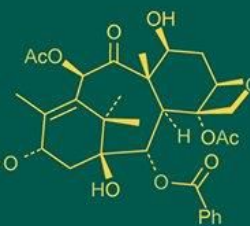
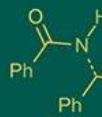


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Response of N-fixing and P solubilizing bacteria with reduced dose of fertilizers on productivity of soybean (*Glycine max* L.)

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Abstract

A field experiment was conducted during the 2022-23 cropping season at the Research Farm, College of Agriculture, IGKV, Raipur, to evaluate the effect of nitrogen-fixing and phosphorus-solubilizing bacteria in combination with reduced fertilizer doses on the productivity of soybean (*Glycine max* L., var. CG SOYA 11-15). The study employed a Randomized Block Design with 9 treatments and 3 replications. Results showed that microbial inoculation significantly improved plant height, nodulation, leghaemoglobin content, chlorophyll content, grain yield, and nutrient use efficiency. The treatment T₅ (75% N + 100% P & K + *Bradyrhizobium daqingense*) recorded the highest grain yield (20.9 q ha⁻¹), maximum nodules (66 plant⁻¹), and highest leghaemoglobin (1.87 mg g⁻¹ FW), achieving 25.5% N savings. Treatment T₇ (*Bacillus aryabhattai*) saved 21.6% P with high yield (20.1 q ha⁻¹), while combined inoculations in T₈ and T₉ saved over 20% N and P. These findings suggest that integrating microbial inoculants with reduced fertilizer inputs enhances soybean productivity and sustainability.

Keywords: Soybean (*Glycine max*), *Bradyrhizobium daqingense*, *Bacillus aryabhattai*, nitrogen-fixing bacteria, phosphorus-solubilizing bacteria, microbial inoculants, leghaemoglobin, sustainable agriculture

Introduction

Chhattisgarh is one of India's agriculturally rich states, where farming is the primary livelihood. Among the major kharif crops, soybean (*Glycine max* L.) has gained prominence due to its high nutritional value and income-generating potential. It contains 40-42% quality protein and 20-22% cholesterol-free oil, along with essential vitamins and minerals like calcium, phosphorus, and iron, making it comparable to meat and dairy in protein content. In India, soybean is cultivated on 11.44 million hectares with a production of 12.04 million tonnes, contributing 43% to the nation's oilseed production and 25% to edible oil output. However, national average productivity (1050 kg ha⁻¹) remains below the global average (1600 kg ha⁻¹), indicating scope for improvement (Rani, D.P. and Singh, C. 2022) [15]. Soybean cultivation in Chhattisgarh covers approximately 0.42 lakh hectares with an average productivity similar to the national average (Ogre *et al.*, 2023) [13]. Despite its potential, sustainable productivity is challenged by over-reliance on chemical fertilizers. Integrating biofertilizers, such as nitrogen-fixing and phosphorus-solubilizing bacteria, offers a promising alternative to improve nutrient use efficiency, crop yield, and environmental sustainability (Lindström and Mousavi, 2020) [10]. Microbial inoculants like *Bradyrhizobium daqingense* and *Bacillus aryabhattai* can enhance soybean growth by fixing atmospheric nitrogen and solubilizing unavailable forms of phosphorus, respectively. These microbes not only supply nutrients but also improve nodulation, chlorophyll content, and leghaemoglobin levels, which are vital for nitrogen fixation. Moreover, phosphate-solubilizing bacteria (PSB) release organic acids and enzymes that mobilize soil phosphorus, reducing the need for chemical inputs. This study evaluates the combined use of *Bradyrhizobium daqingense* and *Bacillus aryabhattai* with reduced fertilizer doses, aiming to enhance soybean yield and assess nutrient savings. The research focuses on promoting integrated nutrient management for more sustainable and cost-effective farming practices.

Experimental details

The present investigation was conducted during Kharif 2023-24 at the Research Farm, IGKV, Raipur (C.G.) using soybean variety CG SOYA 11-15. The crop was sown on July 10, 2023, and harvested on October 30, 2023, with a duration of 93-96 days. The experiment was laid out in a Randomized Block Design (RBD) with 9 treatments and 3 replications, comprising 27 plots in total. Each gross plot measured 20 m², covering a total experimental area of 680 m². The seed rate used was 80 kg ha⁻¹.

Treatments and Experimental Layout

The experiment comprised 9 treatment combinations arranged in a Randomized Block Design (RBD) with 3 replications, totaling 27 plots. Treatments included varying combinations of NPK fertilizer levels and bio-inoculants such as *Bradyrhizobium daqingense*, *Bacillus aryabhataii*, and *Rhizobium japonicum* (IGKV native strain). The recommended dose of fertilizer (RDF) was 25 kg N, 60 kg P₂O₅, and 40 kg K₂O per hectare.

The field was prepared to a fine tilth by ploughing once followed by two harrowings and leveling. Each gross plot measured 4 m × 5 m (20 m²), with a 1 m row-to-row and 0.5 m plot-to-plot spacing. Treatments were randomly allocated within each replication to minimize experimental error.

Plant Height

Plant height was recorded at 30 and 60 DAS from three tagged plants per plot. Measurements were consistently taken from the same plants and expressed in centimetres.

Nodulation Study

At 45 DAS (flowering stage), three plants per plot were carefully uprooted to preserve nodules. Roots were washed under running water, and nodules were counted. Fresh weight was recorded after separation and storage. Nodules were then oven-dried at 60 °C for 18-20 hours (until constant weight), and dry weight was documented.

Chlorophyll Content

Chlorophyll content was measured at the flowering stage using a SPAD-502 meter, which provides rapid, non-destructive estimates of leaf chlorophyll concentration.

Leghaemoglobin Content

Leghaemoglobin concentration was estimated using a modified pyridine hemochromogen method. Leghaemoglobin, a heme-containing protein found in the nodules of leguminous plants, is quantified by converting it into a pyridine-ferrohemochrome complex. The absorbance of this complex is measured spectrophotometrically at a specific wavelength, and the concentration is calculated using its known extinction coefficient.

Procedure

Fresh pink soybean root nodules (~1.0 g) were homogenized in 10 mL cold 0.1 M phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was filtered through Whatman No. 1 filter paper and centrifuged at 10,000 rpm for 10 minutes at 4 °C. The clear supernatant was collected for analysis. Equal volumes (2 mL) of supernatant and cold pyridine were mixed, followed by the addition of sodium dithionite (a pinch or 0.1 mL of 1% solution) to reduce ferric heme to ferrous form. The solution

developed a characteristic pinkish-red colour of reduced leghaemoglobin-pyridine complex. The mixture was then gently mixed and allowed the reaction to stabilize for 5-10 minutes. Spectrophotometric measurement the absorbance was measured at 556 nm using the spectrophotometer and a blank prepared with phosphate buffer and pyridine treated the same manner was used for calibration.

Grain and Straw yield

Crop was harvested and bundles were made plot wise, then allowed to dry in the plot for 2-3 days and weighed. After threshing, plot wise yields for straw and seeds were recorded. Samples were stored to determine the amount of NPK content in seed and straw.

B:C Ratio

The Benefit-Cost (B:C) Ratio is an important economic indicator used in agricultural economics to assess the profitability and economic efficiency of crop production systems. It is defined as the ratio of gross return (total benefits) to the cost of cultivation (total expenditure).

Savings of NPK

Fertilizer savings refer to the reduction in the application of chemical fertilizers (Nitrogen, Phosphorus, and Potassium) without adversely affecting crop yield usually achieved through the use of biofertilizers, organic inputs, or integrated nutrient management (INM) practices. This estimation helps to assess the economic and environmental benefits of such technologies in crop production systems.

Objective

To quantify the savings in terms of N, P, and K fertilizers (in kg ha⁻¹ and ₹ ha⁻¹) achieved through partial replacement of chemical fertilizers using microbial inoculants like N-fixing bacteria (e.g., *Rhizobium*, *Azotobacter*) and P-solubilizing bacteria (e.g., PSB, *Bacillus* spp.)

NPK accumulation

Nitrogen

Nitrogen in plant samples was determined by employing KELPLUS digestion and distillation system (Bremner, J. M. 1960). This essentially involves (1) Digesting organic N compound to produce NH₄-N form through digestion and (2) Using distillation to determine the amount of NH₄-N present in the digested plant. For the digestion 0.25gram sample (seed plant⁻¹) was taken into 100 ml capacity micro digestion tube also 2-2.5gram salt mixture (copper sulphate + potassium sulphate) was added. After this 10 ml. H₂SO₄ was added to the digestion tube and This sample mixture was digested in digestion unit (Kelplus) at 350 420 °C for 90 minutes. After digestion, transferred the above micro test tube containing digested plant material to the distillation unit. Digested sample with digestion tube fix in left chamber of Kel plus machine (Kelplus Elite EX). 10 ml boric acid with mixed indicator was taken in 250 ml volume conical flask and places it under the receiver tube for absorption and ammonia liberated. After this distilled samples were titrated by using 0.02 N H₂SO₄ in burette apparatus till the blue colour changes to pinkish colour. The nitrogen content was calculated by using formula

$$\text{Nitrogen (\%)} = \frac{R(\text{Sample titer} - \text{blank titer}) \times \text{Normality of acid} \times 100}{\text{Sample Weight (g)} \times 1000}$$

Phosphorus

Phosphorus estimation following procedure are first digestion of the sample and second color development and estimation (Jackson 1973) [7]. For the digestion of the sample, we took 0.5 g of dried and ground plant sample in a 50 ml conical flask and added 5 ml of triacid mixture. The sample was kept overnight for pre digestion. The Pre-digested sample was placed in hot plate and heated gently at first. The digestion was carried out at 180° to 200 °C till it gives white fumes and the liquid content is largely volatilized. At the stage of complete digestion, the residue remained in the flask become clear and white, (if the digested residues brown due to organic matter, then again add 0.5 ml of triacid mixture and digest the material.) Transferred the digested material into a 100 ml volumetric flask, dilute it with distilled water up to the mark. The solution was then filtered it through Whatman No. 42 filter paper for further analysis. After the digestion for Color development and estimation. We took 10 ml filtered plant extract into a 50 ml volumetric flask, added 10 ml ammonium molybdate-ammonium vanadate solution and made the volume to 50 ml with distilled water. Yellow color will develop after 15-20 minutes due to the formation of the phosphorus-vanado-molybdate complex. To make standard curve from 50 ppm standard solution of P we took 0, 2, 4, 6, 8, and 10 ml and transfer in 50 ml volumetric flask then add 10 ml of ammonium molybdate-ammonium vanadate solution and make the volume. This contains 0, 2, 4, 6, 8, 10 ppm P. Measured the color intensity of standard solutions at a wave length of 470 nm or by using blue filter in a spectrophotometer. Prepare the standard curve by plotting concentration of P on X-axis and spectrophotometer readings on Y-axis on a graph paper. Take reading of plant samples in the similar manner that of standard. Workout the ppm content of P with help of standard curve. Further P accumulation was calculated.

Potassium

For the potassium estimation we took about 1 g of the plant sample into a 100 ml conical flask and added 15 ml of the triacid mixture and covered the mouth of the flask with funnel. Then the content was then digested over a sand bath till a clear colorless solution is obtained. After cooling, the digest was transferred to a 100 ml volumetric flask and the volume was made up with distilled water and then the solution was then filtered through Whatman No. 42 filter paper and the filtrate was collected for potassium estimation. Potassium estimated through Flame photometer, the red filter (768 nm) was selected for potassium detection and calculation of potassium by using formula

$$\text{Potassium (\%)} = \frac{X \times V}{1000 \times W}$$

Results and Discussion

Plant height

Plant height of soybean was significantly influenced by the different treatments at both 30 and 60 days after sowing (DAS) (Table-1). At 30 DAS, the highest plant height (15.8 cm) was recorded in T₁ (RDF), which was statistically at par with several treatments except T₇. Treatments incorporating microbial consortia, such as T₈ (15.4 cm) and T₉ (14.8 cm), showed competitive growth, indicating that partial nutrient inputs supplemented with beneficial microbes can sustain

early vegetative growth. The lowest height (13.0 cm) was observed in T₇, likely due to reduced phosphorus availability despite microbial inoculation. At 60 DAS, T₂ (33.4 cm), receiving 75% N and 100% P and K, recorded the highest plant height, highlighting the critical role of phosphorus in later growth stages. Microbial treatment T₉ (32.9 cm) also showed significant improvement, suggesting a synergistic effect of *Bacillus aryabhattai* and *Rhizobium japonicum* under reduced fertilizer inputs. These findings align with previous reports by Jaga and Sharma (2015) [8] and Jain and Trivedi (2005) [9], who observed improved plant height with integrated use of microbial inoculants and balanced fertilization.

Nodulation

Nodulation in soybean at the flowering stage (50 DAS) was significantly influenced by the treatments (Table-1). The number of nodules ranged from 43 to 66 per plant, with the highest recorded in T₅ (66 nodules/plant), which received 75% N and 100% P and K along with *Bradyrhizobium daqingense*, followed by T₆ (63 nodules/plant) with *Rhizobium japonicum*. The lowest nodule count (43) was observed in T₃, which received reduced phosphorus without rhizobial inoculation. Fresh nodule weight (Fig-1) was also highest in T₅ (2.66 g), followed closely by T₆ (2.58 g), with T₅-T₉ treatments showing statistically similar results, indicating the positive effect of microbial inoculation. Dry weight of nodules was highest in T₅ (0.90 g), statistically at par with T₆, emphasizing the crucial role of phosphorus and effective *Rhizobium* strains in enhancing nodulation and biomass accumulation (Fig-1). These findings are supported by previous studies (Alagawadi *et al.*, 1993; Bhattacharyya, 2012; Gebremariam and Tesfay, 2021) [1, 6], which highlight the synergistic effects of phosphorus and rhizobial inoculation on legume nodulation.

Leghaemoglobin Content

Leghaemoglobin concentration in soybean nodules at 50 DAS was significantly influenced by treatments. The highest content (1.87 mg g⁻¹) was recorded in T₅ (75% N + 100% P and K + *Bradyrhizobium daqingense*), followed by T₆ (1.82 mg g⁻¹) with *Rhizobium japonicum*. The lowest value (1.18 mg g⁻¹) was observed in T₂, which lacked microbial inoculation. Treatments with Rhizobial inoculants (T₅, T₆, T₈, T₉) consistently showed higher leghaemoglobin content, highlighting the role of biofertilizers in enhancing symbiotic nitrogen fixation. Moderate levels were recorded in T₁ and T₃, which lacked microbial application. The superior performance of T₅ and T₆ reflects the combined effect of effective Rhizobial strains and adequate phosphorus, essential for ATP production and nodule function. These results are consistent with findings by Sahu *et al.* (2023) [16], who reported increased leghaemoglobin content with integrated use of NPK and microbial inoculants in soybean.

Chlorophyll Content

Chlorophyll content in soybean leaves at the flowering stage varied significantly across treatments. The highest SPAD values (46.8) were observed in T₁ (RDF) and T₅ (75% N + 100% P and K + *Bradyrhizobium daqingense*), followed by T₆ (44.0 SPAD) with *Rhizobium japonicum*. The lowest chlorophyll content (32.4 SPAD) was recorded in T₂, which lacked microbial inoculation. Treatments with rhizobial

inoculants (T₅, T₆) consistently showed higher chlorophyll content, indicating improved nitrogen availability and enhanced photosynthetic activity due to effective biological nitrogen fixation. Intermediate SPAD values in treatments T₃, T₄, T₇, T₈, and T₉ (37.7-42.6) suggest a moderate impact of nutrient and microbial combinations. These findings are supported by Nyoki and Ndakidemi (2016) [12], Zhou *et al.* (2006) [18], and Prasad (2021) [14], who reported that rhizobial inoculation, particularly when combined with P and K, enhances chlorophyll content through improved nutrient uptake and symbiotic efficiency.

Grain Yield

Grain yield data (Table-2) showed significant differences across treatments, with the highest yield (20.9 q ha⁻¹) recorded in T₅ (75% recommended N + 100% P and K + *Bradyrhizobium daqingense*), followed closely by T₁ (recommended NPK) and T₆ (75% N + 100% P and K + *Rhizobium japonicum*), each yielding 20.8 q ha⁻¹. The lowest yield (16.7 q ha⁻¹) was observed in T₃ (75% P + 100% N and K). Treatments with rhizobial inoculation outperformed those without, indicating the beneficial role of N-fixing biofertilizers in enhancing yield under reduced nitrogen input. These findings align with Jaga *et al.* (2015) [8] and Debela *et al.* (2021) [5], who also reported improved soybean performance with rhizobial inoculation and integrated nutrient management.

B:C Ratio

Data from Table-2 indicated that the highest B:C ratio (1.83) was achieved in T₅ (75% N + 100% P and K + *Bradyrhizobium daqingense*), followed by T₆ (*Rhizobium japonicum*), highlighting the efficiency of microbial inoculants in improving economic returns. The lowest B:C ratio (1.31) was observed in T₃ (75% P + 100% N and K),

possibly due to phosphorus deficiency affecting root development and nodulation. These findings support the economic benefits of integrating biofertilizers with reduced chemical inputs. Similar results were reported by Biswas *et al.* (2024) [3] and Meena *et al.* (2023) [11], who found higher B:C ratios with integrated nutrient management practices in soybean cultivation.

Saving of NPK

Data from Table-2 highlight nutrient input savings through reduced fertilizer application combined with microbial inoculants. T₅ (75% N + 100% P and K + *Bradyrhizobium daqingense*) recorded the highest yield (20.9 q ha⁻¹) with 25.5% nitrogen saving, outperforming the RDF (T₁: 20.8 q ha⁻¹). T₆, with *Rhizobium japonicum*, yielded similarly (20.8 q ha⁻¹) and saved 25% N, indicating the superior efficacy of *B. daqingense*. For phosphorus saving, T₇ (75% P + 100% N and K + *Bacillus aryabhattai*) yielded 20.1 q ha⁻¹ with 21.6% P saving. In contrast, T₃ (same fertilizer dose without inoculant) had the lowest yield (16.7 q ha⁻¹), saving only 5.3% P, underscoring the role of phosphate-solubilizing bacteria. Dual inoculation in T₉ (75% N and P + 100% K + *R. japonicum* + *B. aryabhattai*) yielded 20.5 q ha⁻¹ with 23.6% combined N and P saving. T₈, with *B. daqingense* + *B. aryabhattai*, showed 19.9 q ha⁻¹ yield and 20.2% N and P saving.

T₂ (75% N + 100% P and K, no inoculant) achieved 18.2 q ha⁻¹ (87.5% of RDF yield), indicating a 12.5% N saving, suggesting that under optimal conditions, reduced N inputs may still maintain productivity. These findings align with those of Wei *et al.* (2023) [17], and Debela *et al.* (2021) [5], who reported comparable yields under reduced chemical inputs when combined with microbial inoculants, confirming their role in improving nutrient use efficiency and maintaining yield under input-reduction strategies.

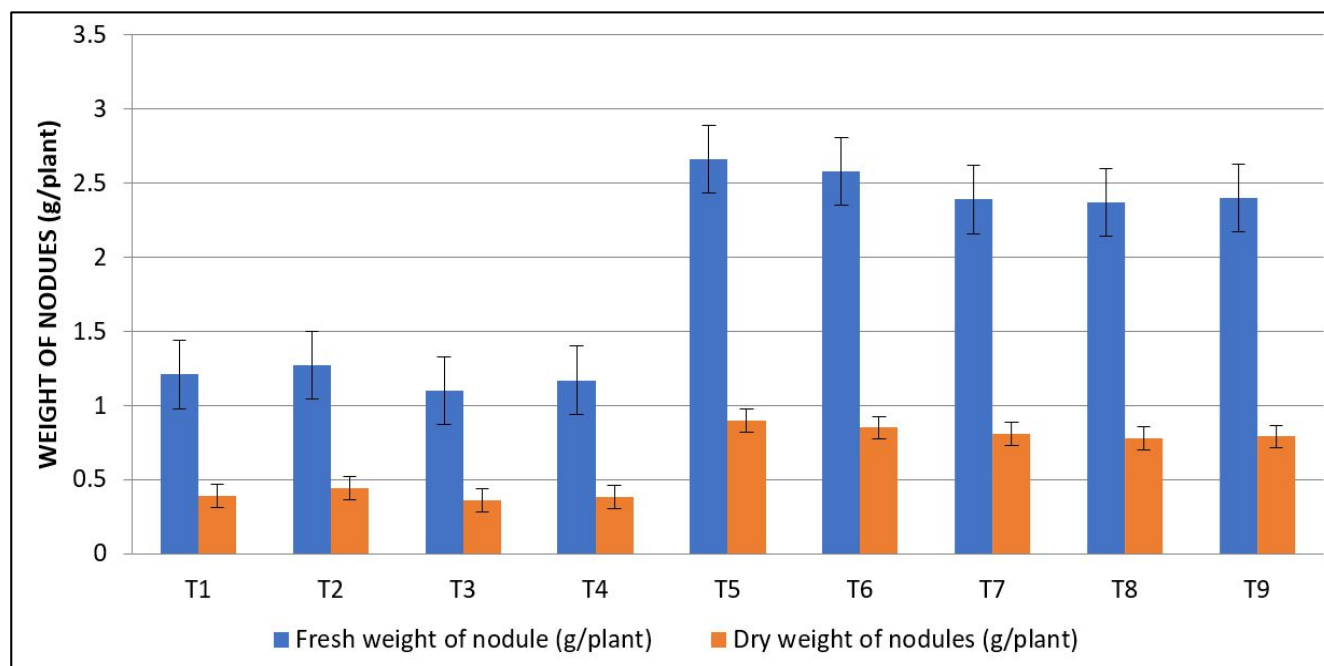


Fig 1: Influence of N-fixing and P solubilizing bacteria (*Bradyrhizobium daqingense* and *Bacillus aryabhattai*) on weight of nodules plant⁻¹

Table 1: Influence of N-fixing and P solubilizing bacteria on plant height (cm plant⁻¹), nodulation, leghaemoglobin content and chlorophyll content (SPAD)

Treatment No	Treatment	30 Days (cm plant ⁻¹)	60 Days (cm plant ⁻¹)	No of Nodules plant ⁻¹	Leghaemoglobin content (mg g ⁻¹)	Chlorophyll Content (SPAD)
T ₁	Recommended dose of fertilizer (25:60:40: NPK)	15.8	33.0	45	1.53	46.8
T ₂	75% N and 100% P and K	15.0	33.4	46	1.18	32.4
T ₃	75% P and 100% N and K	14.3	33.2	43	1.52	37.7
T ₄	75% of N and P and 100% of K	14.1	30.7	44	1.40	39.1
T ₅	75% N and 100% of P and K + <i>Bradyrhizobium daqingense</i>	14.6	29.2	66	1.87	46.8
T ₆	75% of N and 100% of P and K + <i>Rhizobium japonicum</i>	14.4	31.6	63	1.82	44.0
T ₇	75% of P and 100% of N and K + <i>Bacillus aryabhataii</i>	13.0	28.2	53	1.56	41.2
T ₈	75% of N and P and 100% of K + <i>B. daqingense</i> + <i>Bacillus aryabhataii</i>	15.4	30.0	54	1.63	39.1
T ₉	75% of N and P and 100% of K + <i>Bacillus aryabhataii</i> + <i>Rhizobium japonicum</i>	14.8	32.9	58	1.70	42.6
	SE(m)	0.58	0.98	2.00	0.053	1.51
	CD (0.05)	1.75	2.95	5.99	0.112	4.51

Table 2: Response of N-fixing and P solubilizing bacteria on grain yield (q ha⁻¹), B:C Ratio and NPK saving

Treatment No	Treatment	Grain yield (q ha ⁻¹)	Straw yield (q ha ⁻¹)	B:C RATIO	NPK saving
T ₁	Recommended dose of fertilizer (25:60:40: NPK)	20.8	34.3	1.82	-
T ₂	75% N and 100% P and K	18.2	29.1	1.47	12.5% saving of N
T ₃	75% P and 100% N and K	16.7	27.6	1.31	5.3% saving of P
T ₄	75% of N and P and 100% of K	18.6	30.3	1.58	14.4% saving of N and P
T ₅	75% N and 100% of P and K + <i>Bradyrhizobium daqingense</i>	20.9	33.9	1.83	25.5% saving of N
T ₆	75% of N and 100% of P and K + <i>Rhizobium japonicum</i>	20.8	33.7	1.82	25.0% saving of N
T ₇	75% of P and 100% of N and K + <i>Bacillus aryabhataii</i>	20.1	32.8	1.78	21.6% saving of P
T ₈	75% of N and P and 100% of K + <i>B. daqingense</i> + <i>Bacillus aryabhataii</i>	19.8	33.8	1.75	20.2% saving of N and P
T ₉	75% of N and P and 100% of K + <i>Bacillus aryabhataii</i> + <i>Rhizobium japonicum</i>	20.5	32.5	1.85	23.6% saving of N and P
	SE(m)	0.90	1.4		
	CD (0.05)	2.5	4.1		

Conclusion

Among the various treatments, T₅, which included the bio-fertilizer *Bradyrhizobium daqingense*, resulted in the highest plant growth, nodulation (66 nodules plant⁻¹), leghaemoglobin content (1.87 mg g⁻¹), chlorophyll content (46.8 SPAD), grain yield (20.9 q ha⁻¹), and the most favourable benefit-cost ratio (1.83). Furthermore, the treatment received 100% phosphorus dose has a positive impact on plant growth parameters particularly nodulation parameters including yield. This suggests a synergistic interaction between phosphorus availability and microbial activity, particularly involving *Bradyrhizobium japonicum*, *Bacillus aryabhataii*, and native strain (*Rhizobium japonicum*) leading to enhance plant growth and productivity.

In terms of saving of NPK, treatment T₉ (75% of N and P and 100% of K + *Bacillus aryabhataii* + *Rhizobium japonicum*) resulted highest combined saving of N and P. This revealed that the IGKV native *Rhizobial* strain (*Rhizobium japonicum*) showed good compatibility with *Bacillus aryabhataii* than *Bradyrhizobium daqingense*. While treatment T₅ (75% N + 100% P and K + *Bradyrhizobium daqingense*) and T₇ (75% P + 100% N and K + *Bacillus aryabhataii*) were most effective in saving nitrogen and phosphorus individually.

These results suggest that integrating biological inoculants in soybean cultivation can be an effective strategy for improving yield and reducing dependence on synthetic

fertilizers, leading to sustainable agricultural practices with higher economic returns.

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