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Rhizobium and Phosphate Solubilizing Bacteria Influence the Soil Nutrient Availability, Growth, Yield, and Quality of Soybean

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Abstract: Crop production encounters challenges due to the dearth of nitrogen (N) and phosphorus (P), while excessive use of chemical fertilizers causes environmental hazards. Use of rhizobium and phosphate solubilizing bacteria (PSB) can be a sustainable strategy to overcome these problems. Hence, a pot experiment was conducted following a completely randomized design to explore the impact of nitrogen fixing bacteria and PSB on the growth, yield, and quality attributes of soybean alongside soil nutrient availability using *Rhizobium japonicum* and *Pseudomonas striata*. The experiment consisted of two factors—*R. japonicum* (100% N, *R. japonicum alone* or with 50% N and control) and *P. striata* (100% P, *P. striata* with 75% P and control). Results revealed a significant influence of interaction on seed N, yield, protein, oil, and nodules of soybean. Microbial inoculants with or without N and P fertilizers produced a statistically similar yield as 100% N and P. Furthermore, *R. japonicum* and *P. striata* along with 50% N and 75% P increased 7% protein and 19% oil than 100% N and P. *R. japonicum* enhanced soil N content and *P. striata* improved soil phosphorus availability. Overall, *R. japonicum* and *P. striata* inoculation with 50% N and 75% P can potentially improve the yield and the quality of soybean and soil nutrient conditions.

Keywords: biofertilizer; legume crop; nitrogen; nodulation; oil percentage; phosphorus; protein content; soybean

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1. Introduction

Nitrogen and phosphorus play a crucial role in the growth and the development of soybean plants. Nitrogen is an indispensable part of amino acid, protein, chlorophyll, and many essential enzymes critical for photosynthesis and plant growth [1]. It is also necessary for partitioning photosynthate, stimulating root growth and enhancing uptake of other nutrients by plants [2]. On the contrary, another macronutrient, P has the utmost importance for pod formation and seed development in soybean [3]. It also plays an imperative role in photosynthesis, enzyme activation, ATP formation, energy transfer, metabolism of carbohydrates, and cell division [4]. Therefore, farmers use excessive N and P fertilizers to maintain high agricultural productivity. However, plants can use only 30–40% of these fertilizers and the rest is lost to soil and water, which is a big threat to the environment [5]. To check this environmental issue, eco-friendly and sustainable sources of nutrients should be used, reducing the necessity of synthetic fertilizers [6].

Hence, application of microbial inoculants as biofertilizers has emerged as a cost-effective, environment-friendly, remunerative, and sustainable strategy to boost soil fecundity and crop growth [7,8]. The interaction between plants and microorganisms such as rhizobium and phosphate solubilizing bacteria (PSB) can reduce reliance on N and P [9]. Rhizobium and PSB have shown added benefit in legume cultivation by fixing N and solubilizing unavailable P [10]. Being a member of the Fabaceae family, soybean can build a symbiotic association with rhizobium, which converts atmospheric N_2 into a useable form for plants [11]. With the help of symbiosis, the soybean can fix up to 200–250 kg N ha^{-1} [12], which is less prone to leaching loss and volatilization [5]. Biologically fixed N reduces production costs and protects the environment from the harmful effects of nitrogenous chemical fertilizer [13]. Previous studies documented that soybean requires up to 80 kg N per 1000 kg seeds depending on soil conditions to maintain optimum productivity [14] and approximately 80% of it is contributed by biologically fixed N [15]. However, in-built soil N-fixing bacteria is not sufficient to fix enough N [16]. That's why inoculating a rhizobial strain with seeds can be a cost-effective, environment-friendly, profitable, and sustainable strategy to supply N to plants [1]. A significant increase in plant growth and the yield of soybean because of seed inoculation with rhizobium was recorded by Zohaib et al. [16].

Additionally, the left-over nitrogen found in soil after the soybean crop harvest is equal to 30–80 kg N fertilizer per hectare, which is beneficial for the next crop [17]. Moreover, the biologically fixed N is sometimes inadequate for plants' growth and development [18]. To optimize yield, soybean needs biologically fixed N and N uptake by roots [19]. An exogenous supply of N as a starter dose during seedling development until the nodulation stage is critical for soybean to ensure optimal production [20]. Hardy et al. [21] documented the beneficent impact of applying N fertilizer before planting on the early vegetative growth of soybean until root nodules are formed.

Furthermore, rhizobium requires P to drive energy for atmospheric N fixation and nodule formation [22]. Phosphorus also provides enough infection sites for rhizobium by promoting root growth [23]. Phosphorus assists rhizobium in building the mitochondrial and symbiosomal membranes of nodules and assimilation of ammonium as amino acids [24]. Mitran et al. [25] revealed a positive relationship between nodular P content and N fixation in legumes. Insufficient P impedes root growth and hinders photosynthesis and the accumulation and translocation of photosynthates and other functions directly related to biological N fixation [22]. Although soil contains a fair amount of P, it usually forms compounds with calcium, magnesium, and other minerals and becomes fixed in the soil [26]. Only 15–20% of soil applied P remains available to plants [27]. The mineralization and the solubilization of fixed organic P are performed by PSB [28]. PSB solubilizes the organic P by secreting various organic acids and enzymes [29], improves legume yield, and reduces dependency on organic and inorganic sources of P fertilizer [30]. Gaur et al. [31] documented the solubilization of approximately 30–40 kg P_2O_5 ha^{-1} due to inoculation of PSB. In addition, PSB produces plant growth hormone especially auxin and stimulates plant growth [32]. An increment of 23%, 15%, and 16% in nodules number per plant, grain yield and protein content were recorded in chickpea by Ditta et al. [33] due to PSB inoculation, respectively.

Besides, dual inoculation of beneficial microbes was proven to be more effectual than single inoculation considering crop growth and yield [34]. The combined application of rhizobium and PSB has an augmented effect on nodulation, nitrogen fixation, root growth, and in turn the yield of soybean.

In Bangladesh, soybean oil is the most popular edible oil but farmers produce only 5% of total demand [35]. The major reason behind this scenario may be the farmers' preference for other crops. But soybean has great potential in Bangladesh as it is the crop with the richest source of protein [36], and it requires less fertilizer, reducing the input cost. Previously neglected, soybean cultivation is now gaining popularity due to the availability of high-yielding, short-duration varieties and a suitable climatic condition [37]. Farmers will be more encouraged if they can reduce production costs by using biofertilizer such as rhizobium and PSB, which will also benefit the environment. In our experiment, we investigated the efficacy of nitrogen fixing bacteria (*R. japonicum*) and phosphate solubilizing bacteria (*P. striata*) to provide necessary N and P to soybean plants and to improve their growth, yield, and quality. We assumed that *R. japonicum* and *P. striata* with a reduced amount of N and P fertilizers as a starter dose would perform better than recommended N and P fertilizer doses. The present study aimed to explore the comparative effect of *R. japonicum* and *P. striata* inoculants with or without a reduced amount of synthetic N and P fertilizers on the growth, yield, and quality of soybean. The experiment also compared the effectiveness of microbial inoculants with chemical fertilizer on nodulation and the nutritional status of soil and plants.

2. Materials and Methods

2.1. Experimental Setup

A pot experiment was conducted in the net house from January to April of 2021 to evaluate the impact of seed inoculation with *Rhizobium japonicum* and *Pseudomonas striata* on the growth, yield, and quality of soybean as well as soil nutrient availability. The experiment was laid out in a factorial completely randomized design and replicated three times. Twelve treatment combinations were used comprising four rhizobium treatments namely, R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N (as recommended for soybean in FRG [38] without *R. japonicum*, R_2: 50% N with *R. japonicum*, and R_3: only *R. japonicum* inoculant and three treatments for *Pseudomonas viz.* P_0: Control (no P and no *P. striata*), P_1: 100% P (as recommended for soybean in FRG [38]) without *P. striata* and P_2: 75% P with *P. striata*. The pot (88 cm $\times$ 33 cm $\times$ 23 cm) was filled with 35 kg of soil, and BARI Soybean-5 was grown as a test crop. Seeds were collected from Bangladesh Agricultural Research Institute, Gazipur. Seeds were sown at a row to row distance of 30 cm. After 15 days, thinning was done to make the plants in a row approximately 5 cm apart from each other.

2.2. Initial and Post-Harvest Soil Analysis

For the pot experiment, soil from unexploited land in Kodda, Gazipur, Bangladesh, was used. The selected site belongs to the Salna series under the Bangladesh Agro-ecological zone 28, Madhupur Tract. The site has a general soil type of shallow red brown terrace soil with sandy clay loam texture under the taxonomical class of Ultic Ustochrepts [39]. Before the beginning of the experiment, soil samples were collected from 10 random spots of the selected site at a depth of 0–15 cm to analyze chemical properties. Then, a composite soil sample was prepared for analysis by air-drying, grinding, and sieving by a 2 mm sieve. The status of initial soil before beginning the experiment was presented in Table 1. After crop harvest, a soil sample was collected from each pot, and the post-harvest soil nitrogen and phosphorus were estimated from the average of three repetitions of each treatment. The soil pH was detected by the method proposed by Mehmood et al. [40] and soil organic carbon by Walkley and Black [41]. Soil organic matter was calculated by multiplying organic carbon with conversion factor 2 as mentioned by Douglas [42]. Available phosphorus, potassium, and sulphur were determined according to the method described by Olsen et al. [43], Brown and Lilleland [44], and Victor and Nearpass [45], respectively.

Table 1. Chemical properties of soil (initial) used for the study.

Soil Properties	Value
pH	7.2
Soil organic carbon (%)	0.48
Soil organic matter (%)	0.96
Nitrogen (%)	0.057
Available phosphorous (mg kg^{-1})	2.83
Available potassium (mg kg^{-1})	140
Available sulphur (mg kg^{-1})	11

2.3. Application of Bio-Fertilizer and Chemical Fertilizer

Two peat soil based biofertilizers were used in this experiments, one for *Rhizobium japonicum* and another for *Pseudomonas striata.* Both biofertilizers contained approximately 1×10^8 cells per gram of soil. The *R. japonicum* strain was collected from the nodules of a soybean plant and cultured and stored in Yeast Manitol Agar media (1 liter media contains mannitol—10 g, K_2HPO_4—0.5 g, $MgSO_4 \cdot 7H_2O$—0.2 g, NaCl—0.1 g, yeast—0.5 g, and agar—15 g) at 4 °C for 2 months before being used in peat based biofertilizer. On the other hand, *P. striata* strains were isolated from the rhizospheric soil of mungbean and grown and stored in Pikovskaya's Agar media (10 g glucose, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.5 g yeast extract, 15 g agar powder and a trace amount of $MgSO_4$ and $FeSO_4$ for 1 liter) at 4 °C for 1 month before use. Seeds were treated with *Rhizobium japonicum* inoculant and *Pseudomonas striata* inoculant at 20 g kg^{-1} seed before sowing. Except for nitrogen and phosphorus, all the nutrients were supplied as per the recommended fertilizer dose [38]. Nitrogen and phosphorus were applied according to the treatment requirement, where 2.45 g urea and 5.25 g triple superphosphate were equivalent to 100% N and 100% P, respectively. In addition, approximately 3.5 g of muriate of potash, 6.9 g gypsum, 0.16 g zinc sulphate, and 0.10 g boric acid were supplied during the final soil preparation for the recommended dose of 50-18-1-0.5 kg ha^{-1} KSZnB. All the fertilizers were incorporated with soil during final land preparation.

2.4. Data Collection

Data on plant height, leaf area index, total chlorophyll content, number of branches plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, and 100-seed weight were recorded. At maturity, five plants were selected to measure plant height, number of branches plant^{-1}, and number of pods plant^{-1}. Leaf area was determined with the help of a leaf area meter, and the leaf area index was calculated using Watson's formula [46].

$$\text{Leaf area index} = \frac{\text{Leaf area}}{\text{Ground area}}$$

The methods described by Arnon [47] and Zohaib et al. [16] were followed to measure the total chlorophyll content of fresh leaves at 60 days after sowing (DAS). For measuring chlorophyll content, 0.5 g of fresh green leaves was chopped into small pieces and 5 mL of 80% acetone was used to extract chlorophyll. The extract was then centrifuged for 5 min at a speed corresponding to 14,000$\times$ *g*. Finally, the absorbance of a supernatant at 645 nm and 663 nm was recorded and total chlorophyll content was calculated using the formula provided by Zohaib et al. [16]. Two plants were uprooted from each pot to calculate the number of nodules and nodule dry weight (mg plant^{-1}) at 30, 50, and 70 DAS. For determining dry weight, nodules were dried in an oven for 72 h at 65 °C and the constant weight was measured. Seeds were harvested from 60 cm $\times$ 33 cm of each pot and the yield was converted into t ha^{-1}. Meanwhile, the number of seeds pod^{-1} and 100-seed weight were also determined. Shoot was fine-ground and digested in acid to measure the phosphorus content by Jones and Case [48]. The nitrogen percentage of shoot, nodules, and seeds, was determined by the Official Methods of Analysis, AOAC [49] recommended standard: the Micro-Kjeldahl method. In this method, approximately 1 g of ground sample was taken in a digestion tube and 15 mL of concentrated sulfuric acid and 4 g of catalyst mixer were added. Then, the sample was digested at 380 °C for 120 min and nitrogen present in the sample was converted to ammonium sulphate. After digestion, the sample was allowed to cool for 20 min and diluted with 50 mL of distilled water. A total of 60 mL of 50% sodium hydroxide solution was added after putting the digested sample in a distillation unit to convert the ammonium sulfate to ammonia. The distillate was then collected in a flask containing 25 mL boric acid solution and titrated with HCl for quantitative estimation. Nitrogen percentage was then calculated using the following equation:

$$\text{Nitrogen (\%)in the sample} = \frac{1.4 \times \text{mL of HCl used in the titration } \times \text{Normality of HCl}}{\text{Weight of the sample (g)}}$$

The nitrogen content of seeds was multiplied by protein factor 6.25 as described by FAO [50] for estimating seed protein. For determining oil content, 50 soybean seeds from each plot were ground to a fine powder and 20 mg of each powdered sample were taken to a 10 mL glass tube. After that, fatty acid methyl ester solution was prepared by adding 2 mL of 5% sulfuric acid-methanol, 25 µL of 0.2% butylated hydroxytoluene, 300 µL methylbenzene, and 100 µL of methyl heptadecanoate (2.5–5 mg mL^{-1}). The preparation was then kept in a water bath at 90–95 °C for 1.5 h and cooled to room temperature. After adding 1 mL 0.9% sodium chloride and 1 mL n-hexane, the methods and the formula described by Yao et al. [51] were followed to perform a gas chromatography analysis and to measure oil content.

2.5. Statistical Analysis

Statistix 10 (analytical software, 2105 Miller Landing Road, Tallahassee, version 10.0.1.5), a software package, was utilized to accomplish the factorial analysis of variance (ANOVA). The level of significance was determined considering the *p*-value of ANOVA. When the *p* value was <0.05, multiple comparisons of treatment means were performed using Tukey's HSD test.

3. Results

3.1. Growth Attributes

Plant height and number of branches $plant^{-1}$ were significantly influenced by the sole effect of *Rhizobium japonicum* and *Pseudomonas striata* ($p < 0.01$) (Table 2). In contrast, the interaction effect became significant for leaf area index and total chlorophyll content of leaf ($p < 0.05$) (Table 3). Inoculation of *R. japonicum* with 50% of recommended N fertilizer produced the tallest plant and the highest number of branches $plant^{-1}$, which was significantly higher from the data obtained for only *R. japonicum* inoculant and control treatment (no N and *R. japonicum*). On the other hand, 75% of recommended P along with *P. striata* provided maximum plant height and number of branches. At 30 DAS, both R_1P_2 (100% recommended nitrogen and 75% recommended P with *P. striata*) and R_2P_2 (application of 50% N and 75% P and seed inoculation with *R. japonicum* and *P. striata*) provided a maximum leaf area index (LAI), which was statistically similar with R_1P_1, R_2P_1, R_3P_1, and R_3P_2. The lowest LAI was recorded from zero N and P fertilizers and no microbial inoculation, which was statistically similar with other treatments except R_1P_1, R_2P_1, and R_2P_2. Similar findings for an interaction effect on LAI were noted at 60 DAS. A significant difference was observed for total chlorophyll content at 60 DAS due to various treatment combinations. Seed inoculation with *R. japonicum* and *P. striata* and application of 50% N and 75% P produced 47% higher chlorophyll in fresh leaves compared to no inoculation and no N and P.

Table 2. Effect of *Rhizobium japonicum* and *Pseudomonas striata* on plant height and number of branch plant^{-1} of soybean.

Treatment	Plant Height (cm)	Branch Plant^{-1}
Rhizobium japonicum		
R_0	48.10 c	4.41 c
R_1	53.70 ab	6.86 ab
R_2	56.05 a	8.18 a
R_3	50.20 bc	6.36 b
$HSD_{(0.05)}$	3.83	1.48
Level of significance	**	**
Pseudomonas striata		
P_0	47.45 c	4.93 b
P_1	52.17 b	6.92 a
P_2	56.43 a	7.50 a
$HSD_{(0.05)}$	3.00	1.16
Level of significance	**	**
CV (%)	5.66	17.67

** indicates significance at 1% level of probability; mean values with different letters are statistically significant. R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Two-way ANOVA showed significant effects of *R. japonicum* and *P. striata* ($p \leq 0.05$) but no interaction.

Table 3. Interaction effect of *Rhizobium japonicum* and *Pseudomonas striata* on leaf area index (LAI) and chlorophyll content of soybean.

Treatment	Leaf Area Index (LAI)		Total Chlorophyll Content (mg g^{-1} FW)
	30 DAS	60 DAS	
R_0P_0	0.37 c	2.00 d	4.42 e
R_0P_1	0.38 c	1.99 d	5.16 de
R_0P_2	0.39 bc	2.17 cd	5.47 de
R_1P_0	0.38 c	2.34 bc	5.89 cd
R_1P_1	0.42 abc	2.60 ab	6.91 bc
R_1P_2	0.55 a	2.69 a	8.06 ab
R_2P_0	0.38 c	2.23 cd	5.94 cd
R_2P_1	0.52 ab	2.73 a	7.59 ab
R_2P_2	0.55 a	2.73 a	8.39 a
R_3P_0	0.40 bc	2.20 cd	6.24 cd
R_3P_1	0.45 abc	2.59 ab	6.83 bc
R_3P_2	0.49 abc	2.60 ab	7.11 abc
$HSD_{(0.05)}$	0.14	0.31	1.32
Level of significance	*	*	*
CV (%)	6.88	10.07	4.44

* indicates significance at 5% level of probability; mean values sharing similar letters are statistically insignificant. R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*.

3.2. Yield and Yield Contributing Characters

Rhizobium and PSB significantly affected pod length and 100-seed weight ($p < 0.01$, Table 4). Pod length ranged from 3.66 cm to 5.11 cm due to different *Rhizobium* treatments and 4.03 cm to 4.72 cm due to *Pseudomonas* treatments. The longest pod was given by 50% of recommended N with *Rhizobium japonicum* inoculant, while 75% P and *Pseudomonas striata* produced that which was longest. An 18% increase in seed weight was observed due to the application of 50% N with *R. japonicum*, while the increase was 12% for 75% P and *P. striata* inoculation. Treatment combinations significantly impacted the number of pods plant^{-1}, number of seeds pod^{-1}, and seed yield (Figures 1–3). *R. japonicum* and *P. striata* (R_2P_2)

inoculation with 50% N and 75% P produced a maximum number of pods plant^{-1} (28.87) and seeds pod^{-1} (4.36), which was statistically similar to R_1P_2 and R_2P_1. No N and P fertilizers and no microbial inoculation provided a minimum value for these two parameters. Seed yield was significantly varied due to *R. japonicum* and *P. striata* interaction. A combination of 50% N + *R. japonicum* and 75% P + *P. striata* produced a 60% higher yield than control (zero N and P with no *R. japonicum* and *P. striata*), but yield was statistically insignificant with the treatments of 100% N and P and only *R. japonicum* and *P. striata* inoculant.

Table 4. Effect of *Rhizobium japonicum* and *Pseudomonas striata* on pod length and 100-seed weight of soybean.

Treatment	Pod Length (cm)	100-Seed Weight (g)
Rhizobium japonicum		
R_0	3.66 c	9.71 c
R_1	4.55 b	11.13 b
R_2	5.11 a	11.89 a
R_3	4.36 b	10.84 b
$HSD_{(0.05)}$	**	**
Level of significance	0.28	0.58
Pseudomonas striata		
P_0	4.03 b	10.07 c
P_1	4.51 a	11.07 b
P_2	4.72 a	11.54 a
$HSD_{(0.05)}$	0.22	0.46
Level of significance	**	**
CV (%)	4.83	4.10

** indicates significance at 1% level of probability; mean values with different letters are statistically significant. R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Two-way ANOVA showed significant effects of *R. japonicum* and *P. striata* ($p \leq 0.05$) but no interaction.

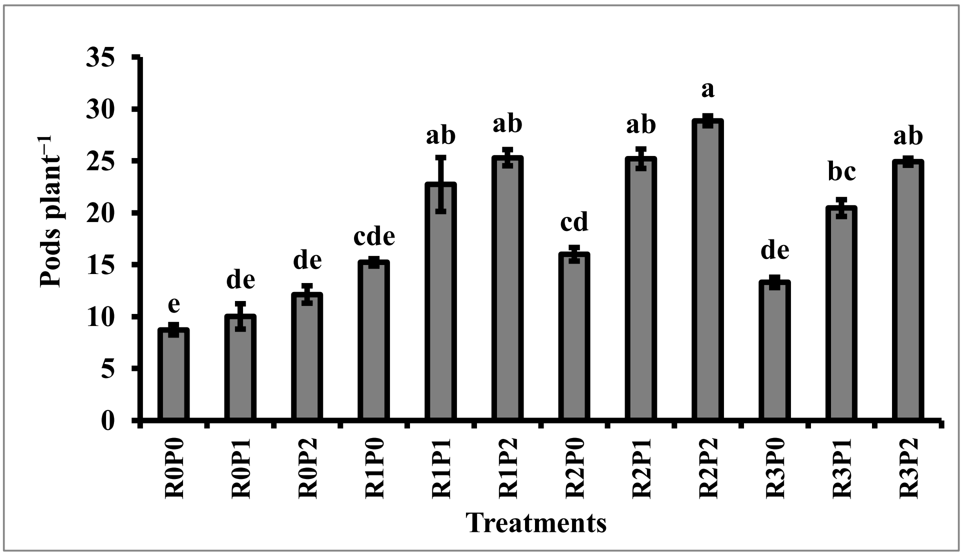

Figure 1. Interaction effect of *Rhizobium japonicum* and *Pseudomonas striata* on pods plant^{-1} of soybean (mean ± SE, *n* = 3); R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Mean values sharing similar letters are statistically insignificant. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*.

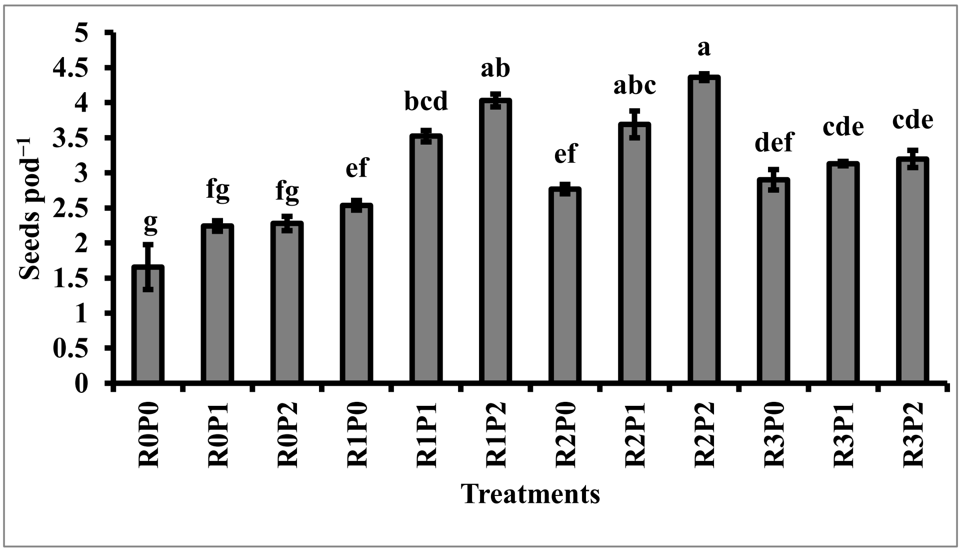

Figure 2. Interaction effect of *Rhizobium japonicum* and *Pseudomonas striata* on seeds pod^{-1} of soybean, (mean ± SE, *n* = 3); R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Mean values sharing similar letters are statistically insignificant. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*.

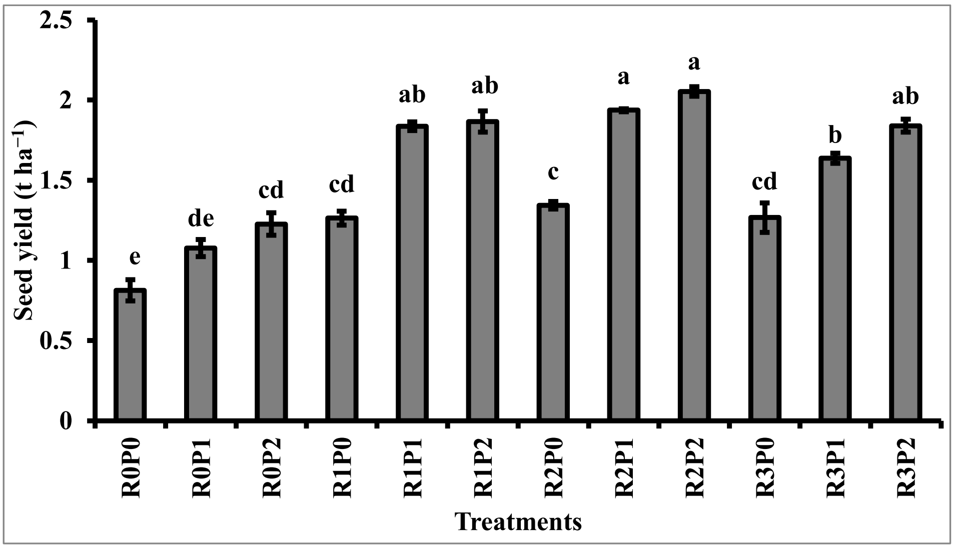

Figure 3. Interaction effect of *Rhizobium japonicum* and *Pseudomonas striata* on seed yield of soybean, (mean ± SE, *n* = 3); R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Mean values sharing similar letters are statistically insignificant. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*.

3.3. Protein and Oil Content

Significant impact of treatment combinations was noted in the case of protein and oil content ($p < 0.01$, Figures 4 and 5). The highest protein content (42.97%) and oil (20.99%) were recorded when the seed was treated with *Rhizobium japonicum* and *Pseudomonas striata* inoculant, and 50% N and 75% P were applied. The minimum protein and oil content was found due to the combined effect of zero nitrogen with no *R. japonicum* and zero phosphorus with no *P. striata*. An approximate increase in protein content of 18% and 7%

was observed due to the application of *R. japonicum* and *P. striata* with 50% N and 75% P compared to the control (no *R. japonicum* and *P. striata* inoculation and no N and P) and the use of a recommended dose of N and P without inoculation, respectively. On the other hand, using *R. japonicum* and *P. striata* with a reduced amount of N and P (50% N and 75% P) provided 19% and 25% more oil than the recommended dose of N and P without inoculation and control treatment.

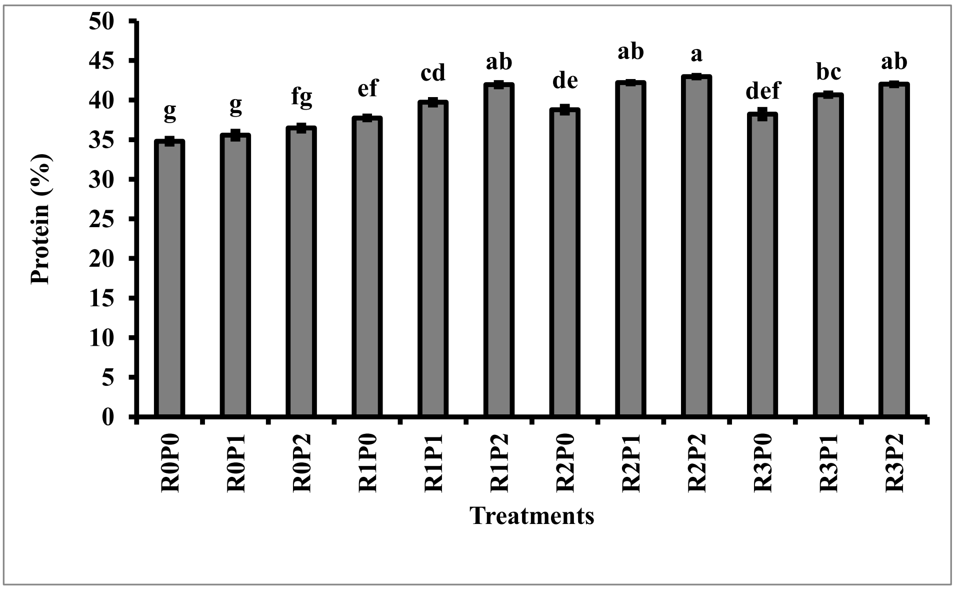

Figure 4. Interaction effect of *Rhizobium japonicum* and *Pseudomonas striata* on protein content of soybean. (mean ± SE, $n = 3$); R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Mean values sharing similar letters are statistically insignificant. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*.

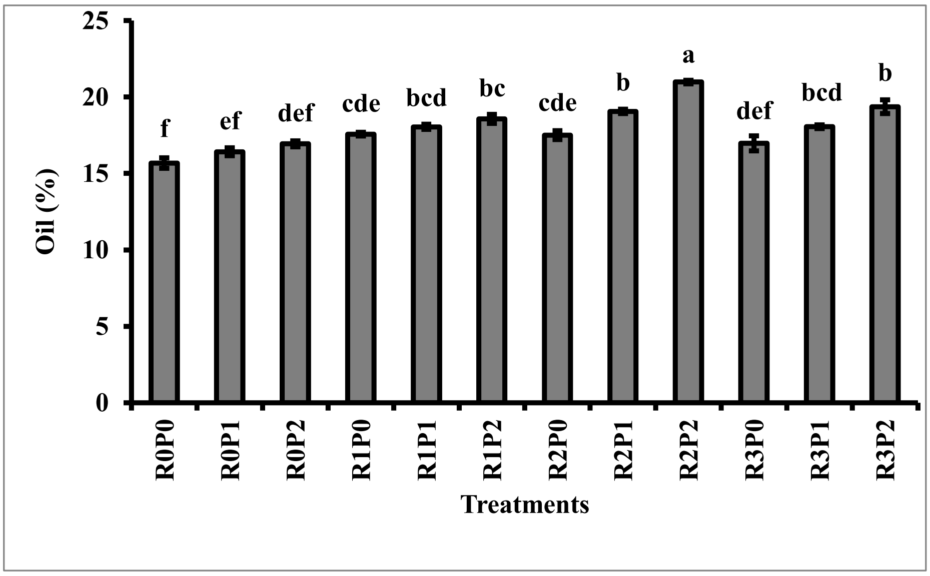

Figure 5. Interaction effect of *Rhizobium japonicum* and *Pseudomonas striata* on oil content of soybean. R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Mean values sharing similar letters are statistically insignificant. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*.

3.4. Nodulation

Interaction of *Rhizobium japonicum* and *Pseudomonas striata* inoculation along with application of N and P brought about notable variations in nodule numbers plant^{-1} (Figure 6), while individual effects became significant for dry weight and the nitrogen content of nodule (Table 5). A gradual increase in nodule numbers and in the dry weight of nodules was observed from 30 DAS to 50 DAS, but a decline for both the parameters was recorded at 70 DAS. At all the sampling date, combined treatments of 50% N + *R. japonicum* and 75% P + *P. striata* provided a maximum number of nodules per plant, which was statistically similar to the nodule numbers produced due to a combination of 50% N + *R. japonicum* and 100% recommended P dose. The minimum number of nodules was observed due to the combination of control treatments (no N + no *R. japonicum* and no P + no *P. striata*). At 30, 50, and 70 DAS, even the recommended N and P dose without *R. japonicum* and *P. striata* inoculation considerably reduced nodule formation, which was 42%, 38%, and 41% lower than the nodules formed due to the application of 50% N + *R. japonicum* and 75% P + *P. striata*. At 30, 50, and 70 DAS, *R. japonicum* inoculation with seeds and 50% N produced the maximum nodule dry weight, followed by the dry weight caused by 100% N and only *R. japonicum* inoculant (Table 5). Nodules contained approximately 28% more nitrogen when *R. japonicum* was inoculated and 50% N was applied compared to the control. In case of *P. striata*, 75% P with *P. striata* inoculation caused maximum dry weight and nitrogen content in nodules, but no significant variations were observed with 100% P.

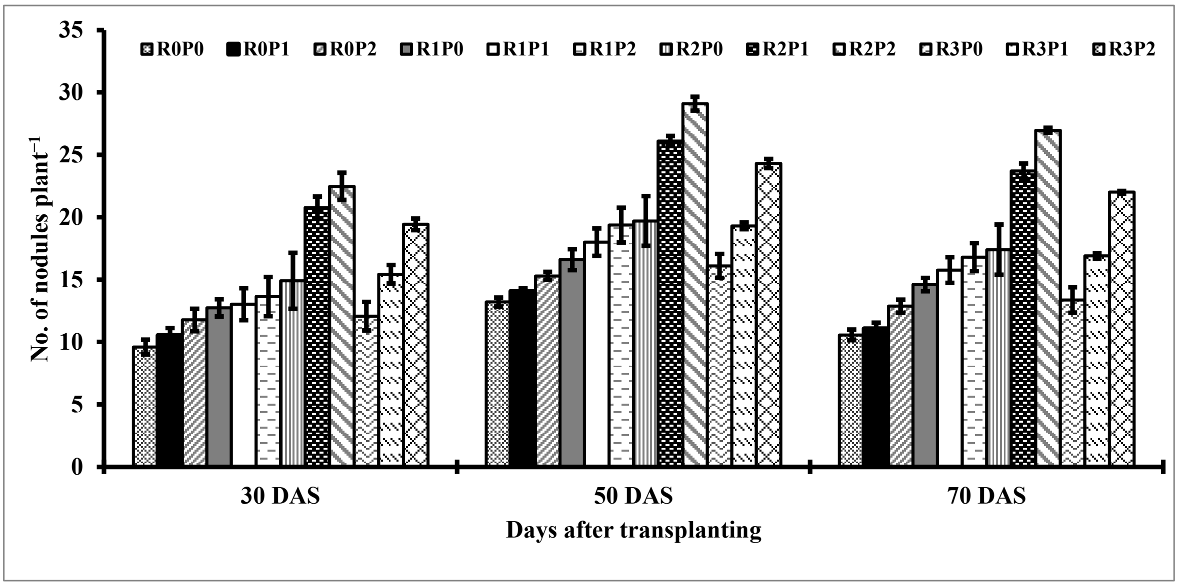

Figure 6. Interaction effect of *Rhizobium japonicum* and *Pseudomonas striata* on number of nodules plant^{-1} of soybean at different days after sowing. (mean ± SE, *n* = 3); R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*. Mean values sharing similar letters are statistically insignificant. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*.

Table 5. Effect of *Rhizobium japonicum* and *Pseudomonas striata* on dry weight and nitrogen content of nodules at different days after sowing.

Treatment	Nodule Dry Weight (mg Plant^{-1})			Nitrogen Percentage of Nodule (60 DAS)
	30 DAS	50 DAS	70 DAS	
Rhizobium japonicum				
R_0	29.82 c	36.05 d	31.97 d	3.54 c
R_1	36.01 b	43.03 c	39.59 c	4.46 b
R_2	41.79 a	66.57 a	61.48 a	4.93 a
R_3	37.08 b	48.56 b	44.62 b	4.72 a
$HSD_{(0.05)}$	2.96	3.47	3.13	0.21
Level of significance	**	**	**	**
Pseudomonas striata				
P_0	34.44 b	46.83 b	42.93 b	4.24 b
P_1	36.39 ab	48.45 ab	44.28 ab	4.49 a
P_2	37.69 a	50.40 a	46.05 a	4.51 a
$HSD_{(0.05)}$	2.32	2.72	2.45	0.16
Level of significance	**	*	*	**
CV (%)	6.29	5.50	5.41	3.62

** indicates significance at 1% level of probability and * indicates significance at 5% level of probability; mean values with different letters are statistically significant. R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Two-way ANOVA showed significant effects of *R. japonicum* and *P. striata* ($p \leq 0.05$) but no interaction.

3.5. Nitrogen and Phosphorus Content in Post-Harvest Soil and Plant

Analysis of post-harvest plant samples revealed a significant sole effect of *Rhizobium japonicum* on shoot N and seed P content ($p < 0.01$). In contrast, a significant impact of *Pseudomonas striata* on shoot P and seed P content was observed ($p < 0.01$, Table 6). The effect of *R. japonicum* on Shoot P was proven insignificant ($p > 0.05$), while shoot N was not significantly influenced by *P. striata* ($p > 0.05$). The highest N content in shoot and P content of seed was recorded for 50% N + *R. japonicum*, which was statistically at par with results found due to inoculation of only *R. japonicum*. In comparison with the control treatment, 2.08 times higher shoot N and 1.38 times higher seed P were recorded due to *R. japonicum* inoculation with 50% N. On the contrary, 75% P + *P. striata* provided approximately 40% and 48% more P in shoot and seed, respectively, than no *P. striata* + no P. Seed nitrogen content was significantly influenced by the combined effect of *R. japonicum* and *P. striata* (Figure 7). A combination of treatments, 50% N + *R. japonicum* and 75% P + *P. striata* gave a maximum nitrogen content in seed which was 23% higher compared with control treatment combinations (no N + no *R. japonicum* and no P + no *P. striata*). The amount of nitrogen in post-harvest soil was significantly influenced by the sole effect of *R. japonicum* treatments, while soil P was impacted due to the individual effect of various *P. striata* treatments (Table 7). In the case of 50% N + *R. japonicum*, the post-harvest soil N content reached its maximum value, which was approximately 60% higher than with no *R. japonicum* inoculation and no N. On the other hand, soil P became 2.06 times more available when *P. striata* was used with 75% P as compared with no *P. striata* and no P.

Table 6. Effect of *Rhizobium japonicum* and *Pseudomonas striata* on nitrogen and phosphorus content of soybean shoot and seed at harvest.

Treatment	Shoot N (%)	Shoot P (%)	Seed P (%)
Rhizobium japonicum			
R_0	1.48 c	0.37	0.48 c
R_1	2.81 b	0.39	0.60 b
R_2	3.08 a	0.39	0.66 a
R_3	2.93 ab	0.39	0.65 a
$HSD_{(0.05)}$	0.19	-	0.05
Level of significance	**	NS	**
Pseudomonas striata			
P_0	2.53	0.28 c	0.38 c
P_1	2.55	0.41 b	0.67 b
P_2	2.65	0.47 a	0.74 a
$HSD_{(0.05)}$	-	0.03	0.04
Level of significance	NS	**	**
CV(%)	5.71	7.00	5.81

** indicates significance at 1% level of probability and NS means non-significant; mean values with different letters are statistically significant. R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Two-way ANOVA showed significant effects of *R. japonicum* and *P. striata* ($p \leq 0.05$) but no interaction.

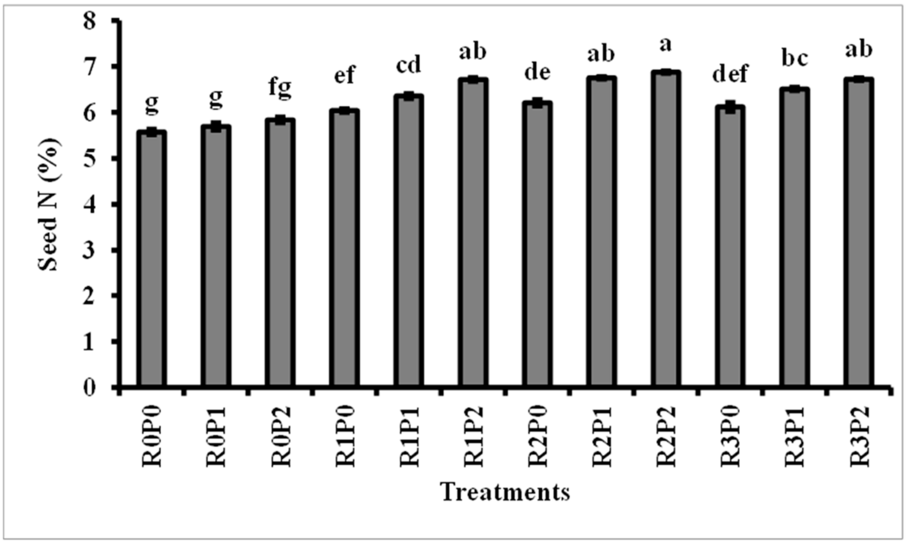

Figure 7. Interaction effect of *Rhizobium japonicum* and *Pseudomonas striata* on seed nitrogen content of soybean (mean ± SE, *n* = 3); R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Mean values sharing similar letters are statistically insignificant. Two-way ANOVA showed a significant interaction ($p \leq 0.05$) between *R. japonicum* and *P. striata*.

Table 7. Effect of *Rhizobium japonicum* and *Pseudomonas striata* on post-harvest soil nitrogen and available phosphorus.

Treatment	Soil N (%)	Soil Available Phosphorus (mg kg^{-1})
Rhizobium japonicum		
R_0	0.11 d	3.81
R_1	0.16 c	3.90
R_2	0.27 a	4.17
R_3	0.22 b	3.88
$HSD_{(0.05)}$	0.02	-
Level of significance	**	NS
Pseudomonas striata		
P_0	0.18	2.88 b
P_1	0.19	2.99 b
P_2	0.20	5.94 a
$HSD_{(0.05)}$	-	0.31
Level of significance	NS	**
CV (%)	8.06	7.69

** indicates significant at 1% level of probability and NS means non-significant; mean values sharing different letters are statistically significant. R_0: Control (no nitrogen and *R. japonicum*), R_1: 100% N without *R. japonicum*, R_2: 50% N with *R. japonicum*, R_3: only *R. japonicum* inoculant, P_0: Control (no P and no *P. striata*), P_1: 100% P without *P. striata* and P_2: 75% P with *P. striata*. Two-way ANOVA showed significant effects of *R. japonicum* and *P. striata* ($p \leq 0.05$) but no interaction.

4. Discussion

Plant-microbe interaction has a crucial impact on various soil biochemical processes related to nutrient availability and uptake by plants, which contributes to plants' growth and yield [52]. Thus, microbial inoculants also curtail chemical fertilizer use and contribute to sustainable agricultural productivity [53]. Our study intended to investigate the impact of nitrogen fixing bacteria (*Rhizobium japonicum*) and phosphate solubilizing bacteria (*Pseudomonas striata*) on the growth, yield, and quality of soybean. In addition, we explored the nodulation and post-harvest soil and the plant's nutrient status. Individually inoculation of *R. japonicum* with 50% N and *P. striata* with 75% P provided maximum plant height, number of branches plant^{-1}, pod length, and 100-test weight (Table 2). In contrast, leaf area index, chlorophyll content, pods plant^{-1}, seeds pod^{-1}, and seed yield were influenced due to their combined effect (Table 3, Figures 1–3). Inoculation of *R. japonicum* and *P. striata* strains with or without N and P containing chemical fertilizers increased the LAI and the chlorophyll. The synergistic effect of these microorganisms triggered chlorophyll production by increasing the supply of nitrogen to the plant, a vital structural unit of chlorophyll [16]. Furthermore, biological nitrogen fixation by Rhizobium and phosphate solubilization by PSB (phosphate solubilizing bacteria) increased plant N and P availability, which escalated the leaf area and, consequently, LAI [54]. Stimulated plant growth was also documented in chickpea [10] and soybean [55] due to plant growth promoting microorganisms, Rhizobium, and PSB. On one hand, the combined effect of Rhizobium and PSB provided an approximately 10% higher yield than the yield obtained from the use of recommended N and P fertilizers and, on the other hand, reduced 50% of N and 25% of P fertilizer requirements. Moreover, seed inoculation with *R. japonicum* and *P. striata* without any N and P fertilizers produced equal yield as recommended N and P, which was evidence that the N fixing ability of *R. japonicum* made legumes less dependent on chemical fertilizers than non-leguminous plants [56]. In actual fact, a higher amount of nitrogen discourages nodulation and N fixation as well as triggers nodule senescence, which ultimately reduces seed yield [57]. On the contrary, a decreased amount of N than the recommended dose as a basal dose may help the early growth stage of soybean before building symbiosis with *Rhizobium japonicum* [58], which in turn unveiled in yield contributing attributes. Singh et al. [12] reported a 14.9% increase in the seed yield of soybean due to the application of *Bradyrhizobium japonicum* and stated the N-fixation and growth regulators (auxin, gibberellin and cytokinin etc.) production ability of Rhizobium as contributor to the growth and yield of soybean. On the other hand, PSB also stimulates the auxin and cytokinin production and makes inaccessible P available to plants, confirmed by Qureshi et al. [59] and Figueiredo et al. [60]. Enhanced cell division and cell elongation due to growth hormone auxin and gibberellin contribute to pod length, biomass production and yield [61]. Mahanty et al. [62] also reported enhanced plant growth and yield due to *Rhizobium* and *Pseudomonas* inoculation. Rhizobium strains fix atmospheric nitrogen by forming nodules and contribute to soil fertility and crop yield as biologically fixed nitrogen is more sustainable and less prone to leaching and volatilization loss [63]. Basu et al. [64] opined that N fixing and PSB improved plant growth and yield by providing nutrients without additional chemical fertilizers input. Yousefi et al. [65] observed significant positive impact on wheat plant growth and yield parameters due to the application of PSB with approximately 52%, and 26% increase in shoot biomass and yield, respectively compared to no PSB. PSB increases plant's P use efficiency and makes it available as inorganic form [66]. Post-harvest plant sample analysis revealed significant influence of combined effect of *R. japonicum* and *P. striata* with or without chemical N and P fertilizers on seed nitrogen, protein and oil content (Figures 4, 5 and 7) which was also confirmed by Waghmare et al. [67], Jaga and Sharma [68]. Seed nitrogen and protein content increased due to *R. japonicum* and *P. striata* inoculation compared to uninoculated treatments because *P. striata* provided available P required for physiological and developmental process of plants and consequently increased the uptake of N by seed [69]. In a point of fact, *R. japonicum* and *P. striata* stimulate root growth which results in an enhanced root system with more root hairs, greater surface area and consequently higher uptake and acquisition of nutrients by plants [70–72]. Ahmad et al. [30] showed enhanced nodulation and nutritional status of mungbean due to co-inoculation of Rhizobium and PSB. However, interaction was not significant for shoot N and P and seed P (p value > 0.05). Filipini et al. [73] observed higher nitrogen content in common beans due to Rhizobium inoculation. Estrada-Bonilla et al. [74] reported the presence of higher amount of P in sugarcane shoot due to the inoculation of PSB compared to no inoculation. In contrast, in a field experiment, significant increase in seed yield, nitrogen and protein percentage of seed due to PSB inoculation was also documented by Wang et al. [75] in case of peanuts. Ditta et al. [33] reported approximately 42% and 16% increase in protein and P content of chickpea seed due to PSB. An increment in soil N and available P was also noted due to the addition of *R. japonicum* and *P. striata*, respectively (Table 7), which might be the consequence of better nodulation and improved rhizospheric environment [76]. In general, nutrient elements, N and P persist in soil as organic forms or are lost by volatilization and leaching [52]. Plants rely on microorganisms whose active metabolic activity initiates the mineralization and makes these nutrients available [53]. Sindhu et al. [77] found N content in post-harvest soil equivalent to approximately 30–80 kg of N fertilizer application, while Gour et al. [31] reported the solubilization of approximately 30–35 kg P_2O_5 and increased P uptake by plant due to PSB. These results also corroborate with Tagore et al. [10], who found 2.35 times more release of P from rock phosphate and higher soil available P when PSB was applied.

The interaction effect of *R. japonicum* and *P. striata* significantly influenced the nodule numbers plant^{-1} (Figure 6), while nodule dry weight and N content were influenced by their individual effect (Table 5). Tindwa et al. [78] also observed a positive influence of available soil P on nodulation, such as nodule numbers and dry weight, which consequently impacted N fixation. Ditta et al. [33] reported a 23% increase in the nodule formation of chickpea due to PSB. However, nodule number and dry weight increased from 30 to 50 DAS but declined at 70 DAS. The reason might be the breakdown of nodules and the use of nitrogen by plants for pod formation [10]. Moreover, on an average, an approximately 57% and 40% reduction in nodule formation was observed due to the control treatment and the application of recommended N and P fertilizers without *Rhizobium japonicum* and

Pseudomonas striata inoculation, respectively, compared to the inoculation of *R. japonicum* and *P. striata* with 50% N and 75% P. Actually, N and P are essential for microbial abundance in soil and their performance [79]. However, surprisingly, excessive nitrogen desists plants from forming nodules, and a dearth of sufficient N in soil forces plants to go for an energy expensive biological N fixation process, which results in more nodule formation and an accumulation of nitrogen in plants [80]. Henceforth, a reduced amount of nitrogen as a basal dose might be helpful for microbial growth and biological N fixation. The phenotypic characteristics of legumes, such as N fixing ability and nodule dry weight, depend upon Rhizobium and the function of the gene-regulating Nod-factor structure [69]. Rhizobium fixes atmospheric N and PSB helps in root development by providing more available P, which combinedly triggers nodule formation [81]. Furthermore, P is essential for the formation of nodule tissue and ATP, which activates the nitrogenase enzyme [12,82]. In addition, Anand et al. [83] documented the influence of PSB on the production of plant growth hormones and root growth stimulation by making previously unavailable P accessible to plants, which indirectly encourages biological N fixation. The growth of a root system due to PSB-induced cytokinin increases the infection sites and in turn nodule formation [84].

5. Conclusions

Inoculation of nitrogen fixing bacteria, *Rhizobium japonicum*, and phosphate solubilizing bacteria, *Pseudomonas striata*, improved soybean growth, yield, and quality. Maximum seed yield and protein and oil content were found by using 50% of recommended nitrogen and 75% of recommended phosphorus when *R. japonicum* and *P. striata* were inoculated with seeds before sowing. Again, these beneficial microorganisms also provided higher nitrogen and available phosphorus in soil which might supplement the fertilizer requirement of the next crop. Thus, *R. japonicum* and *P. striata* benefited the environment by reducing the need for chemical fertilizers. So, *R. japonicum* and *P. striata* biofertilizers with 50% N and 75% synthetic P fertilizer can be used to produce soybean in a profitable and environmentally friendly way. However, further detailed experimentations under different soil conditions and agro-ecological zones on the capacity of these biofertilizers to fix atmospheric nitrogen and solubilize phosphorus will provide more profound knowledge of their potential in sustainable soybean production.

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