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Nodule bacteria and cyanobacterial symbionts: Biological drivers of sustainable crop production and healthy nutrition

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ABSTRACT

Background: Chemical fertilizers negatively impact soil health and biodiversity, highlighting the urgent need for sustainable biological alternatives. Nodule-forming bacteria are well recognized for their ability to establish symbiotic associations with leguminous crops, enabling biological nitrogen fixation and promoting environmentally sustainable agriculture. At the same time, legumes represent a cornerstone of functional food science, providing high-quality proteins, bioactive compounds, and micronutrients essential for human health.

Objective: This study aimed to evaluate the effects of co-inoculating legume crops with native strains of nodule bacteria and cyanobacteria on plant growth in humus-deficient soils, yield performance, grain nutritional quality, and their potential contribution to functional food development.

Methods: Field trials were conducted using pea, soybean, and peanut crops inoculated with **microbial consortia comprising symbiotic nodule bacteria** (*Rhizobium leguminosarum*, *Bradyrhizobium arachidis*, *B. japonicum*) and cyanobacteria (*Nostoc* PCC 7807, *Arthrospira platensis*). Key agronomic parameters, including biometric traits, pod and seed weight, nodulation efficiency, grain protein content, and yield components, were measured and subjected to statistical analysis under humus-deficient soil conditions.

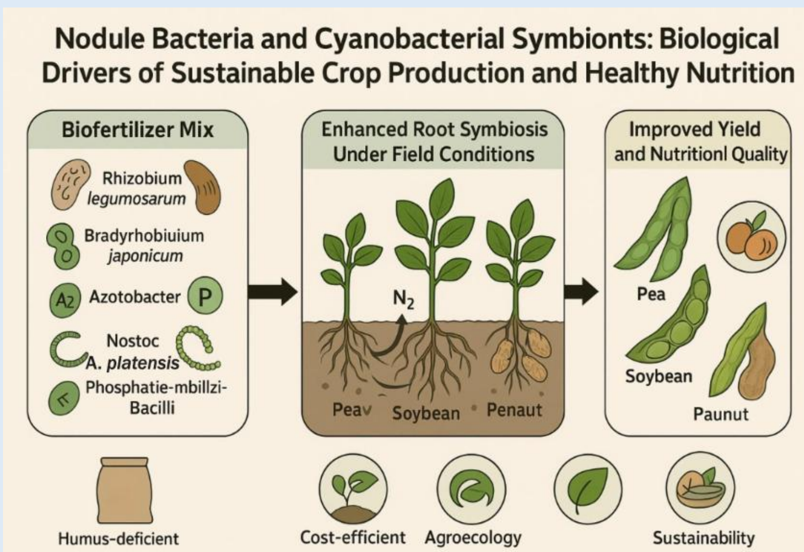
Results: Co-inoculation significantly enhanced plant performance across all tested crops. Peanut pod weight increased by up to 120%, while soybean nodulation improved by 60% compared to the uninoculated control. Grain protein content increased by 3–7%, with improved amino acid balance, directly strengthening the functional food value of legumes. Total yield rose by as much as 67%, highlighting the strong synergistic potential of microbial consortia for sustainable crop production and nutritionally enriched food sources.

Novelty: This study presents a novel biofertilizer formulation combining four functionally distinct bacterial taxa with nitrogen-fixing and nutrient-mobilizing capabilities. In addition to symbiotic nodule bacteria, the formulation includes free-living *Azotobacter*, phosphate-solubilizing bacilli, and two cyanobacterial species (*Arthrospira platensis*, *Nostoc* sp.), linking soil microbiology innovations with functional food science by enhancing protein density and nutritional quality in legumes.

Conclusion: The results confirm that synergistic interactions between cyanobacteria and nodule bacteria significantly enhance legume productivity, grain quality, and functional food potential. The developed biofertilizer based on nitrogen-fixing, phosphate-solubilizing, and photosynthetic microbes proved effective in improving yields of soybean, peanut, and pea under low-humus soil conditions, offering a sustainable strategy to support agroecology, food security, and the production of health-promoting plant-based foods.

Keywords: nodule bacteria, cyanobacteria, symbiosis, legumes, humus-deficient soils, sustainable agroecology and functional foods, protein enrichment, healthy nutrition

Graphical Abstract: Synergistic role of nodule bacteria and cyanobacteria in legume productivity, functional foods, and sustainable agriculture



Graphical Abstract: Nodule bacteria and cyanobacterial symbionts

INTRODUCTION

The widespread use of synthetic pesticides and agrochemicals in intensive farming poses significant risks to human health and agroecosystem sustainability. These inputs disrupt soil microbial communities, reduce biodiversity, and degrade soil fertility. In contrast, beneficial microorganisms, particularly nodule-forming bacteria, play a vital role in nitrogen fixation and soil restoration. Their ability to establish symbiotic relationships with leguminous crops makes them indispensable for sustainable crop production.

Legume–rhizobia symbiosis is a cornerstone of sustainable agriculture, as it enhances soil fertility and reduces reliance on nitrogen (N) fertilizers [1–4]. Consequently, legumes such as soybeans, beans, and peas are widely cultivated not only as food crops but also for their agronomic benefits in crop rotations, intercropping systems, and as green manure or winter cover crops. Importantly, legumes are also recognized as key components of functional food science due to their high protein density, balanced amino acid profile, and richness in dietary fiber, minerals, and phytochemicals. These compounds, including isoflavones, saponins, and antioxidants, are linked to cardiovascular protection, glycemic control, and gut microbiome health [5]. Therefore, innovations that enhance legume yield and nutritional quality directly support the development of plant-based functional foods with broad implications for human health and sustainable diets [6,7].

However, the efficiency of symbiotic nitrogen fixation is influenced by complex interactions involving both plant and microbial genetics [8-12]. The success of these associations is further challenged by environmental stressors such as degraded soils, limited organic matter, and climate variability. In this context, cyanobacteria, photoautotrophic nitrogen-fixing microorganisms, are gaining attention for their multifunctional roles. Beyond their agronomic effects, cyanobacteria such as *Arthrospira* (commonly known as Spirulina) and *Nostoc* spp. are valued as functional food organisms themselves, being rich in proteins, polyunsaturated fatty acids, vitamins, pigments (phycocyanin, carotenoids), and bioactive compounds with antioxidant and immunomodulatory properties [13–15]. When applied to agricultural systems, they not only improve soil structure, moisture retention, and nutrient cycling but also contribute to the nutritional enhancement of edible crops [16-18].

When used together, nodule bacteria and cyanobacteria create a synergistic effect that restores soil function, increases nutrient availability, and promotes crop resilience in degraded or humus-deficient soil conditions. Such interactions not only improve agronomic performance but also influence grain protein content, amino acid balance, and mineral accumulation, thereby strengthening the functional food value of legumes [19-21]. Understanding their combined effects in field conditions is therefore critical not only for advancing ecologically sound agricultural practices but also for aligning crop production with modern nutrition and health needs.

Several studies have demonstrated the nutritional and agronomic benefits of microbial coinoculation. For instance, soybean seed inoculation with *Bradyrhizobium japonicum* and/or cyanobacterial strains (e.g., *Anabaena oryzae, Nostoc muscorum, N. calcicola*) significantly enhanced nitrogen uptake and overall productivity. Dual inoculation (rhizobia + cyanobacteria) increased nodule formation, biomass, and protein content more than either microorganism applied alone, suggesting strong synergistic interactions. Moreover, nitrogen accumulation was higher in seeds than in vegetative tissues, underscoring the role of microbial inoculants in nutrient partitioning toward edible parts of the crop [22-23].

Similarly, soil inoculation with cyanobacterial consortia (e.g., *Nostoc entophytum* and *Oscillatoria angustissima*) improved wheat germination and enhanced pea growth and metabolism compared to single-species treatments [24]. Such biofertilizers can reduce chemical fertilizer requirements by up to 50% while contributing to the nutritional fortification of harvested grains. However, the long-term impacts of cyanobacterial inoculation on soil health remain insufficiently studied, and a complete replacement of chemical fertilizers is not yet feasible [19,25,26].

Cyanobacteria such as *Anabaena* also produce secondary metabolites— including polysaccharides, vitamins, hormones, and amino acids—that stimulate plant growth, enhance protein synthesis, and positively influence soil microbial activity [27,28]. These attributes link microbial inoculation not only to soil fertility management but also to the design of biofertilizers that enrich food crops with functional compounds.

Therefore, this study aimed to evaluate the potential of co-inoculating legume crops with native strains of nodule bacteria and cyanobacteria under humus-deficient soil conditions. Field experiments were conducted to assess plant growth, biomass production, and yield performance in pea, soybean, and peanut. In addition, the study sought to develop a cost-effective biofertilizer formulation that advances agroecological practices while enhancing the nutritional and functional food value of legume crops, thus linking soil microbiology, sustainable agriculture, and human health.

METHODS

Microorganisms: Original strains of nodule and free-living nitrogen-fixing bacteria were isolated from diverse soil types and climatic regions of Armenia and selected based on their high nitrogen-fixation potential, ecological adaptability, and beneficial plant interactions.

- *Bradyrhizobium japonicum* MDC 5789 was isolated from soybean nodules grown in claycarbonate soils of the Tavush region. This Gram-negative bacterium significantly enhances nitrogen fixation, increasing soybean yield by 22.8–35.0%.
- *Bradyrhizobium arachidis* was newly isolated from root nodules of *Arachis hypogaea* (peanut) collected in the Vagharshapat area, Armavir region. This strain can form both large and numerous small nodules on peanut roots, indicating strong symbiotic compatibility and nitrogen-fixing capacity.
- *Rhizobium leguminosarum* MDC 5609 was obtained from pea nodules in brown soils at 1000 m elevation in the Noragavit region. It is a highly active nitrogen fixer, increasing pea grain yield by 15.0–24.0%, and is notable for its robust nitrogenase activity.
- *Azotobacter chroococcum* MDC 6111 is a free-living nitrogen-fixing bacteria isolated from the rhizosphere of wheat in chestnut soils of Mkhchyan village (845 m a.s.l.), Artashat region. This strain demonstrates superior efficiency, fixing up to 13.5 mg of nitrogen per gram of glucose consumed, surpassing the average for most *A. chroococcum* strains (typically <10 mg/g carbon source).
- *Paenibacillus polymyxa* MDC 280 is a Gram-positive, spore-forming, free-living bacteria isolated from Armenian black soil. It exhibits both nitrogen-fixing and phosphate solubilizing activities, releasing up to 280 mg/L of $Ca_3(PO_4)_2$ per day. This salt-tolerant strain withstands NaCl concentrations up to 7.0% and contributes to improved nutrient availability in soils.
- *Arthrospira platensis* and *Nostoc* PCC 7807 are filamentous cyanobacteria with distinct structural features, ecological niches, and functional roles. *A. platensis* is known for its high protein content and application as a nutritional supplement, while *Nostoc* PCC 7807 is notable for its symbiotic

capabilities and contribution to soil fertility through biological nitrogen fixation.

Arthrospira platensis serves as a potential plant biostimulant due to its high content of proteins, vitamins, essential amino acids, and antioxidants. In agriculture, its application enhances seed germination, increases chlorophyll concentration, and improves overall plant vigor. Although it does not fix nitrogen, its biomass contributes organic matter and vital micronutrients (N, P, K) to the soil, functioning as both a soil conditioner and a growth enhancer. Importantly, *A. platensis* is already recognized as a functional food for humans, widely used as “spirulina” for its high protein content, γ-linolenic acid, and bioactive compounds with antioxidant and immunomodulatory properties. When applied in crop systems, these same bioactive metabolites, such as phycocyanin, carotenoids, and phenolics, not only improve plant performance but also enrich the nutritional and functional quality of grains, legumes, and vegetables. This dual role strengthens the bridge between soil microbiology and functional food science, as crops grown with A. platensis inputs may provide enhanced dietary protein, vitamins, and antioxidants for human nutrition. Moreover, its antifungal and antibacterial metabolites reduce the need for pesticides, lowering chemical residues in food and promoting cleaner, healthier functional food products.

Nostoc, in contrast, is a nitrogen-fixing cyanobacterium that enriches soil fertility through atmospheric nitrogen fixation via specialized heterocyst cells. This is particularly valuable for nutrient-deficient or degraded soils, including rice paddies and arid agricultural systems. In addition to improving nitrogen balance, *Nostoc* enhances soil organic carbon and microbial diversity, creating healthier and more resilient agroecosystems. From a functional food perspective, the contribution of *Nostoc* to improved nutrient uptake in legumes and cereals translates into higher grain protein content, enriched amino acid balance, and elevated mineral bioavailability (iron, zinc, magnesium). These are critical attributes for addressing global nutritional deficiencies and expanding the portfolio of plant-based functional foods. Furthermore, the gelatinous sheath of *Nostoc* improves soil water retention and drought tolerance, supporting consistent production of nutritionally enriched crops even under stress conditions.

Together, *A. platensis* and *Nostoc* represent complementary microbial tools in advancing sustainable, low-input agricultural systems that not only improve soil fertility and crop productivity but also directly enhance the functional food value of agricultural products. By increasing protein density, antioxidant content, and micronutrient levels in staple legumes and grains, their integration into biofertilizer formulations offers a climate-smart strategy for producing healthier foods that contribute to disease prevention, nutritional security, and human well-being.

Cyanobacterial cultures were grown in 250 mL Erlenmeyer flasks at 30°C under continuous illumination provided by fluorescent lamps, with a light intensity of 45 μmol photons m^{-2} s^{-1} (nutrient medium composition as described in references [15, 29–31]). After 14 days of cultivation, the dry biomass yield of *Nostoc* PCC 7807 (OD = 2.08 ± 0.05 mL^{-1}) reached 2.33 g/L, while that of *Arthrospira platensis* (OD = 1.070 ± 0.03 mL^{-1}, measured at λ = 740 nm) was 1.60 g/L.

Prior to application, cyanobacterial suspensions were diluted fivefold and applied at a dose of 50 mL per plant. Field trials were conducted on 150 m^2 plots characterized by semi-desert gray soil. Inoculation treatments included various combinations of host-specific nodule bacteria, *Azotobacter chroococcum*, *Paenibacillus polymyxa* (a phosphate-mobilizing bacteria), and cyanobacteria. All treatments were arranged in a randomized design with three replicates, and standard agronomic practices were followed throughout the experiment.

Host Plants: Peanut (*Arachis hypogaea* L., Lia» variety**).** Commonly known as groundnut, peanut is a widely cultivated and economically important legume. Named by Carl Linnaeus due to its unique characteristic of developing pods underground, it is valued for its high protein and oil content. Seeds of the 'Lia' variety were obtained from the Armenian Center for Agriculture and Seed Production.

Soybean (*Glycine max* L., «Lidia»-variety**).** Soybean is a major leguminous crop recognized for its high nutritional value, particularly in terms of protein and oil content. The 'Lidia' variety used in this study was sourced from the All-Russian Soybean Research Center located in the Far East of Russia.

Pea (*Pisum sativum* L., «Kangun» variety**).** Pea is a traditional and culturally significant crop in Armenia, historically grown in agricultural regions such as the Sevan basin, Lori, and Syunik (Zangezur). The 'Kangun' variety is known for its content of essential amino acids and vitamins B1 and B9. Peas perform best on fertile soils, whereas yields are significantly reduced on sandy or marshy soils. In addition to their nutritional importance, peas contribute ecologically and agronomically through their ability to fix atmospheric nitrogen, making them beneficial in crop rotation systems. Initial fertilization for peas should involve liquid or water-soluble formulations containing phosphorus in a readily available form.

Soil Analysis: Soil **humus content** was determined using the method of I.V. Tyurin.

Total nitrogen was measured using the Kjeldahl method.

Mobile nitrogen forms were analyzed according to the method of I.V. Tyurin and M.M. Kononova.

Available phosphorus and potassium were determined using Machigin's method [32-33].

Protein Content of Seeds: Protein content in seeds was determined using the Kjeldahl method and the Folin–Ciocalteu phenol reagent method, as described in "Protein measurement with the Folin phenol reagent" [34-35].

Phosphatase Activity: Soil phosphatase activity was assessed following the method proposed by Pikovskaya [36].

Inoculation Procedure: Pre-sowing inoculation involved treating seeds with a bacterial suspension at a concentration of $\times 10^9$ CFU/mL. The inoculant mixture consisted of strains of *Azotobacter chroococcum*, *Paenibacillus polymyxa*, *Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, and *Bradyrhizobium arachidis*, in a volumetric ratio of 10:1:1, respectively.

Field Experiment Design: Field experiments were conducted without the application of chemical fertilizers or herbicides. Weed control was carried out manually. Irrigation was applied 6 to 10 times throughout the growing season.

Grain Yield Measurement: Grain yield was assessed by weighing threshed grain from 1 m^2 plots in three replicates and extrapolating to a per-hectare basis, adjusting for a standard grain moisture content of 14%. The yield was calculated using the formula:

$$\text{Yield (q/ha)} = (\text{Grain weight (g)} / \text{Area (m}^2)) \times 0.1$$

Yield data were statistically processed using analysis of variance (ANOVA), and the least significant difference ($LSD_{0.5}$) and relative experimental error (SX, %) were calculated [37].

Host Biomass and Plant Growth Parameters: Biometric characteristics were recorded at the full maturity stage. Parameters included plant height (cm), biomass (g), number and weight of pods, number of nodules, and other relevant traits [38].

Statistical Analysis: All statistical analyses were conducted using one-way ANOVA in the web trial version of SigmaStat 3.5. Differences between treatments were considered significant at a 95% confidence level ($p < 0.05$). Figures were prepared using Microsoft Office 2016 [39].

RESULTS

The objective of this study was to evaluate the effectiveness of nodule-forming bacterial and cyanobacterial communities under field conditions on key food legume crops, including pea (*Pisum sativum*), soybean (*Glycine max*), and peanut (*Arachis hypogaea*). Two field trials were conducted: the first on a 34 m^2 plot and the second on a 70 m^2 plot. Yield parameters from both experiments are presented in the corresponding tables.

Pre-sowing agrochemical analysis revealed that the experimental soil was characterized by relatively low organic matter content and classified as a brown semi-desert type (Table 1).

Table 1. Agrochemical characteristics of the experimental soil (pre-sowing analysis).

Soil Parameter	Value
Organic matter (%)	2.10 – 2.75
Salinity (%)	0.092
Total carbon (mg per 100 g soil)	0.67
Available nitrogen (NO_3^-) (mg per 100 g soil)	30.2
Available phosphorus (PO_4^{3-}) (mg per 100 g soil)	14.0
Available potassium (K^+) (mg per 100 g soil)	14.7
Available sulfur (mg per 100 g soil)	0.07
pH	6.8 – 7.7

Synergistic Effect of Cyanobacteria on the Efficiency of Symbiotic Nodule Bacteria: Co-inoculation of peanut (Arachis hypogaea) seeds with Bradyrhizobium arachidis followed by Arthrospira platensis significantly enhanced plant growth compared to both the control and singleinoculant treatments. Plants in the co-inoculated group reached an average height of 38.0 ± 2.4 cm and exhibited a dry biomass increase of 55.0 g per plant over the control and 41.1 g per plant over the group inoculated with B. arachidis alone.

Pod yield was also markedly improved in the *A. platensis* co-inoculated group, reaching 100.8 ± 0.7 g per plant, which was more than double the yield of the control group (45.8 ± 1.0 g per plant) and 59.7 g per plant higher than in plants inoculated solely with *B. arachidis*.

Co-inoculation with *Nostoc sp.* strain 7807 also resulted in enhanced pod weight (72.5 ± 0.9 g per plant) compared to both the control and *B. arachidis* monoculture treatments (Table 2, Fig. 5), although its effect was less pronounced than that of *A. platensis*.

Furthermore, nodulation efficiency, assessed by the number and color of nodules, was highest in the *A. platensis* treatment, with a pink, large-nodule index of 14.3 ± 2.2, followed by *Nostoc sp.* (9.3 ± 1.8) and the *B. arachidis* monoculture (8.7 ± 1.9), indicating enhanced symbiotic performance in the cyanobacteria co-inoculated treatments.

Table 2. Growth Performance of Peanut (*Arachis hypogaea* L.) Variety 'Lia' Under the Influence of Symbiotic Bacteria–Cyanobacteria Associations.

Treatment	Plant Height (cm·plant^{-1})	Dry Biomass (g·plant^{-1})	Pod Weight (g·plant^{-1})	Number of Pink Nodules (plant^{-1})
Control (untreated)	25.5 ± 1.8	32.0 ± 1.7	45.8 ± 1.0	–
B. arachidis	28.1 ± 2.6	60.4 ± 1.5	59.7 ± 1.0	8.7 ± 1.9
B. arachidis* + *A. platensis	38.0 ± 2.4	90.4 ± 2.0	100.8 ± 0.7	14.3 ± 2.2
***B. arachidis* + *Nostoc* sp. 7807**	29.7 ± 2.3	75.6 ± 2.3	72.5 ± 0.9	9.3 ± 1.8

The height of soybean plants treated with *Bradyrhizobium japonicum* strain MDC 5789 reached 50.7 ± 2.4 cm per plant. Co-inoculation with *A. platensis* increased plant height to 59.6 ± 2.7 cm, while treatment with *Nostoc* sp. in combination with *B. japonicum* resulted in the highest average height of 64.4 ± 2.0 cm per plant. Compared to the untreated control group, these values represent increases of 14.9 cm, 23.8 cm, and 28.6 cm, respectively.

Pod weight also showed a significant improvement in all treated groups. Compared to the control, increases in pod weight were observed at 3.7% with *B. japonicum* alone, 9.0% with *B.*

japonicum + *A. platensis*, and 10.24% with *B. japonicum* + *Nostoc* sp.

The number of effective nodules per plant also increased under cyanobacterial co-inoculation. While *B. japonicum* alone resulted in an average of 35.3 ± 2.9 nodules per plant, the addition of *A. platensis* and *Nostoc* sp. further enhanced nodulation to 37.1 ± 2.5 nodules per plant (Table 3).

Table 3. Growth Performance of Soybean (*Glycine max* L.) Variety 'Lydia' Under the Influence of Symbiotic Bacteria–Cyanobacteria Associations.

Treatment	Plant Height (cm·plant^{-1})	Dry Biomass (g·plant^{-1})	Weight of 100 Pods (g)	Number of Nodules (plant^{-1})
Control (untreated)	35.8 ± 2.1	20.2 ± 1.7	155.3 ± 1.8	–
***B. japonicum* MDC 5789**	50.7 ± 2.4	26.9 ± 1.6	161.1 ± 2.4	23.2 ± 5.2
B. japonicum* MDC 5789 + *A. platensis	59.6 ± 2.7	28.5 ± 2.0	167.1 ± 2.6	35.3 ± 2.9
***B. japonicum* MDC 5789 + *Nostoc* sp. 7807**	64.4 ± 2.0	30.8 ± 2.9	169.0 ± 2.5	37.1 ± 2.5

The height of soybean plants treated with *Bradyrhizobium japonicum* strain MDC 5789 reached 50.7 ± 2.4 cm per plant. Co-inoculation with *A. platensis* increased plant height to 59.6 ± 2.7 cm, while the combination with *Nostoc* sp. 7807 resulted in the highest growth, reaching 64.4 ± 2.0 cm per plant. These values represent increases of 14.9 cm, 23.8 cm, and 28.6 cm, respectively, compared to the control.

Pod weight also improved under all treatments, exceeding the control by 3.7% with *B. japonicum* alone, 9.0% with *A. platensis*, and 10.24% with *Nostoc* sp. The number of effective nodules increased to 35.3 ± 2.9 and 37.1 ± 2.5 nodules per plant following co-inoculation with *A. platensis* and *Nostoc* sp., respectively (Table 3).

These results demonstrate that *A. platensis* had the most pronounced effect on the biometric parameters of peanut plants, increasing pod weight by 120.0% and the number of pink nodules by 14.3 nodules per plant compared to the control. In contrast, co-inoculation with *Nostoc* sp. 7807 resulted in a 58.3% increase in pod weight, while inoculation with the rhizobia strain alone increased plant weight by only 30.3%.

It is noteworthy that, for soybean, the co-inoculation of *Nostoc* sp. 7807 with the host-specific *B. japonicum* MDC 5789 strain was slightly more effective than the combination with *A. platensis*, particularly in terms of plant height, dry biomass, and pod yield.

Table 4. Influence of Symbiotic Bacteria–Cyanobacteria Associations on Soybean and Pea Productivity.

Treatment	**Soybean (*Glycine max* L.) 'Lydia'**		**Pea (*Pisum sativum* L.) 'Kangun'**	
	Weight of 1000 Seeds (g)	Protein (%)	Weight of 1000 Seeds (g)	Protein (%)
Control (untreated)	150.3	31.2	155.0	20.9
***B. japonicum* MDC 5789**	164.8	35.3	–	–
B. japonicum* MDC 5789 + *A. platensis	167.4	35.5	–	–
***B. japonicum* MDC 5789 + *Nostoc* sp.**	169.0	37.8	–	–
***R. leguminosarum* MDC 5609**	–	–	196.8	21.6
R. leguminosarum* MDC 5609 + *A. platensis	–	–	272.0	24.5
***R. leguminosarum* MDC 5609 + *Nostoc* sp.**	–	–	276.0	26.0

The most pronounced improvement in pea productivity was observed with seed treatment using a combination of *Rhizobium leguminosarum* MDC 5609 and *Nostoc* sp., where the weight of 1000 seeds reached 276.0 g and the protein content increased to 26.0%. In contrast, similar coinoculation treatments applied to soybean (*B. japonicum* MDC 5789 + *Nostoc* sp.) did not result in significant changes in seed yield, despite a modest increase in protein content (Table 4).

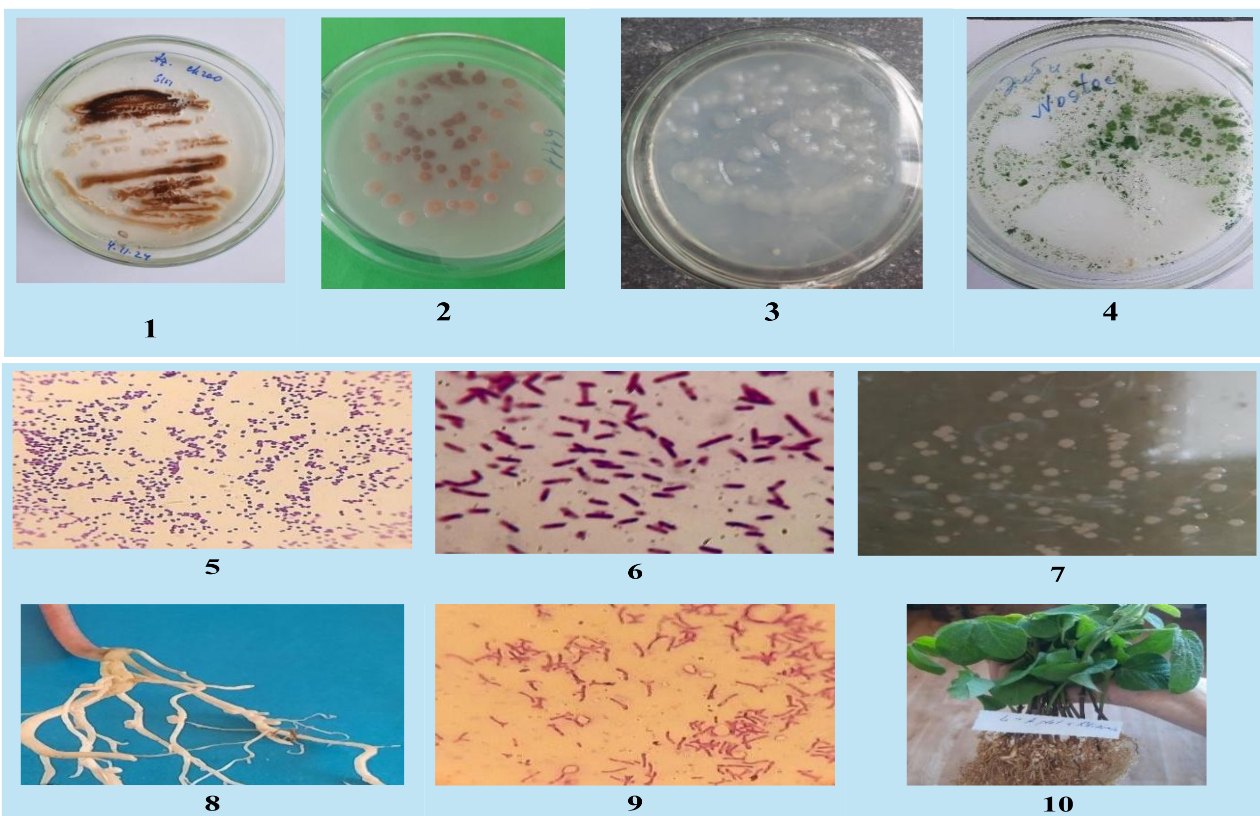


Figure 1. Morphology and growth characteristics of bacterial and cyanobacterial strains used in the study. (1 and 2) Colonies of *Azotobacter chroococcum*; (3) Growth pattern of *Bradyrhizobium arachidis*; (4) Colony of *Nostoc* sp. on agar medium; (5) Cells of *A. chroococcum* (microscopy); (6) Cells and (7) colonies of *Paenibacillus polymyxa* on meat-peptone agar; (8) Root nodules and (9) bacteroids in pea plant nodules; (10) Root nodules formed on soybean plants treated with Rhizomix and *A. platensis*.

During the cultivation of bacterial strains on Pikovskaya's medium supplemented with bromophenol blue and insoluble calcium phosphate [$Ca_3(PO_4)_2$], a distinct color shift from purple to cream-white was observed. This change indicated acidification of the medium due to the release of free H^+ ions (Figure 2). Comparative phosphatase activity of the strains was assessed on days 3 to 5 of incubation, based on the extent and intensity of the color change. The most active phosphate-solubilizing strains *Azotobacter chroococcum* MDC 6111 and *Paenibacillus polymyxa* MDC 280 were selected for inclusion in the biofertilizer formulations.

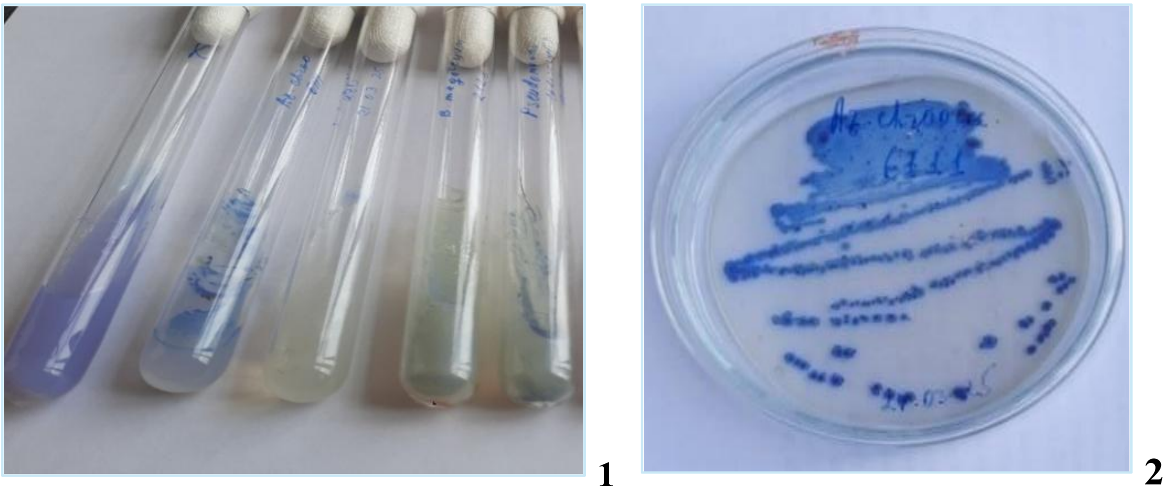


Figure 2. Phosphate-solubilizing activity of bacterial (PGPR) strains on Pikovskaya's medium containing bromophenol blue. Image 1 (test tubes), from left to right: pure Pikovskaya medium, Azotobacter chroococcum; Paenibacillus polymyxa, Priestia megaterium; Pseudomonas tritici. Image 2 (Petri dish): Phosphatase activity profile of Azotobacter chroococcum grown in Pikovskaya nutrient medium to assess its potential for inorganic phosphate solubilization.

Synergistic Effect of Two Cyanobacterial Species on the Performance of Symbiotrophic Nodule Bacteria: The present study investigated the yield performance of soybean, peanut, and pea crops inoculated with the Rhizomix biofertilizer - a composite preparation containing host-specific root nodule bacteria, free-living nitrogen-fixing bacteria, and phosphate-solubilizing bacteria. Additionally, the synergistic effects of co-inoculating two distinct cyanobacterial species on crop yield were evaluated.

Rhizomix biofertilizer comprises host-specific symbiotrophic root nodule bacteria *Bradyrhizobium japonicum* MDC 5789, *Bradyrhizobium arachidis*, and *Rhizobium leguminosarum* MDC 5609 at a concentration of 10^8–10^9 cells per seed, combined with free-living nitrogen-fixing bacteria such as *Azotobacter chroococcum* and *Paenibacillus polymyxa* in a ratio of 10:1:1. This formulation has demonstrated high efficacy in multiple trials [2].

Under field conditions, yield data for soybean variety "Lydia" (Table 5) showed that coinoculation with two cyanobacteria resulted in an average yield increase of 11.8 centners per hectare (c ha^{-1}), compared to a 7.1 c ha^{-1} increase achieved by the Rhizomix biofertilizer alone.

For the peanut variety "Lia," yield increased by 8.9 c ha^{-1} relative to the control, while the Rhizomix treatment alone accounted for a 5.7 c ha^{-1} increase. Pea yields increased by 7.2 c ha^{-1} over the control and 2.7 c ha^{-1} more than with Rhizomix alone.

These data confirm the reliability and significance of the observed yield improvements. The study highlights the positive synergistic effect of combined inoculation with *Arthrospira platensis* and *Nostoc* sp. 7807 on soybean, peanut, and pea productivity grown on humus-deficient soils. Notably, both co-inoculation treatments demonstrated significant increases in grain weight and protein content relative to the control. Figures 3 and 4 visually illustrates this synergistic effect attributable to cyanobacterial co-inoculation.

Table 5. Synergistic Effect of Cyanobacteria on the Effectiveness of Symbiotrophic Nodule Bacteria.

Investigated Crop	**Soybean 'Lidia'**	**Peanut 'Lia'**	**Pea 'Kangun'**
Treatment			
Control (untreated)			
Weight of green mass (g)	65.5	84.7	92.4
1000-grain weight (g)	197.4	511.5	224.0
Protein (%) [34]	30.3	23.1	23.7
Grain yield (c ha^{-1})	17.3	19.6	14.6
Treated with Rhizomix			
Weight of green mass (g)	85.9	148.7	129.9
1000-grain weight (g)	234.0	522.1	242.4
Protein (%)	34.7	24.5	25.2
Grain yield (c ha^{-1})	24.4	25.3	19.1
Treated with Rhizomix + *A. platensis* + *Nostoc* sp.			
Weight of green mass (g)	89.1	158.9	134.5
1000-grain weight (g)	240.0	538.3	278.8
Protein (%)	35.3	26.7	27.4
Grain yield (c ha^{-1})	29.1	28.5	21.8

Figure 3. Peanut plant (var. *Lia*) with developed pods and root nodules from a field experiment, treated with a co-inoculant of *Arthrospira platensis* and *Nostoc* sp.

Figure 4. Peanut seeds after treatment, from left to right: control (untreated); inoculated with *Bradyrhizobium arachidis*; co-inoculated with *B. arachidis* and *Arthrospira platensis*; coinoculated with *B. arachidis* and *Nostoc* sp.

DISCUSSION

The symbiotic relationship between legume plants and rhizobia is a cornerstone of sustainable agriculture and a fundamental model of plant–microbe interaction. In this mutualistic association, rhizobia provide fixed atmospheric nitrogen in exchange for photosynthetically derived carbon compounds, thereby enhancing soil fertility and reducing reliance on synthetic nitrogen fertilizers [8,9]. Rhizobia induce the formation of specialized structures—nodules—on legume roots, within which they differentiate into bacteroids capable of nitrogen fixation. These bacteroids are highly abundant in young nodules and can be observed microscopically due to their motility and structural distinctiveness. This process significantly improves the productivity and nutritional value of leguminous crops.

Advances in next-generation sequencing and metagenomics have revealed that nodules are not exclusively inhabited by rhizobia but function as complex micro-ecosystems enriched with diverse plant growth-promoting (PGP) bacteria [40-42]. These microbial partners contribute to phosphate solubilization, phytohormone production, and pathogen suppression, complementing the activity of rhizobia and further enhancing legume yield and seed quality [2,32,43,44].

Legumes such as peas, soybeans, and peanuts are not only agronomically important but also represent critical functional food sources, providing plant-based proteins, essential amino acids, and bioactive compounds that support human health. Improving their protein content and micronutrient density directly strengthens the nutritional quality of the food supply and contributes to the development of functional foods.

To address the challenges of cultivating legumes on humus-poor soils, a cost-effective biofertilizer formulation, Rhizomix, was developed. This preparation combines host-specific nodule bacteria (*Bradyrhizobium japonicum* MDC 5789, *Bradyrhizobium arachidis*, and *Rhizobium leguminosarum* MDC 5609) with free-living nitrogen-fixing and phosphatesolubilizing bacteria (*Azotobacter chroococcum*, *Paenibacillus polymyxa*) in a 10:1:1 ratio. Coinoculation with two cyanobacteria, *Arthrospira platensis* and *Nostoc* sp. 7809, further enhanced the performance of Rhizomix.

Nostoc contributes atmospheric nitrogen fixation through heterocysts and improves soil organic carbon and microbial diversity, while *A. platensis*, though non-nitrogen-fixing, provides organic matter, micronutrients, and bioactive metabolites with antioxidant and antimicrobial properties [4,15,26,27,43,45]. Importantly, *A. platensis* is already consumed as a functional food ("spirulina") due to its high protein, vitamin, and antioxidant content. Its agricultural application not only improves crop vigor and resilience but also enriches the protein density and bioactive compound content of food crops, linking soil microbiology directly to human nutrition and functional food science.

Key findings of this study include:

- Soybean (*Glycine max* 'Lydia'): 1000-seed weight increased from 197.4 g (control) to 240.0 g (Rhizomix + cyanobacteria). Grain yield rose from 17.3 c ha^{-1} (control) to 29.1 c ha^{-1} (co-inoculation). Protein content reached 35.3%.
- Peanut (*Arachis hypogaea* 'Lia'): 1000-seed weight rose from 511.5 g (control) to 538.3 g (co-inoculation). Yield increased from 19.6 c ha^{-1} (control) to 28.5 c ha^{-1}. Protein content increased to 26.7%.
- Pea (*Pisum sativum* 'Kangun'): 1000-seed weight increased from 224.0 g (control) to 278.8 g (co-inoculation). Yield improved from 14.6 c ha^{-1} (control) to 21.8 c ha^{-1}. Protein content increased to 27.4%.

Across all crops, co-inoculation with Rhizomix and cyanobacteria significantly improved yield, seed protein levels, and nutritional quality. These enhancements demonstrate not only the agronomic benefits of microbial consortia but also their relevance to functional food production, as protein-enriched legumes provide superior dietary value and contribute to healthy nutrition.

In conclusion, integrating rhizobia, PGP bacteria, and cyanobacteria offers a viable, ecologically sustainable strategy for enhancing legume productivity under poor nutrient conditions. Beyond yield gains, this approach strengthens the link between soil health and functional food science, ensuring the production of protein-rich, bioactive compound-enriched legumes that support both agricultural sustainability and human well-being.

To summarize, when sustainable agriculture and functional food come together, a powerful effect emerges producing products that are both safe for nature and good for people. This interaction creates a food future where food is not only affordable, but also healthy. Developing such a system requires the collaboration of farmers, scientists, technologists, entrepreneurs and consumers. However, the fundamental change lies in shifting our mindset and prioritizing food choices that promote both human health and environmental sustainability [33,45-47].

CONCLUSION

The present study demonstrated the effectiveness of a multifunctional biofertilizer, Rhizomix, formulated with host-specific nodule bacteria (*Rhizobium leguminosarum*, *Bradyrhizobium japonicum*, *Bradyrhizobium arachidis*), free-living nitrogen-fixing bacteria (*Azotobacter chroococcum*), and phosphate-solubilizing bacteria (*Paenibacillus polymyxa*), in enhancing the productivity and nutritional quality of economically important legumes such as soybean, peanut, and pea.

A major innovation was the co-inoculation with two ecologically and functionally distinct cyanobacteria, *Arthrospira platensis* (spirulina) and *Nostoc* sp., which provided synergistic improvements across all agronomic traits, including green biomass, 1000-seed weight, seed protein content, and total yield. *A. platensis*, recognized as a functional food ingredient rich in proteins, vitamins, and antioxidants, contributed organic matter and bioactive compounds that promoted plant vigor and enhanced the nutritional quality of harvested seeds. *Nostoc*, as a heterocystous nitrogen-fixer, reinforced soil fertility and improved nutrient cycling. Together, their integration into the Rhizomix system amplified nitrogen fixation, nutrient uptake, and stress resilience, especially under humus-deficient soil conditions.

This biologically based approach aligns with the principles of sustainable agriculture and functional food science by producing legume crops that are higher yielding and richer in proteins and bioactive nutrients relevant to human health. The findings highlight the value of microbial consortia, combining symbiotic, free-living, and cyanobacterial partners in advancing both agroecological sustainability and the nutritional potential of plant-based diets.

Future research should investigate the long-term effects of such complex inoculants on soil health, microbiome stability, and their adaptability to different agro-climatic zones, thereby paving the way for broader application of microbial technologies in the dual pursuit of food security and functional nutrition.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and consented to its submission to the journal, reviewed all the results and approved the final version of the manuscript. SH – writhing – original draft, methodology, investigation and data analysis; TS - methodology and investigation; GK - formal analysis and investigation; VG- projects supervision, investigation, data analysis and validation, writhing – original draft.

Conflict of Interest Statement

The authors declare that they are not aware of any competing relationships that could have influenced the work presented in this article.

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