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Research Article

Rhizobial Mitigation of Potyvirus Enhances Plant Growth and Biomass Resilience

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ABSTRACT

Potyvirus infection can severely impair legume growth and productivity, whereas beneficial rhizobacteria may enhance plant performance and resilience under biotic stress. This study evaluated the growth-promoting and stress-mitigating effects of *Rhizobium leguminosarum* in cowpea challenged with potyvirus. The bacterial isolate exhibited characteristic *Rhizobium* morphology and biochemical properties and was molecularly identified as *R. leguminosarum* based on phylogenetic analysis. Potyvirus infection was confirmed through characteristic symptoms on indicator plants and positive ACP-ELISA reactions. Viral infection markedly suppressed plant growth, reducing shoot and root biomass, whereas rhizobial inoculation substantially alleviated these effects. At 100 days after planting, virus-infected rhizobium-inoculated plants produced 13.54 g shoot fresh weight plant^{-1} compared with 5.98 g plant^{-1} in virus-infected uninoculated plants. Similarly, shoot dry weight increased from 1.02 to 2.62 g plant^{-1}, while root fresh weight increased from 3.24 to 7.70 g plant^{-1} following rhizobial inoculation. Rhizobial inoculation also markedly enhanced nodulation, with approximately 39 nodules plant^{-1} in virus-infected inoculated plants compared with 1.6 nodules plant^{-1} in virus-infected uninoculated plants. Shoot nitrogen concentration reached 2.502% in virus-free rhizobium-inoculated plants and remained relatively high under viral infection. Overall, *R. leguminosarum* substantially improved biomass production, root development, nodulation, and nitrogen status of potyvirus-infected cowpea, indicating its potential as a component of sustainable strategies for enhancing crop resilience to viral stress. Further multi-location studies are required to validate its effectiveness under diverse agroecological conditions.

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Introduction

The increasing global population is a significant challenge to food security, demanding increased food production on available arable land in a sustainable manner (Adhab and Alkuwaiti, 2022). Deteriorating soil fertility, adverse climatic conditions, and destructive insect pests and diseases are increasingly limiting agricultural production systems. In addition, the excessive application of chemical fertilizers and pesticides in conventional farming practices to sustain high crop yields has led to environmental issues and concerns about potential health risks (Chen, 2025). In this context, pulses are an essential and sustainable part of food and nutrition security in the world, especially in developing countries. They also have the ability to establish symbiotic relationships with soil microorganisms, such as rhizobia,

which significantly boost soil fertility and plant productivity (Kumar et al., 2026).

Pulses are a group of leguminous crops such as chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), and soybean (*Glycine max*) that can form symbiotic relationships with the nitrogen-fixing bacteria known as rhizobia (Kumar et al., 2026). These bacteria attach themselves to the roots of plants and cause them to form special structures called root nodules. Biological nitrogen fixation inside these nodules transforms the N2 in the atmosphere into plant-assimilable forms, contributing to the growth and development of plants (Chiurazzi et al., 2025). In addition to nitrogen fixation, rhizobia and other plant growth promoting rhizobacteria (PGPR) protect plants by different mechanisms such as induced systemic resistance (ISR) that activates systemic defense mechanisms against pathogens, and the production of siderophores which bind Fe ions and make them unavailable for invading pathogens (Chiurazzi et al., 2025).

The rhizobial inoculation has been proven to enhance soil fertility as well as crop productivity in previous studies. It is well documented that plant growth, nutrient acquisition, biomass accumulation, and yields have been enhanced in numerous crops by the application of compatible microbial inoculants (Al-Ani et al., 2012; Al-Ani and Adhab, 2013; Adhab et al., 2025). For example, inoculation of soybean has resulted in an increase in grain production of about 12–19%, while inoculation of common bean with effective strains of *Rhizobium tropici* has been reported to increase the yield by up to 66% (Kebede, 2021). Likewise, the pods per plant increased by 13.7% in cowpea treated with *Bradyrhizobium japonicum* in Tanzania resulting in a significant enhancement of 100-seed weight (Kebede, 2021). Besides biological nitrogen fixation, PGPR can also increase the availability of vital micronutrients such as calcium, magnesium, phosphorus, and potassium involved in several biochemical processes. This improvement can be achieved by generating low molecular weight organic acids and other metabolites which allow the release of inorganic phosphorus in forms that plants can readily absorb or by increasing the uptake of inorganic phosphorus itself (Chiurazzi et al., 2025).

Cowpea is vulnerable to several viral diseases and about 140 viruses have been documented to infect this crop, causing significant yield reductions (Abd El-Aziz, 2024). Of these, members of the genus Potyvirus are of special significance with wide geographical distribution and prevalence. Viral infections in plants can cause variety of symptoms such as chlorosis, distortion of the leaf blade, leaf curling, mosaic or variegation, mottling, and vein clearing. Besides their direct damage to plant growth and development, viral infections can predispose plants to environmental stresses and negatively influence root nodulation (Sun et al., 2025).

In addition to their ability to promote plant growth and nutrition, PGPR have also been widely studied and utilized as bioagents for the biocontrol of a wide range of plant pathogens, such as bacteria, fungi, nematodes, and viruses. They are thought to exert beneficial effects for viral diseases by inducing or priming plant defense mechanisms, regulating plant growth, and improving physiological resilience. For instance, cowpea aphid-borne mosaic virus (CABMV), one of the most important viral pathogens of cowpea vectored by aphids, has been found to cause high losses or complete crop failure when it infects cowpea cultivars such as 'OLO II' and 'OLOYIN' at early stages, especially within 10 days after planting (Kareem and Taiwo, 2007). These results indicate the potential significance of beneficial microorganisms in alleviating virus related stresses and ensuring productivity of crops.

Although rhizobial inoculations have been demonstrated to have positive effects, there are significant gaps in the understanding with respect to their effectiveness, consistency, and applications under various environmental conditions. The application of microbial inoculants also shows significant differences among production systems, partially due to variations in farmers' awareness, quality of inoculants, compatibility of bacterial strains with host genotypes, and environmental factors. Thus, the present study was conducted, under field conditions in Iraq, to assess the efficacy of using rhizobial inoculants to improve growth and performance of cowpea under Potyvirus infection.

Materials and Methods

Isolation and preparation of potyvirus

Cowpea plants (*Vigna unguiculata* L.) showing natural infection were collected from a cowpea field located in Abu Ghraib, west of Baghdad, Iraq (33.2976 N, 44.0818 E) and an isolate of potyvirus was obtained according to the protocol described by Al-Ani and Adhab (2013). Enzyme linked immunosorbent assay (ELISA) was used to confirm virus infection, using the PathoScreen Potyvirus Test Kit (Agdia, USA) which utilizes antigen

coated plates. The confirmed virus isolate was then inoculated mechanically into healthy cowpea plants grown in muslin-covered cages in the greenhouse. Plants showing typical virus symptoms were used as inoculum for subsequent experiments. ELISA confirmed virus-free cowpea plants were kept as controls.

Isolation of *Rhizobium* and preparation of inoculum

Cowpea rhizobia were isolated from the root nodules of cowpea grown at a field in Abu Ghraib. The nodules were surface-sterilized with 1% sodium hypochlorite for 2 min followed by rinsing several times with sterile distilled water. Aseptically, the disinfected nodules were crushed in sterile Petri dishes and a part of the crushed suspension was streaked on yeast extract–mannitol agar (YEMA) medium. The composition of the medium as described by Senthilkumar et al. (2020) is given below:

K_2HPO_4 = 1 g
KH_2PO_4 = 1 g
NaCl = 0.2 g
$MgSO_4.7H_2O$ = 0.2 g
$CaSO_4.2H_2O$ = 0.1 g
$FeCl_2.2H_2O$ = 0.08 g
KNO_3 = 1 g
Yeast extract = 1 g
Mannitol = 10 g
Agar = 15 g
Distilled water = 1 L

The plates were set up in an upside down position and were incubated for 4 days at 28 °C.

Characteristic bacterial colonies were cultivated on a liquid yeast extract–mannitol medium in 250-mL Erlenmeyer flasks for 4 days at 28°C. Standard plate count method was used to determine the concentration of viable bacterial cells. Tenfold serial dilutions (10^{-1} to 10^{-8}) were made, and 1 mL of each dilution was aseptically placed into sterile Petri dishes. To each plate, about 20 mL of molten nutrient agar was then added, mixed and allowed to solidify. The plates were then inverted and incubated for 4 days at 28°C and the colonies were counted. For each dilution, three plates were used. Viable-cell concentration was determined using the average number of colonies and the dilution factor. The mean numbers of viable bacteria in the nodules of the root were 2.193×10^7 cells cm^{-3}.

Morphological and biochemical identification of the *Rhizobium* isolate

Colony and cellular morphology, biochemical characteristics and molecular analysis were used to characterize the *Rhizobium* isolate. After 48 h of incubation at 30°C, the morphology of the colonies was determined by observing the form, texture, color, margin, opacity, and elevation of the colonies while cellular morphology was examined using Gram staining. Biochemical characteristics examined were oxidase, catalase, methyl red and Voges–Proskauer reactions, hydrolysis of starch and casein, fermentation of maltose and galactose and utilization of citrate. All the biochemical analyses were carried out three times following the standard procedures.

Molecular identification of the *Rhizobium* isolate

Genomic DNA was extracted using a Zymo DNA Extraction Kit (Zymo Research, USA). The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the universal bacterial primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). The PCR program consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min kb^{-1}, followed by a final extension at 72°C for 7 min. PCR products were examined by agarose gel electrophoresis to verify amplification and were subsequently purified by ethanol precipitation. Purified amplicons were sequenced using the dideoxy chain-termination method on an ABI PRISM® 3500 XL DNA Sequencer.

The resulting sequences were edited and aligned using BioEdit software. Homologous sequences were identified through BLAST searches against the National Center for Biotechnology Information (NCBI) database. Phylogenetic relationships were inferred using the maximum-likelihood method based on the Tamura–Nei model in MEGA X, using 511 aligned nucleotide positions.

Preparation of bacterial inoculum, seed treatment, and viral inoculation of *V. unguiculata* under nitrogen-free conditions

The bacterial inoculum was prepared in a liquid medium containing river clay supplemented with 0.5% potassium dihydrogen phosphate (KH_2PO_2), 1% mannitol, and 1% Arabic gum powder as a binding agent. The medium was prepared at a ratio of 500 mL per 300 g of soil and sterilized by steam sterilization at 1.5 kg cm^{-2} and 121°C for 2 h.

Healthy, uniform, and well-developed local cowpea seeds were washed thoroughly with water and surface-sterilized with sodium hypochlorite containing 1% free

chlorine. The seeds were subsequently rinsed several times with sterile distilled water and thoroughly mixed with the prepared bacterial inoculum in a sterile metal container. The inoculated seeds were left uncovered for 2 h and then sown at a rate of four seeds per pot. The soil used was sandy loam and was sterilized by autoclaving at 121°C for 60 min on two consecutive occasions separated by 24 h to minimize microbial contamination.

Four treatments were established:

1. R1 + V1 = Rhizobial inoculation + viral infection
2. R1 + V0 = Rhizobial inoculation + virus-free condition
3. R0 + V1 = Non-inoculated + viral infection
4. R0 + V0 = Non-inoculated + virus-free condition (absolute control)

Following germination, three uniform seedlings were retained in each pot, and each treatment was replicated five times. Plants were irrigated weekly until the end of the experiment with a nitrogen-free nutrient solution prepared by dissolving 1 g of a nutrient mixture in 1 L of sterile distilled water. The nutrient mixture consisted of 31.7 g potassium chloride, 18 g calcium phosphate, 13.7 g calcium sulfate dihydrate, 5.5 g magnesium sulfate heptahydrate, 2.7 g ferric sulfate, 0.5 g copper sulfate pentahydrate, 0.6 g manganese sulfate hydrate, 0.5 g boric acid, and 26.8 g potassium dihydrogen phosphate, following Burton (1984).

Leaves from confirmed virus-infected cowpea plants were macerated in 0.01 M neutral phosphate buffer and used for viral inoculation. The homogenate was passed through double layers of sterilized muslin or gauze and the resultant sap was mechanically applied to leaves of cowpea plants, two weeks after sowing. There were four treatments replicated five times making a total of 20 pots. In each replication, three plants per pot were maintained after thinning. The same nitrogen free nutrition regime was applied to all plants during the experiment.

Data collection and determination of nitrogen content

The experiment was conducted following the completely randomized design with five replications for each treatment. The data were taken at three growth stages viz., pre-flowering stage (60 days after sowing), flowering stage (85 days after sowing) and pod formation stage (100 days after sowing). Fresh and dry weights of shoots and roots as well as tissue N concentration were measured at each sampling period.

Aboveground plant material was oven-dried and pulverized with the help of an electric mill to determine nitrogen. A sub-sample of 0.2 g was transferred to a 250 mL Erlenmeyer flask and then 1 g of selenium, 8 mL of sulfuric acid and 3 mL of hydrogen peroxide (H_2O_2) were added. The mixture was then heated for 24 h at 100°C in a sand bath and incubated at 30°C for 1 h 15 min. The digest was then cooled and the volume was made up to 250 mL in a volumetric flask. An aliquot of 100 mL was taken for analysis. The total nitrogen concentration was measured with an automated analyzer following the method of Steenekamp et al. (2024).

Assessment of nodulation

Nodulation was evaluated destructively after 60, 85 and 100 days after sowing. Roots were gently pulled from the soil at each sampling stage, washed carefully and observed for nodulation. The number of nodules per plant was counted and the fresh weight of the nodules was measured immediately. Nodules were then dried and weighed to measure nodule dry biomass. Nodule functionality was determined visually by observing the color of the internal tissues of freshly cut nodules. The pinkish to reddish coloration was regarded as the evidence of activity of leghemoglobin and biological nitrogen fixation.

All the assays were performed in controlled environments and standard procedures were used throughout the study to ensure consistency of experiments, measurement accuracy and reproducibility.

Statistical analysis

The experiment was conducted following the completely randomized design with five replications for each treatment. Percentage data were arcsine square-root transformed prior to statistical analysis. The Fisher's least significant difference (LSD) test (P = 0.05) was used to compare treatment means.

Results and Discussion

Rhizobium isolation, morphological and biochemical characterization, and molecular identification

The isolated bacterial strain produced colonies having morphological and cellular features typical to genus *Rhizobium*. The colonies appeared to be milky-white, translucent, mucoid, circular with entire margins and elevated. When examined under the microscope, the cells were found Gram-negative and rod-shaped (Table 1). The observed morphological features were similar to those described for *Rhizobium leguminosarum*.

All the biochemical tests performed to confirm the identity of the bacterial strain yielded positive results. The strain was found to be positive for oxidase and catalase

activities and also for starch and casein hydrolysis. On the other hand, the strain showed negative result for Methyl Red test while the Voges–Proskauer test was positive, suggesting differences in its utilization in carbohydrates and fermentation processes. The isolate was also able to use citrate and gave a positive reaction on Triple Sugar Iron (TSI) agar. In addition, it fermented maltose and galactose (Table 1). All of these morphological and biochemical features were representative of the phenotype of *R. leguminosarum*.

Table 1. Morphological and biochemical characterization of *R. leguminosarum*.

Character	Results	Character	Results
Gram stain	-	Oxidase	+
Shape	Circular	Starch hydrolysis	+
Margin	Entire	Casein hydrolysis	+
Texture	Mucoid	Methyl red	-
Opacity	Translucent	Voges proskaur	+
Colour	Milky white	Citrate	+
Elevation convex	Raised	Triple sugar iron	+
Bacterium shape rods	Rods	Galactose fermentation	+
Catalase	+	Maltose fermentation	+

The isolate was then molecularly identified to confirm its taxonomic position. The query strain was identified as belonging to the Alphaproteobacteria by phylogenetic analysis and identified with known *Rhizobium* species. The isolate had the highest sequence similarity and phylogenetic association with *Rhizobium leguminosarum* (CP171844.1) and *R. leguminosarum* bv. *viciae* (CP022564.1) and adjacent branches were formed by the *R. indicum* strains (CP054031.1). The correlation among the colony morphology, the cell characteristics, the biochemical properties and the phylogenetic homology based on the sequence data strongly suggests that the isolate is a strain of *R. leguminosarum* (Figure 1).

Biological characterization and symptomatology of the potyvirus

Characteristic symptoms of potyvirus infection were produced when sap from infected plants was mechanically inoculated on indicator plants. Chlorotic mottling appeared 4-9 days after inoculation on *V. unguiculata* and then resulted in systemic vein clearing and mild mosaic symptoms. However, inoculation caused pale chlorotic local lesions in 14 days in *Chenopodium amaranticolor* which later on became distinct necrotic lesions. The biological reactions and positive serological results from a potyvirus-specific ACP-ELISA kit verified the virus identification as a member of the genus potyvirus.

Infected, non-inoculated plants (R0 + V1) showed the characteristic symptoms of the physiological responses typical of potyvirus infection. Viral infections might cause damage to chloroplast function and structure, impair cellular metabolism and decrease photosynthetic efficiency, which can result in chlorosis, mosaic formation and growth suppression (Chen et al., 2024).

Effects of *Rhizobium* inoculation on shoot biomass

Formation of shoots biomass was significantly affected by the interaction between the inoculation of *Rhizobium* and infection by potyvirus throughout the plant development (Figure 2). The analysis revealed that in the absence of rhizobial inoculation (V1 + R0), virus infection significantly reduced shoot growth, as evidenced by shoot fresh weights of 2.54, 4.56 and 5.98 g plant^{-1} at 60, 85 and 100 days after planting (DAP), respectively. On the contrary, the growth suppression due to viral infection was significantly reduced by rhizobial inoculation. The fresh weight of the plants grown under V1 + R1 was 13.54 g plant^{-1} while that of under V1 + R0 treatment was 5.98 g plant^{-1} at 100 DAP. The highest fresh weight of 21.38 g plant^{-1} was obtained with the virus free rhizobium inoculated treatment (V0 + R1) compared to the healthy uninoculated control (V0 + R0) with fresh weight of 11.96 g plant^{-1}.

The present results showed that rhizobial inoculation significantly enhanced growth of cowpea plants when challenged with potyvirus. The increased biomass might be linked with biological nitrogen fixation, better nutrient uptake and rhizobium induced plant growth. Viral infection, on the other hand, can impose significant metabolic costs by diverting host resources to be used for viral multiplication, infection and dissemination, thereby impeding host cell growth and biomass production (Owaresat et al., 2023; Adhab and Schoelz, 2024).

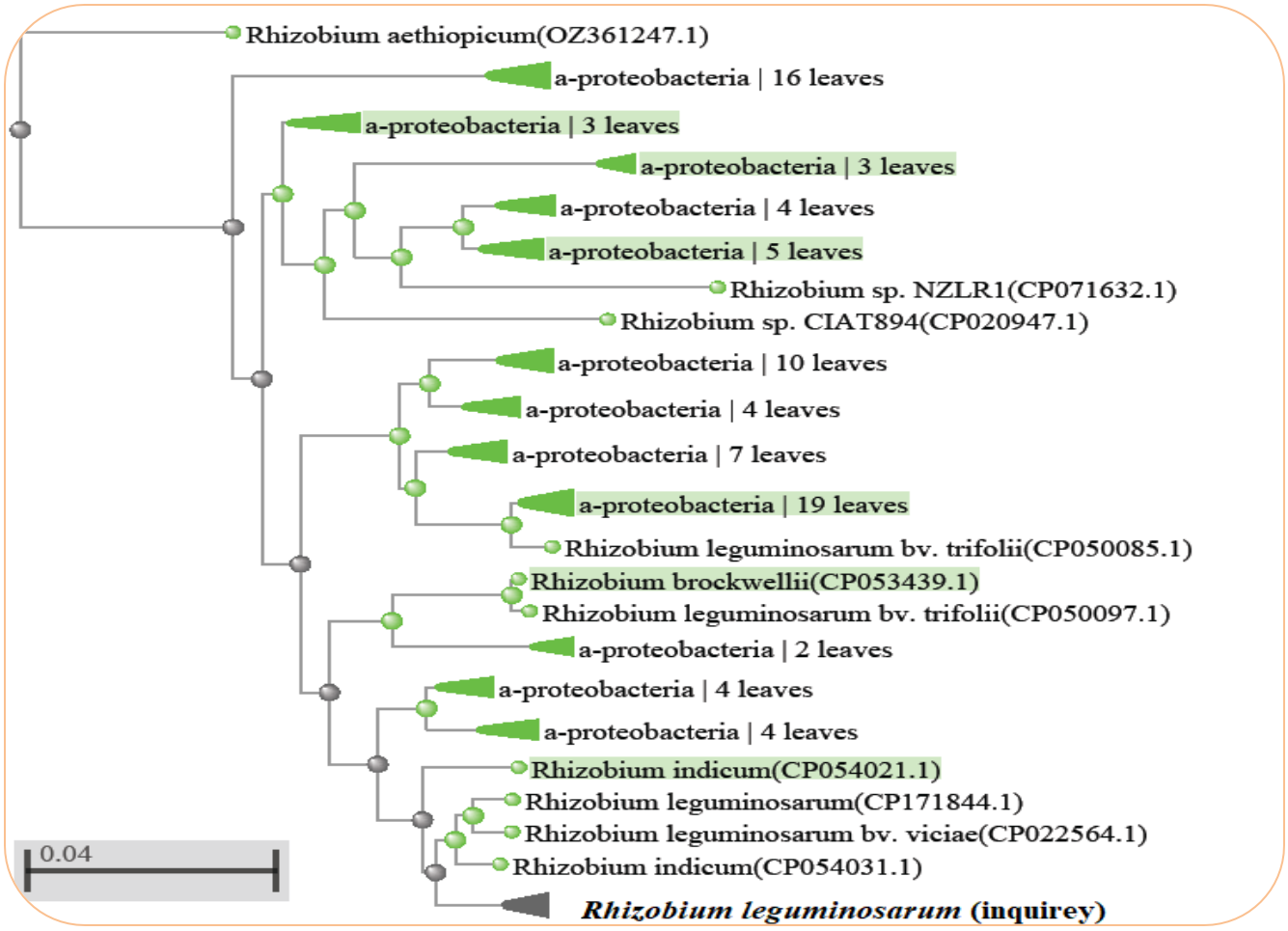

Figure 1. Phylogenetic analysis of *. leguminosarum*.

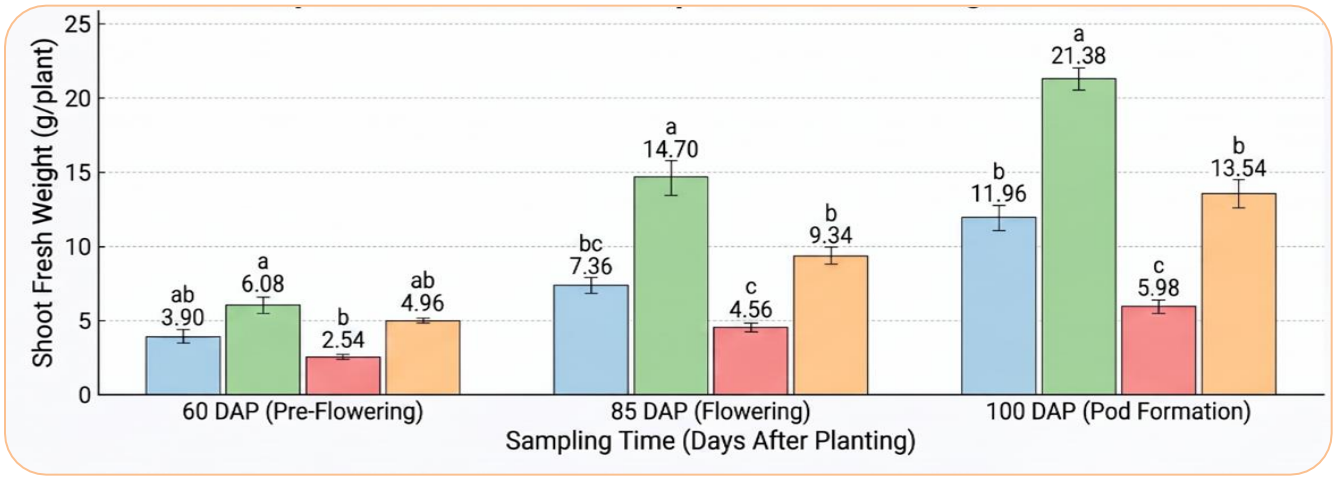

Figure 2. Temporal dynamics of shoot fresh weight accumulation in cowpea under rhizobial inoculation and potyvirus stress. Data represent the mean fresh weight (g/plant) recorded at 60, 85, and 100 DAP. Treatments: V0 + R0 (Absolute Control: Blue), V0 + R1 (Rhizobia only: Green), V1 + R0 (Virus only: Red), and V1 + R1 (Virus + Rhizobia: Orange). Error bars represent the standard error (n = 5). Different lowercase letters above the bars indicate significant differences within each sampling stage according to Fisher's LSD test ($p < 0.05$; LSD = 2.7629). Interaction effects (Rhizobia × Virus × Time) were significant at $p < 0.01$.

The similar trends were observed with the accumulation of dry matter (Figure 3). The plant dry matter accumulated under the virus inoculation with rhizobial inoculation (V1 + R1) was 2.62 g plant^{-1} while the plants inoculated with the virus without rhizobial inoculation (V1 + R0) accumulated only 1.02 g plant^{-1} at 100 DAP. The maximum dry weight of 4.40 g plant^{-1} was observed for V0 + R1 treatment whereas the healthy uninoculated control treatment (V0 + R0) gave a dry weight of 2.28 g plant^{-1}. Therefore, inoculation with rhizobium significantly enhanced dry matter production in plants infected with potyvirus, however, under the present experimental conditions; biomass production was equal to or even greater than that of the uninoculated healthy plants.

The enhancement of biomass production by *R. leguminosarum* is congruent with its ability to fix nitrogen biologically and to enrich plant nutrition and plant growth (Adhab, 2021). The higher nitrogen availability might aid in the production of proteins and in the synthesis of photosynthetic components, which would help to build biomass. The present findings, however, should not be interpreted as a proof that biological nitrogen fixation completely offsets the physiological costs of viral infection, but as an evidence that growth was improved under the prevailing experimental conditions.

Root system resilience and development

Root development also followed a similar trend as observed for shoot biomass, which indicated the positive effects of rhizobial inoculation under viral stress (Figure 4). The suppression of root growth was more distinct in the non-rhizobial inoculated plants challenged with potyvirus (V1 + R0) which had significantly reduced root fresh weight to 3.24 g plant^{-1} at 100 DAP. This suppression was significantly reduced by rhizobial inoculation, leading to fresh weight of roots in V1 + R1 of 6.78 g plant^{-1} at 85 DAP and 7.70 g plant^{-1} at 100 DAP. The root fresh weight of V1 + R1 at 100 DAP was similar to healthy uninoculated control (V0 + R0; 6.96 g plant^{-1}), while irus-free inoculated treatment (V0 + R1) had the highest root fresh weight of 10.90 g plant^{-1}.

The beneficial effect of the rhizobial inoculation was further substantiated by the root dry matter accumulation (Figure 5). V1 + R0 treatment had a lower root dry weight of around 0.34 g plant^{-1} between 85 and 100 DAP. Conversely, the amount of root dry matter, gradually increased in V1 + R1 from 0.66 g plant^{-1} at 60 DAP to 0.99 g plant^{-1} at 100 DAP. V1 + R1 plant dry weight was more than the healthy uninoculated plant (V0 + R0; 0.69 g plant^{-1}) and V0 + R1 had the highest root dry weight of 2.40 g plant^{-1} at the final assessment.

The improved root development following rhizobial inoculation might be associated with enhanced nutrient acquisition and rhizosphere interactions. Rhizobial colonization can also influence root architecture through the production of phytohormones, including indole-3-acetic acid (IAA), which may stimulate lateral root formation and increase the absorptive capacity of the root system. In addition, beneficial microorganisms can induce a primed physiological state that enhances plant responses to biotic stress. Such mechanisms could contribute to the improved root development observed in rhizobium-inoculated plants under viral infection (Al-Ani et al., 2011).

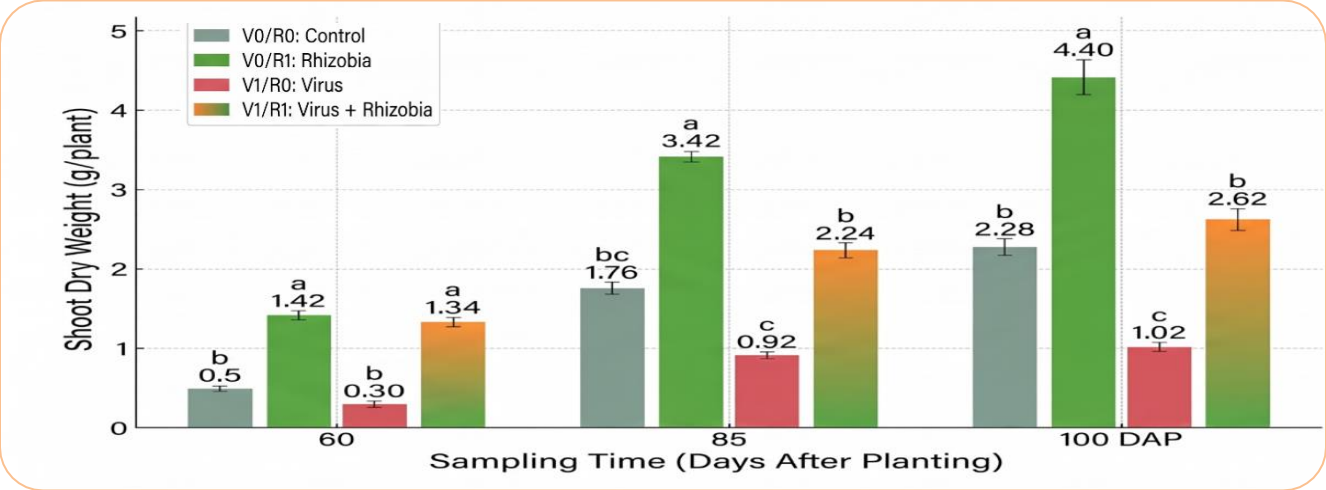

Figure 3. Effect of Rhizobia inoculation and infection with potyvirus on the dry weight of the green parts of cowpea plants over time.

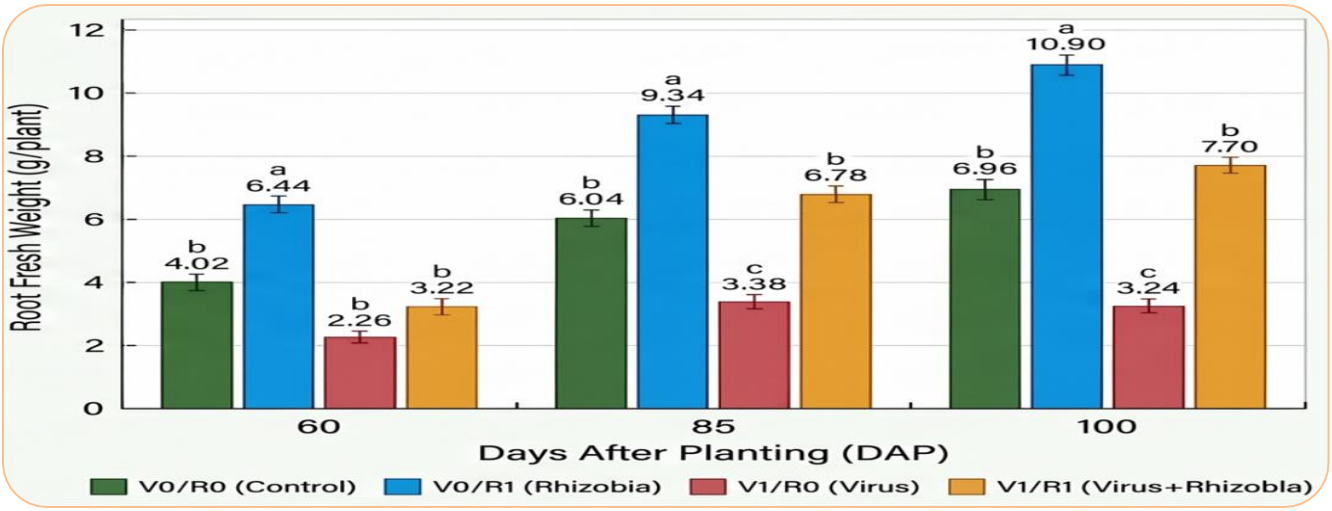

Figure 4. Effect of Rhizobia inoculation and potyvirus infection on the fresh weight of the root system of cowpea plants over time.

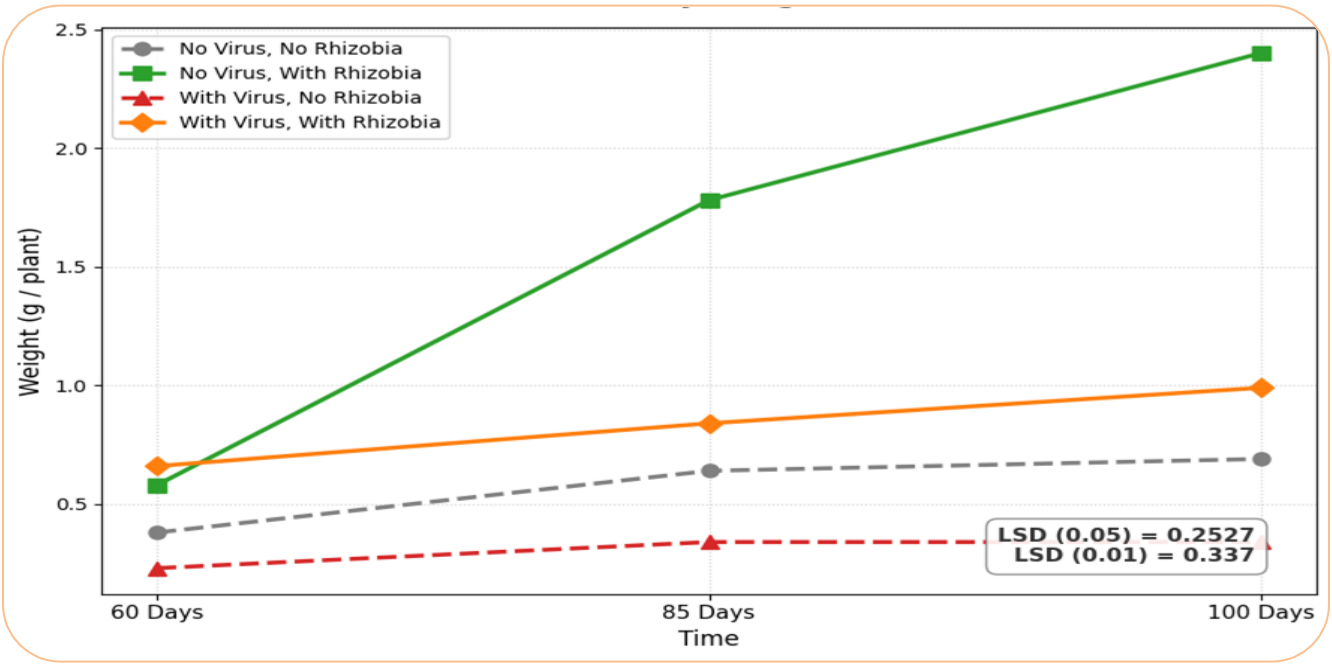

Figure 5. Effect of Rhizobia inoculation and potyvirus infection on the dry weight of the root system of cowpea plants over time.

The present findings are also consistent with previous observations that viral infection can interfere with root development through systemic movement and disruption of host physiological processes. However, the proposed effects on hormone balance and viral movement into root tissues require direct physiological or molecular evidence and should therefore be considered potential mechanisms rather than definitive explanations.

Quantitative dynamics of root nodulation

The establishment of rhizobial association in cowpea was confirmed directly by the presence of root nodules (Figure 6). The number of nodules was found to be significantly higher in *R. leguminosarum* inoculated plants (RI) than in non-inoculated plants (R0) during the experimental period. The number of nodules per plant in V0 + RI treatment increased steadily to 86.6 plant^{-1} at 100 DAP from 29.5 plant^{-1} at 60 DAP under virus free conditions.

Potyvirus infected plants had fewer nodules than virus-free plants; however the inoculated and virus-infected plants had significantly more nodules than the non-inoculated plants. V1 + RI treatment yielded around 39 nodules plant^{-1} at 100 DAPS, whereas V0 + R0 and V1 + R0 treatments produced 2.4 and 1.6 nodules plant^{-1}, respectively. The findings of the present study confirm the ability of the inoculated *R. leguminosarum* strain to establish a symbiotic association with cowpea while retaining a high nodulating potential under viral stress.

The ability of the rhizobium bacteria to maintain nodulation throughout the infection might, in part, account for the increased biomass and nitrogen captured by plants inoculated with rhizobium. However, the number of nodules does not necessarily reflect the level of nitrogen fixation, other factors such as measurements of nitrogenase activity, nodule acetylene reduction, or isotopic nitrogen incorporation would be better indicators of functional nitrogen fixation.

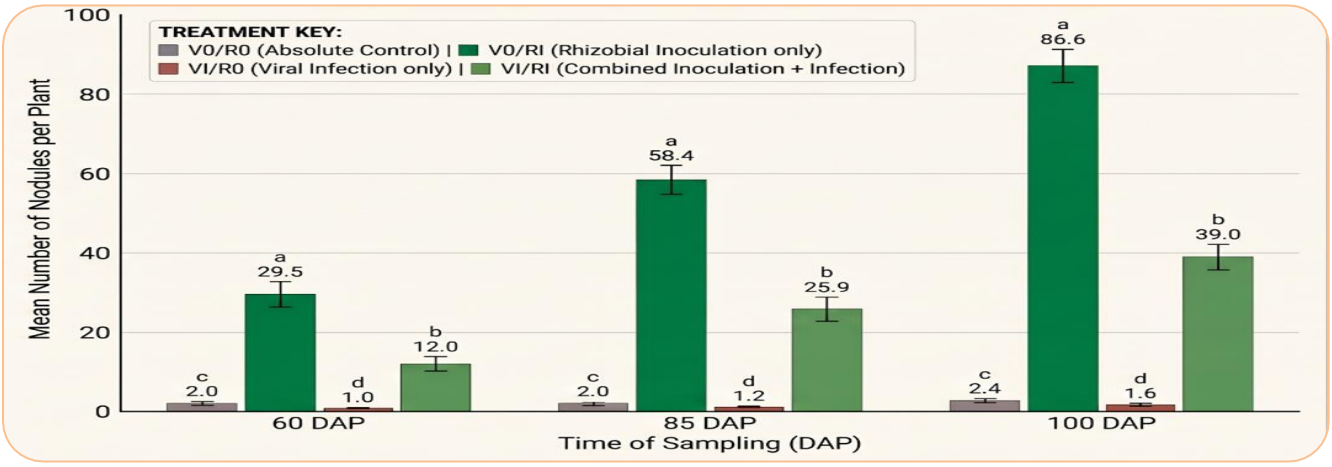

Figure 6. Dynamics of root nodulation in cowpea under *R. leguminosarum* inoculation and potyvirus stress. Mean number of nodules per plant recorded at 60, 85, and 100 DAP.

Foliar nitrogen accumulation

Nitrogen analysis of shoots showed significant effects of rhizobial inoculation and viral infection (Figure 7). The shoot N concentration of virus-free plants rose from 1.29% for plants not inoculated with rhizobia (V0 + R0) to 2.502% for plants that were inoculated (V0 + R1), suggesting greater N accumulation in the plants after rhizobial inoculation. It is interesting to note that the viral infection, without rhizobial inoculation, also raised the shoot N to 1.788% compared with 1.29% in healthy control.

The rise in tissue nitrogen concentration in viral infection has to be handled with care. Increased N concentration does not necessarily reflect higher N nutrition and/or higher N fixation. Changes in biomass accretion and the composition of nitrogen-containing compounds such as defence-related metabolites and proteins might lead to changes in tissue N concentration due to viral infection. The observed increase should, therefore, not be linked only to improved nitrogen uptake or fixation without directly measuring nitrogen fluxes.

Viral infection of rhizobium-inoculated plants maintained relatively high nitrogen concentrations, indicating that the symbiotic association was not affected by the pathogen pressure. Therefore, rhizobial inoculation may help to enhance the nutritional status and tolerance to viral stress in the plant. Earlier investigations have also highlighted the importance of beneficial microorganisms in enhancing plant growth and resistance in response to biotic stress (Al-Ani et al., 2013; Adhab et al., 2025).

Overall, the biological nitrogen fixation, enhanced nutrient uptake and plant growth promotion suggest that *R. leguminosarum* could be useful for sustainable crop production systems. Rhizobial inoculants can reduce synthetic nitrogen fertilizer usage, and can help enhance resource-use efficiency. Nonetheless, their effectiveness depends on native rhizobial population, edaphic factors such as soil moisture, soil temperature, soil structure and texture, host genotype, and other environmental conditions. Based on the present results, it is therefore not recommended that rhizobial inoculants should be used as substitutes for synthetic fertilizers or pesticides in general.

The multifunctional aspects of rhizobial inoculation have been summarized in Figure 8. Apart from biological nitrogen fixation, rhizobia stimulate nutrient acquisition and plant growth through various mechanisms including phosphorus solubilization, production of phytohormones, and improved root development. Some strains can also inhibit plant pathogens in direct antagonistic ways such as production of siderophores, antibiosis and activities of hydrolytic enzymes as well as indirectly through induced systemic resistance (ISR) (Sheoran et al., 2025).

Rhizobial inoculation therefore represents a promising component of sustainable legume production. Biological nitrogen fixation provides a renewable source of plant-available nitrogen, while rhizobia may also contribute to the acquisition of phosphorus and other nutrients and stimulate root development through compounds such as IAA (Adhab, 2021; Mitra et al., 2025). These combined effects can potentially improve plant growth and yield while reducing dependence on external inputs.

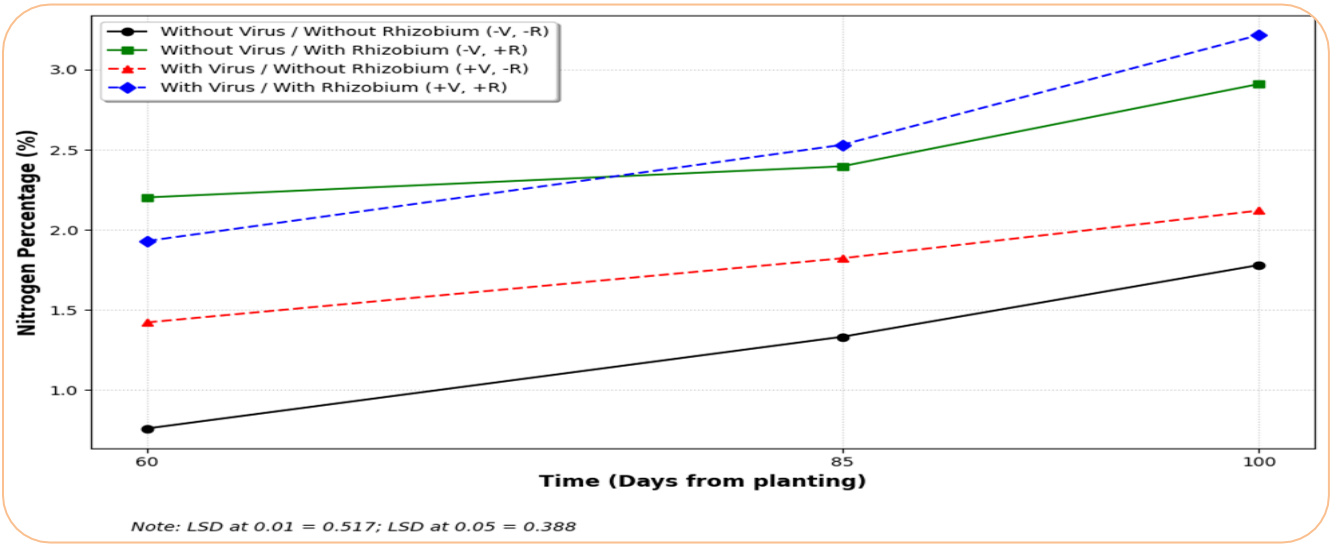

Figure 7. Dynamics of nitrogen percentage in the vegetative parts of cowpea plants.

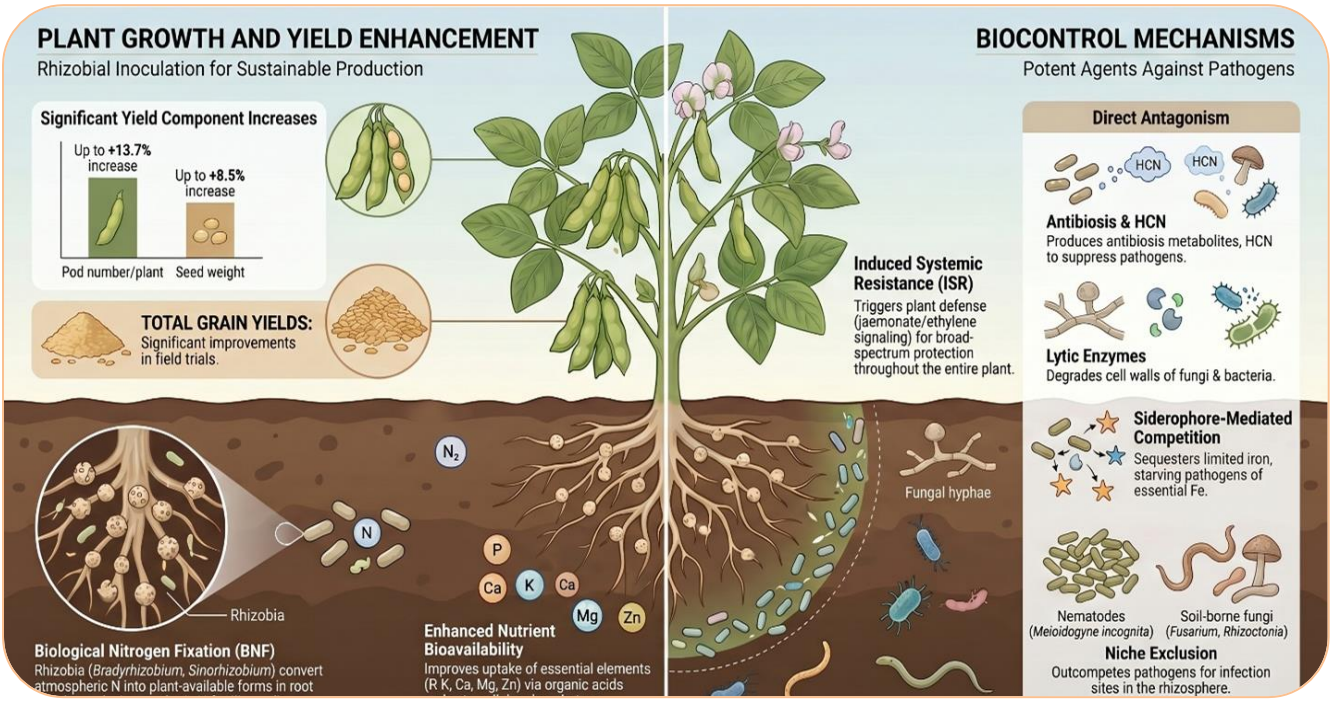

Figure 8. Benefits and mechanisms of rhizobial inoculation for sustainable legume production.

The economic and environmental advantages of rhizobial inoculation are potentially substantial because inoculants can provide biological functions without the environmental costs associated with excessive use of synthetic fertilizers. Nevertheless, inoculation efficiency is often site-specific and may be constrained by competition with indigenous rhizobial populations, soil acidity, temperature, moisture, and inoculant quality. Future research should therefore focus on strain selection, rhizobial consortia, and improved formulation technologies, including polymer-based formulations, to enhance inoculant survival, establishment, and consistency under diverse agroecological conditions.

More broadly, rhizobial technology represents a promising strategy for sustainable intensification of legume production. Beyond nitrogen fixation, rhizobia can improve nutrient availability through organic-acid-mediated phosphorus solubilization and may enhance the acquisition of macro- and micronutrients, including P, K, Ca, Mg, Zn, and Fe. Certain strains can additionally contribute to pathogen suppression through direct antagonism and ISR (Al-Ani and Adhab, 2013; Al-Ani et al., 2013). These multifunctional properties may make rhizobial inoculants useful components of integrated crop-management systems.

Although the present findings demonstrate substantial benefits in cowpea, extrapolation to non-leguminous crops or other production systems should be made cautiously because the effectiveness of rhizobial symbiosis depends strongly on host compatibility and environmental conditions. Further multi-location and multi-season experiments are therefore required to determine the stability, economic feasibility, and broader applicability of rhizobial inoculation.

Conclusion

The application of *Rhizobium leguminosarum* substantially alleviated the adverse effects of potyvirus infection on the growth and development of cowpea under the conditions evaluated in this study. Viral infection alone markedly reduced shoot and root biomass, whereas rhizobial inoculation considerably improved fresh and dry matter accumulation in infected plants. The beneficial effect of inoculation was accompanied by enhanced root nodulation and increased shoot nitrogen concentration, indicating that the rhizobial association remained functional under viral stress.

The greater biomass production observed in rhizobium-inoculated, virus-infected plants suggests that improved nitrogen acquisition and other plant-growth-promoting effects associated with *R. leguminosarum* can substantially enhance plant resilience to viral infection. However, the present study did not directly quantify nitrogen fixation; therefore, the contribution of biological nitrogen fixation should be confirmed through direct physiological or isotopic measurements in future studies.

Overall, *R. leguminosarum* shows considerable promise as a biological input for improving cowpea growth and resilience under potyvirus pressure. Its potential dual role as a biofertilizer and plant-growth-promoting microorganism could contribute to more sustainable crop production and reduced dependence on synthetic inputs. Future research should focus on elucidating the molecular mechanisms underlying rhizobium-mediated antiviral responses and evaluating selected strains, consortia, and advanced inoculant formulations across diverse genotypes, environments, and field conditions.

Authors' Contributions

WMA and FAA conceptualized and designed the study. WMA conducted the field investigations and experiments, while FAA provided overall supervision and ensured methodological and analytical consistency. FAA supervised the research, contributed to experimental design, data interpretation, and manuscript preparation. All authors critically reviewed and refined the manuscript, verified the data and analyses, and approved the final version for submission.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

Policy Addressed

Agricultural policies should promote validated rhizobial bioinoculants as components of integrated nutrient and plant-health management, supported by quality standards, locally adapted strain development, farmer

extension, and multi-location validation to strengthen sustainable and resilient cowpea production.

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