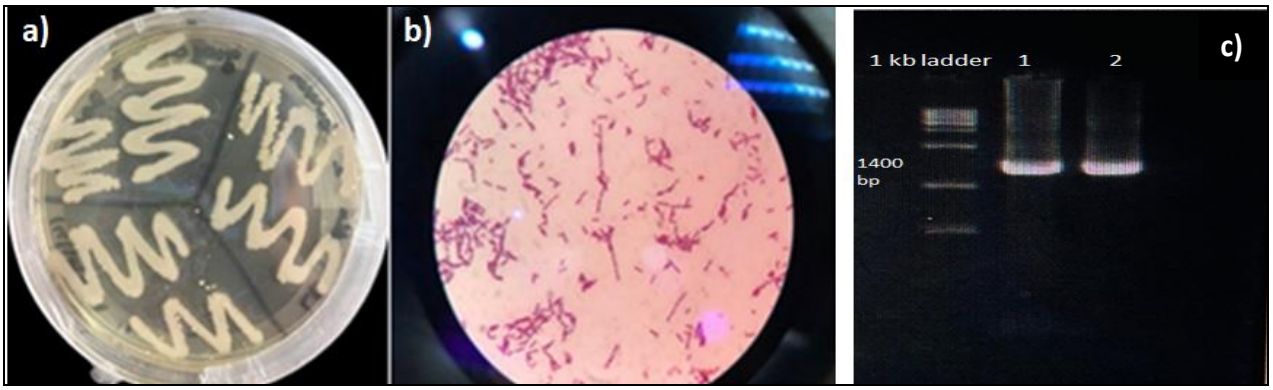

Figure 1. Confirmation of bioinoculant. (a) Growth of creamy-white-colored bacterial colonies on LB media, (b) Gram staining result of purple rod-shaped bacterial strain under microscope 100x, (c) Molecular confirmation of bacterial strain through 16S rRNA gene amplification. Lane 1: 1kb DNA ladder; Lanes 1 and 2: PCR amplified 16S rRNA gene (~1400 bp) of bacterial isolates showing consistent band size for both replicates of the same sample.

Add the soil test results

Soil chemical analysis indicated that the soil had a pH of 7.45 and contained 420 mg/kg total nitrogen, 4.35 mg/kg available phosphorus, and 80.5 mg/kg available potassium.

Phosphate solubilization test

In the phosphate solubilization test, the bioinoculant bacteria (*B. subtilis* strain) with an OD_{600} of 2 were cultured on Pikovskaya's agar medium and incubated to assess their activity. Gulonic acid was used as a positive control to compare phosphate-solubilizing efficiency. After incubation, distinct clear zones appeared around the bacterial colonies, similar to those observed around the positive-control disc (Figure 2). The formation of these clear halos indicates the ability of the bacteria to solubilize the insoluble phosphate present in the medium, demonstrating their potential to enhance phosphorus availability for plants.

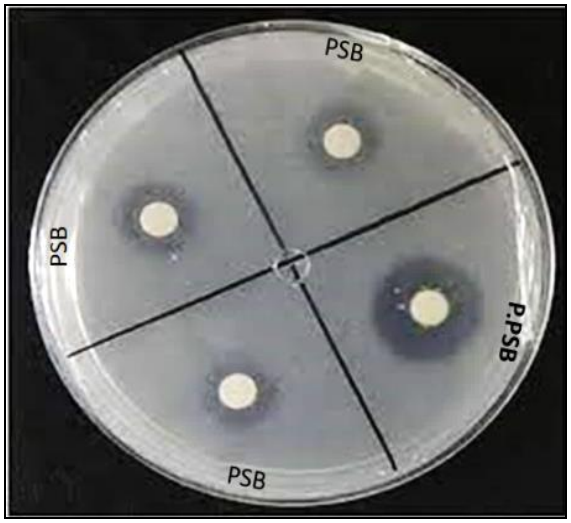

Figure 2. Phosphate-solubilizing activity of the bacterial isolate, *B. subtilis*, on Pikovskaya's agar medium. Three isolates of *B. subtilis* (labeled PSB) and one positive control, gluconic acid (labeled as P.PSB), were inoculated to assess their phosphate solubilization potential.

Effects of PSB bioinoculant on root length

The data in Figure 3 shows that PSB application by seed dipping, granules, and spray methods significantly elevated root length as compared to the negative control. In the sand–soil granule method, Gulzar-19 and Zarghon-21 exhibited significantly greater root lengths of 9.03 cm and 10.14 cm, respectively (Figure 3). In contrast, the roots of the untreated control plants were the shortest, measuring 5.1 cm and 5.0 cm, respectively. Root elongation was higher in the bioinoculant-treated plants, which aligns with previous studies reporting enhanced phosphorus solubility and improved root system development due to increased nutrient availability (Soumare et al., 2020; Ghanaim et al., 2025).

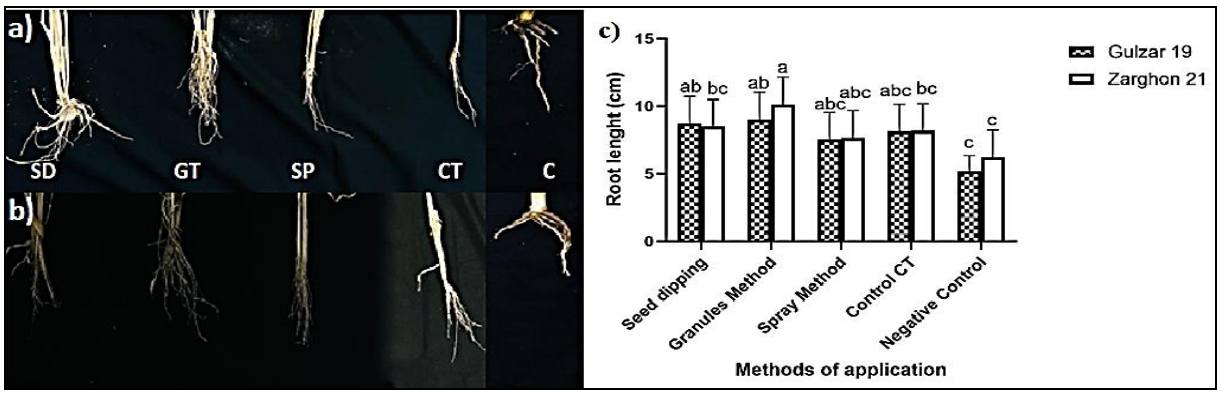

Figure 3. Effect of PSB bioinoculants on root length of wheat cultivars under different application methods. a) Roots architecture of Gulzar-19 under seed dipping (SD), granules method (GT), spray method (SP), control with chemical fertilizer treatment (CT), and control (C); b) Roots architecture of Zarghon-21 under the same respective treatment; c) Average root length (cm). Different letters above the bars indicate statistically significant differences among treatments according to Duncan's multiple range test at $p<0.05$.

Effects of PSB bioinoculant on spike length

Figure 4 illustrates that application of PSB through seed dipping, granule, and spray methods significantly enhanced spike length relative to the negative control. Among the treatments, seed dipping produced the greatest improvement, followed by granule and spray applications. These findings indicate that PSB inoculation positively influences reproductive growth, likely by improving phosphorus availability and

enhancing physiological processes that support spike elongation. The longest spike length was recorded in the seed-dipping treatment, measuring 15.3 cm in Gulzar-19 and 16.83 cm in Zarghon-21. Spike elongation was also enhanced in the granule and spray application methods compared with the control, as shown in Figure 4. The improved spike length may be attributed to enhanced phosphorus absorption and increased photosynthetic activity, which support flower development and promote reproductive success (Fathi and Mehdiniya, 2023).

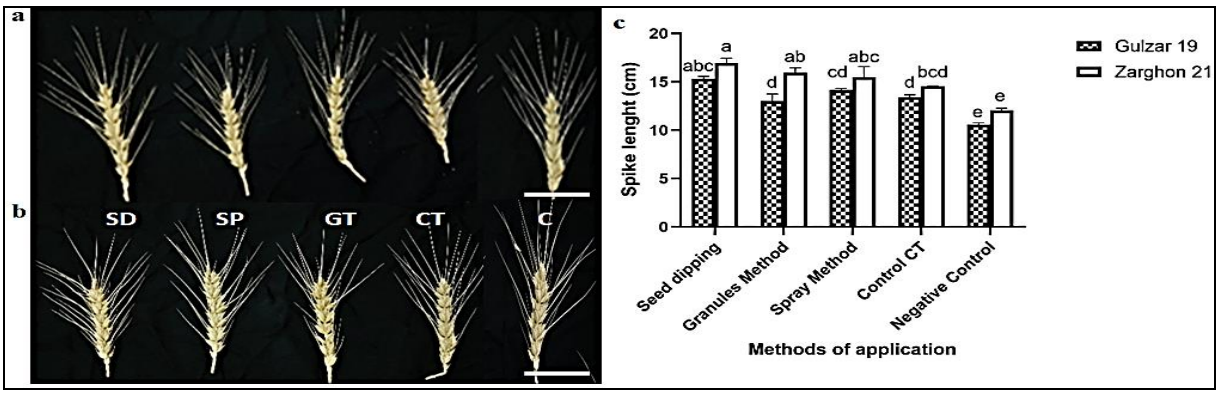

Figure 4. Effect of PSB bioinoculants on spike length of wheat cultivars under different application methods. a) spike length of Gulzar-19 under seed dipping (SD), granules method (GT), spray method (SP), control with chemical fertilizer treatment (CT), and control (C); b) spike length of Zarghon-21 under the same respective treatment; c) Average spike length (cm). Different letters above the bars indicate statistically significant differences among treatments according to Duncan's multiple range test at $p<0.05$.

Effects of PSB bioinoculant on plant height

Figure 5 shows PSB application by seed dipping, granules, and spray methods, with significantly elevated plant height as compared to the negative control. The application of the PSB bioinoculant significantly increased plant height, particularly with the seed-dipping method. The tallest plants reached 81.25 cm in Gulzar-19 and 82.29 cm in Zarghon-21 (Figure 5). Plants treated with the bioinoculant via spray and granule applications also exhibited greater height compared with the control (55.57 cm). The enhanced plant height can be attributed to improved energy metabolism and cell elongation resulting from increased phosphorus bioavailability, which promotes overall plant vigor (Han et al., 2022).

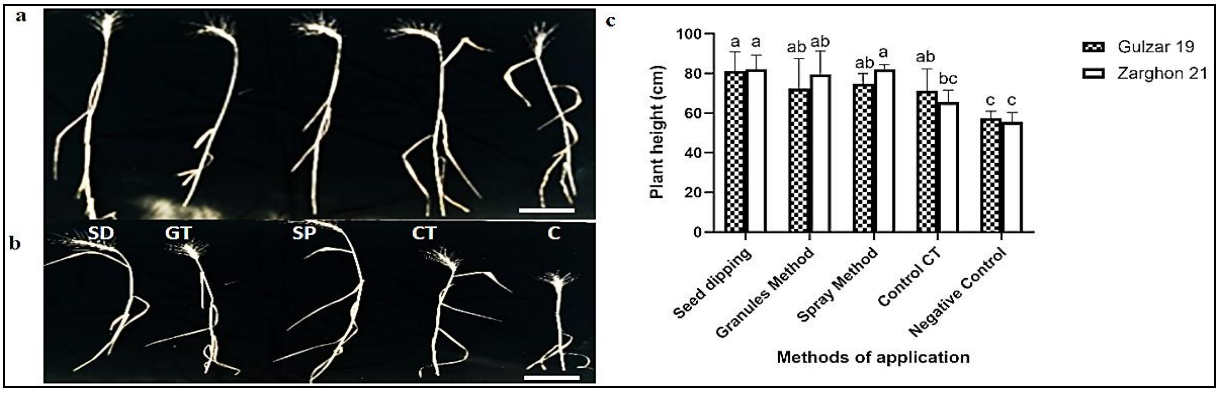

Figure 5. Effect of PSB bioinoculants on plant height of wheat cultivars under different application methods. a) Plant height of Gulzar-19 under seed dipping (SD), granules method (GT), spray method (SP), control with chemical fertilizer treatment (CT), and control (C); b) Plant height of Zarghon-21 under the same respective treatment; c) Average plant height (cm). Different letters above the bars indicate statistically significant differences among treatments according to Duncan's multiple range test at $p<0.05$.

Effects of PSB bioinoculant on grains per spike

Among the different PSB application methods, the granule treatment produced the highest number of grains per spike in Gulzar-19 (43.67 grains), while the seed-dipping method resulted in the highest number of grains per spike in Zarghon-21 (40.83 grains), as shown in Figure 6. The untreated control produced the lowest number of grains per spike, with 24.68 in Gulzar-19 and 27.24 in Zarghon-21. These results are consistent with previous reports indicating that PSB inoculation enhances reproductive efficiency by improving nutrient allocation to developing grains (Cardarelli et al., 2022).

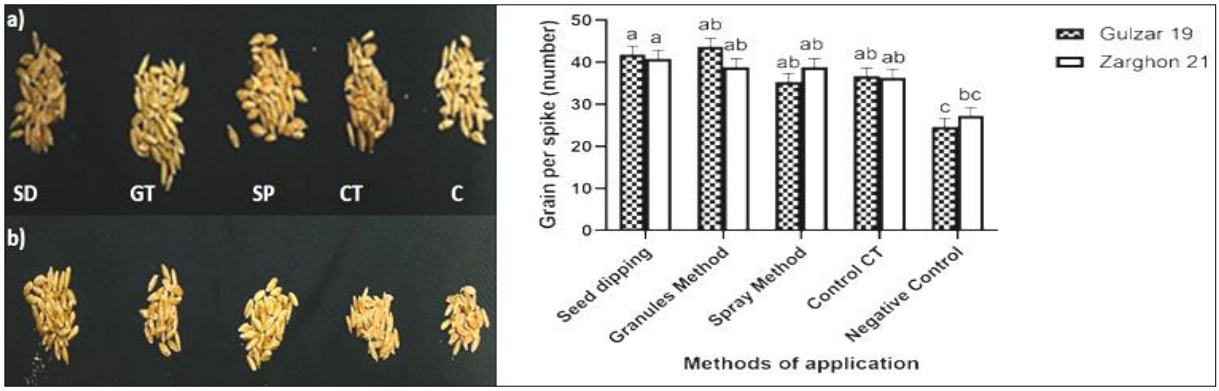

Figure 6. Effects of PSB bioinoculant on grain per spike under different application methods.
a) Grain per spike of gulzar-19 under seed dipping (SD), granules method (GT), spray method (SP), control with chemical fertilizer treatment (CT), and control (C); b) Grain per spike of Zarghon-21 under the same respective treatment;
c) Average grain per spike (No.). Different letters above the bars indicate statistically significant differences among treatments according to Duncan's multiple range test at $p<0.05$.

Effects of PSB bioinoculant on grain weight per spike

Seed dipping of bioinoculant application showed best results in grain weight per spike, where Gulzar-19 and Zarghon-21 showed significantly higher weight per spike 1.67 g and 1.59 g, respectively. Whereas in positive control it was 1.22g and 1.20g as shown in Figure 7. The enhancement of grain weight indicates the contribution of PSB towards promoting the availability of phosphorus, which results in the development of grain filling and grain yield quality in general. (Allouzi et al., 2022) has also reported similar trends, where their findings reveal that there is higher productivity after the application of bioinoculants in the cereal.

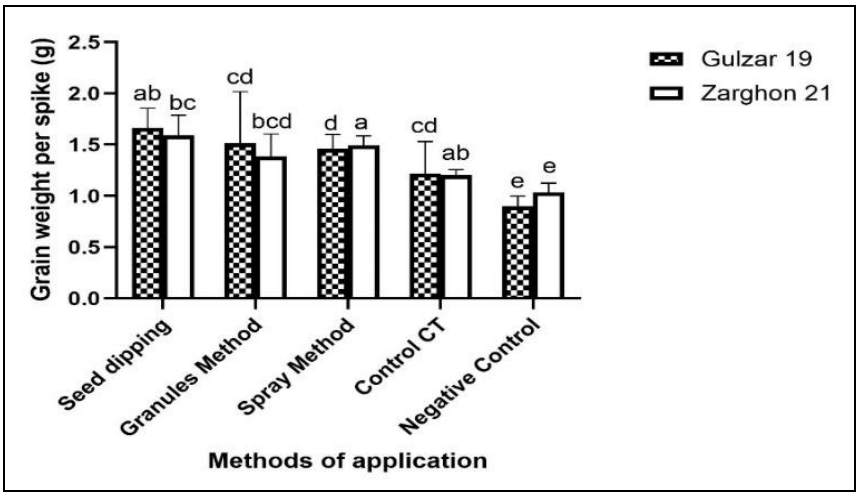

Table 7. Effect of PSB bioinoculants on grain weight per spike of wheat cultivars under different application methods. Different letters above the bars indicate statistically significant differences among treatments according to Duncan's multiple range test at $p<0.05$.

Effects of PSB bioinoculant on total chlorophyll content (TCC)

Chlorophyll content, an important indicator of photosynthetic efficiency, was also enhanced in both wheat cultivars by the seed-dipping method. The highest total chlorophyll levels recorded using this method were 60.88 mg/g in Gulzar-19 and 65.15 mg/g in Zarghon-21. Both the granule and foliar spray methods also showed significant improvements over the control. Interestingly, Zarghon-21 responded better to the granule method, whereas Gulzar-19 showed a stronger response to the spray method (Figure 8A). These findings suggest that PSB inoculation enhances chlorophyll production, particularly via seed dipping, likely by improving phosphorus availability and nitrogen metabolism, which are critical for chlorophyll synthesis and photosynthetic activity. Similar results were reported by Fathi and Mehdiniya (2023), who observed increased chlorophyll content in plants inoculated with phosphate-solubilizing microbes.

Effects of PSB bioinoculant on protein content

The PSB bioinoculant also influenced protein content in wheat grains. The highest protein levels were observed with the seed-dipping method, reaching 8.29 mg/g in Zarghon-21 and 7.87 mg/g in Gulzar-19, indicating that PSB enhanced nitrogen assimilation and amino acid synthesis. Overall, Zarghon-21 exhibited increased protein content across all treatments, suggesting a favorable genotype–microbe interaction (Figure 8B). Enhanced phosphorus availability associated with PSB inoculation likely supports ATP production and protein biosynthesis (Ajeethan et al., 2026). These findings confirm that PSB inoculation boosts metabolic activity and improves the nutritive value of wheat grains.

Effects of PSB bioinoculant on sugar content

Sugar accumulation reflects the photosynthetic performance and carbohydrate metabolism of the plant. In Gulzar-19, the foliar spray treatment produced the highest sugar content (165.69 mg/g), followed by the seed-dipping method (158.62 mg/g). In Zarghon-21, sugar levels also increased under bioinoculant treatments, although they were comparatively lower than in Gulzar-19. Sugar content in the untreated control was significantly lower in both cultivars (Figure 8C). The enhanced sugar levels likely result from increased photosynthetic activity and carbon assimilation, facilitated by improved nutrient availability through PSB inoculation. These findings align with Allouzi et al. (2022), who reported that PSB stimulates carbohydrate production by enhancing root activity and phosphorus uptake.

Effects of PSB bioinoculant on phenol content

Phenolic compounds play a crucial role in plant defense and stress tolerance. In Gulzar-19, the highest phenol levels were observed with the granule (1.39 mg/g) and seed-dipping (1.26 mg/g) treatments, while untreated plants showed considerably lower phenol content (Figure 8D). The elevated phenolic levels suggest that PSB inoculation enhances systemic resistance and secondary metabolism, likely by activating plant defense mechanisms. These findings are consistent with previous studies reporting that bioinoculants stimulate phenolic compound synthesis, thereby increasing plant resistance to abiotic and biotic stresses (Devi et al., 2022).

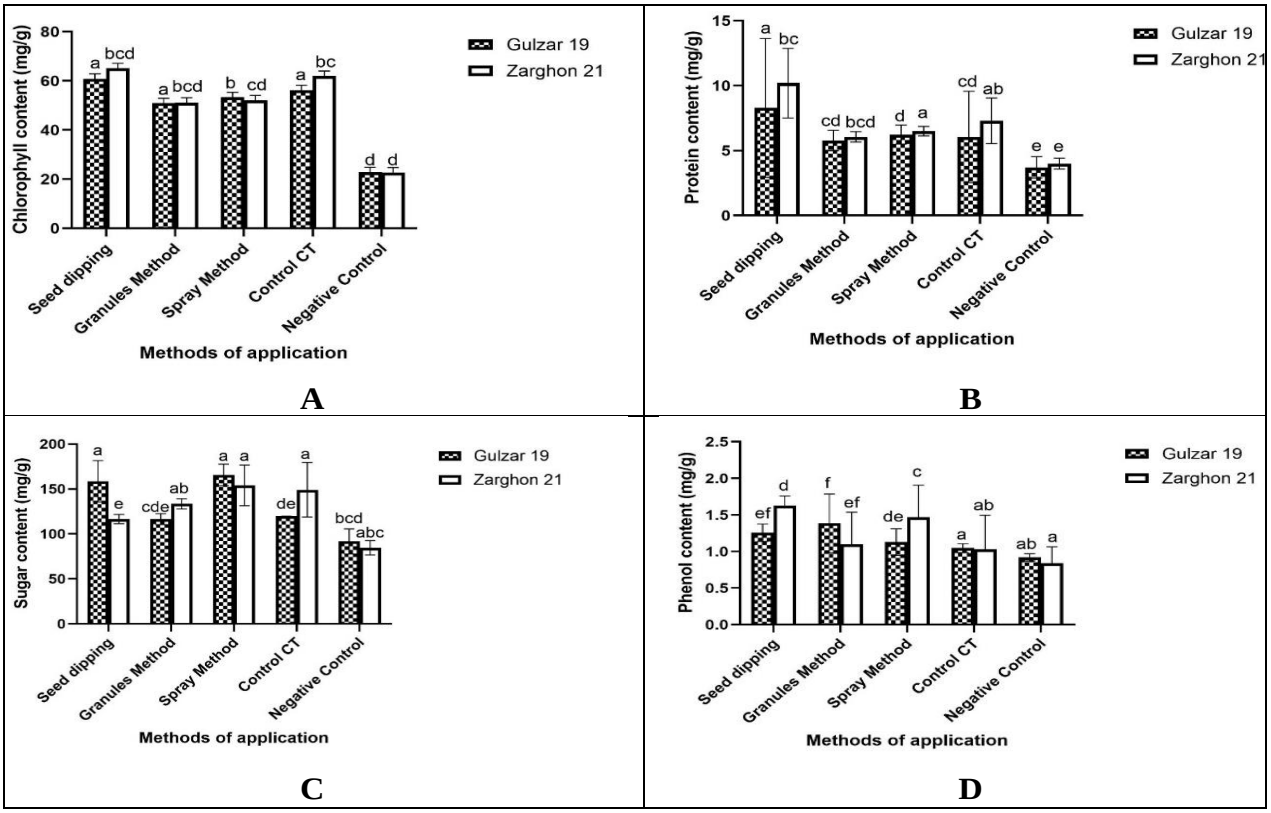

Figure 8. Effect of PSB bioinoculants on total chlorophyll (A), protein (B), sugar (C), and phenol (D) content of wheat cultivars (Gulzar-19 and Zarghon-21) under different application methods. Different letters above the bars indicate statistically significant differences among treatments according to Duncan's multiple range test at $p<0.05$.

CONCLUSIONS

The application of phosphate-solubilizing *Bacillus subtilis* bioinoculant has been shown to effectively enhance the growth, physiological attributes, and yield of wheat. Plants treated with the bioinoculant exhibited increased plant height, root and spike length, and grain weight, as well as higher chlorophyll, protein, sugar, and phenol contents compared to the untreated control. Among the application methods, seed dipping and granule treatments were most effective in promoting rhizosphere colonization and nutrient uptake, while foliar spraying primarily improved biochemical traits. Overall, the B. subtilis bioinoculant represents an environmentally safe, economically viable and sustainable alternative to chemical fertilizers for enhancing wheat productivity.

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