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RESEARCH ARTICLE

Synergistic Effects of *Rhizobium* Inoculation and Organic Manure on Growth, Yield, and Soil Microbial Dynamics in Green Gram (*Vigna radiata* L.)

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ABSTRACT

Continuous use of synthetic fertilizers degrades soil health, necessitating sustainable alternatives like Integrated Nutrient Management (INM). A three-year field experiment (Kharif) in the middle Gujarat agro-climatic zone evaluated the performance of promising *Rhizobium* isolates (*Rhizobium selenitireducens* AAU M1, *R. cellulosilyticum* AAU PP16, and *R. pusense* AAU P16) combined with Farm Yard Manure (FYM) and Recommended Dose of Fertilizer (RDF) on green gram (*Vigna radiata* L. cv. GAM 5). Pooled data revealed that combining 2 t FYM/ha with *R. selenitireducens* AAU M1 seed treatment, and integrating 2 t FYM/ha + 50% RDF + *R. selenitireducens* AAU M1, produced seed yields (980 kg/ha and 1042 kg/ha, respectively) statistically at par with the 100% synthetic RDF treatment (1103 kg/ha). Crucially, the 100% chemical regime suppressed biological symbiosis, whereas the organic-biological approach maximized root nodulation (41 nodules/plant) and exponentially increased soil *Rhizobium* and phosphate-solubilizing bacteria populations up to 1,000-fold. While 100% RDF generated the highest net return, the integrated and organic-biological treatments delivered highly competitive Benefit-Cost Ratios of 4.00 and 3.80, respectively. The study concludes that substituting synthetic nitrogen with *R. selenitireducens* AAU M1 and FYM is an economically viable and ecologically restorative strategy for green gram cultivation, offering comparable yields while regenerating soil microbial health.

Keywords: *Green gram, Rhizobium selenitireducens, Integrated nutrient management, Biofertilizer, Soil microbial population, Nodulation*

INTRODUCTION

Green gram (*Vigna radiata* L.), globally recognized as mung bean, is a staple grain legume of prime importance cultivated extensively for its high nutritional value. Pulses are colloquially known as “heartbeats” because they are vital for all living entities, serving four essential roles: food, feed, fuel, and fertilizer. Green gram provides an affordable, highly digestible source of dietary protein (ranging from 20% to 32%), essential amino acids, carbohydrates, vitamins, and minerals, which makes it critical for maintaining global food security (Diatta *et al.,* 2024; Mehta *et al.,* 2021). Beyond basic nutrition, the crop contains significant amounts of bioactive compounds—including polyphenols, polysaccharides, and peptides—that offer antioxidative and immunomodulatory benefits (Hou *et al.,* 2019; Mehta *et al.,* 2021).

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To achieve optimal growth, yield, and productivity, green gram requires a steady supply of major macronutrients, particularly nitrogen and phosphorus. Historically, the agricultural sector has relied heavily on synthetic chemical fertilizers to meet these nutrient demands. However, to overcome the deleterious environmental and economic effects of continuous chemical fertilizer application, there is an urgent need to evolve and adopt sustainable agricultural practices. While continuous genetic evaluations help identify promising, high-yielding pulse genotypes (Joshi *et al.,* 2021), realizing their full yield potential sustainably requires alternatives to chemical farming. The excessive use of synthetic nitrogen sources not only degrades soil health and beneficial microbial populations but also increases the economic burden on farmers.

A highly effective alternative to mitigate the reliance on synthetic inputs is the implementation of Integrated Nutrient Management (INM) and organic farming systems (Suddala Prem Kumar *et al.,* 2024). Green gram possesses the inherent ability to form a symbiotic relationship with *Rhizobium* bacteria, facilitating the biological fixation of atmospheric nitrogen directly into the root nodules. The strategic application of efficient biofertilizers—such as specific *Rhizobium* inoculants, combined with organic manures like Farm Yard Manure (FYM) provides a sustainable mechanism to enrich soil fertility, improve plant nutrient uptake, and reduce dependency on chemical fertilizers.

Based on previous screening experiments under pot conditions, three promising *Rhizobium* cultures were identified for further evaluation. Building on those preliminary findings, this field study was conducted to assess the performance of these selected isolates rigorously (*Rhizobium selenitireducens* AAU M1, *R. cellulosilyticum* AAU PP16, and *R. pusense* AAU P16) on green gram cv. GAM 5.

MATERIALS AND METHODS

Experimental Site and Agro-Climatic Conditions

The field experiment was conducted over three consecutive *Kharif* (monsoon) seasons at the agronomy research farm of B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat, India. The experimental site is situated in the Middle Gujarat Agro-climatic Zone III. The region is characterized by a semi-arid tropical to subtropical climate with hot summers and moderate winters, receiving the majority of its annual precipitation via the southwest monsoon during the crop growth period (July to October).

Crop Cultivar and Microbial Inoculants

- Host Crop: Green gram (*Vigna radiata* L.) cv. GAM 5 was used as the test crop.
- Bacterial Strains: Three promising *Rhizobium* isolates evaluated were:

1. *Rhizobium selenitireducens* AAU M1
2. *Rhizobium cellulosilyticum* AAU PP16
3. *Rhizobium pusense* AAU P16

These isolates were originally screened and identified based on prior two-year pot evaluation trials. Liquid *Rhizobium* cultures were applied as a seed treatment at the rate of 5 mL/kg seed uniformly before sowing.

Treatment Details and Experimental Design

The experiment was laid out in a Randomized Block Design (RBD) with three replications. The gross plot size was 3.6 m × 4.5 m, and the net plot size harvested for yield estimation was 2.4 m × 3.9 m.

A uniform basal application of phosphorus (40 kg P_2O_5/ha) was applied to all experimental units through Single Super Phosphate (SSP). Nitrogen was applied through Urea as per treatment specifications. Farm Yard Manure (FYM) was incorporated into the soil during field preparation as a basal dose.

The experiment comprised eight nutrient management treatments:

- T_1: 100% Recommended Dose of Nitrogen (100% RDF (N) @ 20 kg N/ha)
- T_2: 4 t FYM/ha
- T_3:2 t FYM/ha + 50% RDF (N) (10 kg N/ha) + seed treatment with *Rhizobium selenitireducens* AAU M1
- T_4: 2 t FYM/ha + 50% RDF (N) (10 kg N/ha) + seed treatment with *Rhizobium cellulosilyticum* AAU PP16
- T_5: 2 t FYM/ha + 50% RDF (N) (10 kg N/ha) + seed treatment with *Rhizobium pusense* AAU P16
- T_6: 2 t FYM/ha + seed treatment with *Rhizobium selenitireducens* AAU M1
- T_7: 2 t FYM/ha + seed treatment with *Rhizobium cellulosilyticum* AAU PP16
- T_8: 2 t FYM/ha + seed treatment with *Rhizobium pusense* AAU P16

Agronomic and Cultural Practices

- Sowing and Plant Density: Seeds were

sown by drilling at a seed rate of 12-16 kg/ha, maintaining an inter-row spacing of 30 cm.

- Crop Maintenance: Two supplemental irrigations were provided in each crop season to avoid moisture stress. Periodic weed management was maintained through hand weeding along with two interculturing operations per season.
- Harvesting: Harvesting was carried out upon physiological maturity.

Sampling and Observations

Growth and Symbiotic Parameters

- Plant Height (cm): Five representative plants were randomly selected and tagged in each net plot to record plant height at 30 days after sowing (DAS), 60 DAS, and at harvest.
- Nodule Count: At 45 DAS (peak flowering/ nodulation phase), five plants from the sampling rows of each plot were carefully uprooted with intact root systems. Roots were gently washed under a slow stream of water over a wire sieve to count the number of active root nodules per plant.
- Plant Population: Total plant population per net plot was recorded at 45 DAS to verify uniform crop establishment.

Yield and Yield Attributes

- Seed and Haulm Yield: Crops from net plot areas were harvested, sun-dried, threshed, and cleaned separately. Seed yield and haulm yield were measured and converted to kg/ha.
- Test Weight (g): A representative sample of 1,000 clean seeds was randomly counted from the harvest of each plot and weighed using an electronic precision balance to record the 1000-seed test weight.

Plant Nutrient Analysis

Representative haulm samples collected at harvest were oven-dried at 65 °C until a constant weight was achieved, and subsequently ground using a stainless-steel mill.

- Total Nitrogen Content (%): Determined using the micro-Kjeldahl digestion and distillation method.
- Total Phosphorus Content (%): Determined using the vanadomolybdophosphoric yellow colour method via spectrophotometry following tri-acid digestion HNO_3: H_2SO_4:$HClO_4$).

Soil Microbial Population Dynamics

Rhizosphere soil samples were collected from the root zone (15 cm depth) at harvest. Enumeration of microbial populations was carried out using standard serial dilution and spread-plate techniques:

- Soil Rhizobium Population: Enumerated on Yeast Extract Mannitol Agar (YEMA) containing Congo red and expressed as log10 CFU/g dry soil.
- Phosphate Solubilizing Bacteria (PSB): Enumerated on Pikovskaya's agar medium by monitoring clear halo zone formation and expressed as log10 CFU/g dry soil.

Economic Analysis

Cost of cultivation, gross returns, net returns, and Benefit–Cost Ratio (BCR) were calculated for each treatment based on the prevailing regional market input costs and output prices:

Gross Return (₹/ha) = (Seed Yield × Price of Seed) + (Haulm Yield × Price of Haulm)

Net Return (₹/ha) = Gross Return - Total Cost of Cultivation

Benefit-Cost Ratio (BCR) = Gross Return (₹/ha)/Total Cost of Cultivation (₹/ha)

Statistical Analysis

Data obtained for each parameter over the three experimental years (2018, 2019, and 2020) were tested for homogeneity of variance and subjected to Analysis of Variance (ANOVA) for a Randomized Block Design. A pooled analysis over the three years was performed to evaluate the main effects of treatments (T), years (Y), and their interaction (Y × T).

Differences among treatment means were compared using the Critical Difference (CD) / Least Significant Difference (LSD) test at a 5 % level of significance (p=0.05). Standard error of the mean (S.Em.±) and Coefficient of Variation (CV %) were computed to describe the precision of the trial.

RESULTS AND DISCUSSION

Crop Growth Parameters: The experimental data indicate that nutrient management practices significantly influenced the vegetative growth of green gram. Plant height, a primary morphological indicator of crop vigor, was

highest under resource-intensive treatments such as 100% RDF (T1) and 4 t FYM/ha (T2) at 30 days after sowing (DAS), 60 DAS, and at harvest. However, treatment T6, which integrated a lower dose of organic manure (2 t FYM/ha) with seed inoculation of *Rhizobium selenitireducens* AAU M1, produced plant heights (20.8 cm at 30 DAS, 40.9 cm at 60 DAS, and 66.9 cm at harvest) that remained statistically at par with both T1 and T2 (Table 1). The sustained vegetative growth observed in T6 can be attributed to the balanced, slow-release nutrient supply from FYM acting synergistically with the biofertilizer. Recent field trials validate this dynamic; Sartaj *et al.,* (2025) demonstrated that integrating farmyard manure with biological inoculants supports sustained vegetative growth and biomass accumulation equivalent to heavy synthetic applications.

Root Nodulation and Symbiotic Efficiency: Root nodulation serves as a critical metric for evaluating the efficiency of symbiotic biological nitrogen fixation. During the active vegetative phase, the application of high-dose chemical nitrogen profoundly suppressed biological symbiosis, with T1 (100% RDF) recording a significantly lower count of 23 nodules per plant. In striking contrast, the purely organic-microbial treatment T6 (2 t FYM/ha + *R. selenitireducens* AAU M1) stimulated robust root-bacterial interactions, yielding the highest count of 41 nodules per plant (Table 1). This aligns perfectly with established physiological models where high concentrations of exogenous inorganic nitrogen inhibit nodule organogenesis. Recent investigations by Sasmal *et al.,* (2024) corroborate these findings, establishing that organic soil amendments like FYM, when combined with specific *Rhizobium* strains, create a highly conducive rhizosphere that maximizes nodulation parameters and biological nitrogen contribution compared to entirely inorganic regimens.

Yield Attributes and Crop Establishment: Uniform crop establishment is essential to ensure that yield variations are strictly treatment-driven. The plant population per plot showed non-significant variations across all treatments, ranging narrowly from 254 to 259 plants/plot (Table 1).

Regarding yield attributes, the 1000-seed test weight exhibited a trend parallel to plant growth. Treatments T1 through T6 were statistically at par, maintaining high test weights ranging from 38.5 g (T6) to 39.8 g (T1). Conversely, treatments relying on alternative *Rhizobium* strains alongside 2 t FYM/ha (T7 and T8) experienced a sharp, significant decline in test weight (28.2 g and 28.4 g, respectively). The sustained seed weight in T6 implies that *R. selenitireducens* AAU M1 provided an adequate nutrient sink for proper grain filling (Table 1). Studies by Menaria *et al.,* (2024) emphasize that efficient biological inoculants supported by the carbon matrix of FYM not only fix atmospheric nitrogen but directly enhance assimilate partitioning, thereby maintaining robust seed weights and overall yield attributes.

Plant Nutrient Uptake: Analysis of the harvested haulm demonstrated non-significant differences across treatments regarding total nitrogen (N) and phosphorus (P) content. The haulm N content ranged from 1.17% (T1) to 1.29% (T4), while P content fluctuated marginally between 0.15% and 0.19%. This lack of statistical disparity highlights a crucial agronomic victory: green gram cultivated using integrated nutrient management (T3, T4, T5) or entirely organic/biological inputs (T6) can assimilate macronutrients just as efficiently as crops grown under a 100% synthetic fertilizer regime (T1) (Table 1). Sasmal *et al.,* (2024) recently established that reducing RDF by 50% while incorporating FYM and *Rhizobium* significantly improves N and P uptake in green gram, fully supporting the viability of transitioning away from full chemical dependence without sacrificing plant nutrition.

Seed and Haulm Yield: Crop yield is the ultimate determinant of the efficiency of any agricultural management practice. The data presented in Table 2 indicate that the various nutrient management strategies significantly influenced seed and haulm yields of green gram. The maximum seed yield (1103 kg/ha) and haulm yield (1460 kg/ha) were recorded under T1, which received 100% RDF (N). However, treatments integrating organic amendments and biofertilizers, specifically T2 (4 t FYM/ha), T3 (2 t FYM/ha + 50% RDF + *R. selenitireducens* AAU M1), T4 (2 t FYM/ha + 50% RDF + *R. cellulosilyticum* AAU PP16), and T6 (2 t FYM/ha + *R. selenitireducens* AAU M1), produced seed yields (1068, 1042, 990, and 980 kg/ha, respectively) that were statistically at par with the 100% synthetic treatment (Table 2).

Similarly, the haulm yields for T1 through T6 ranged from 1425 kg/ha to 1496 kg/ha, showing no significant statistical divergence among

Table 1: Effect of Nutrient Management on Growth, Nodulation, Yield Attributes, and Plant Nutrient Uptake

Treatment Details	Plant Height 30 DAS (cm)	Plant Height 60 DAS (cm)	Plant Height at Harvest (cm)	Nodules / Plant (Nos.)	Test Weight (g)	Plant Population / Plot	Haulm N Content (%)	Haulm P Content (%)
T1: 100% RDF (N)	22.6 a	43.3 a	70.5 a	23 e	39.8 a	257	1.17	0.15
T2: 4 t FYM/ha	22.7 a	42.6 a	70.8 a	28 de	39.5 a	256	1.21	0.16
T3: 2 t FYM/ha + 50% RDF (N) + *R. selenitireducens* AAU M1	22.1 a	41.7 a	67.6 a	34 bc	39.2 a	256	1.21	0.16
T4: 2 t FYM/ha + 50% RDF (N) + *R. cellulosilyticum* AAU PP16	21.6 a	40.6 a	67.7 a	33 bcd	38.8 a	259	1.29	0.19
T5: 2 t FYM/ha + 50% RDF (N) + *R. pusense* AAU P16	21.3 ab	40.4 a	66.2 a	32 cd	38.8 a	259	1.28	0.16
T6: 2 t FYM/ha + *R. selenitireducens* AAU M1	20.8 abc	40.9 a	66.9 a	41 a	38.5 a	254	1.23	0.17
T7: 2 t FYM/ha + *R. cellulosilyticum* AAU PP16	18.7 bc	33.5 b	57.7 b	37 ab	28.2 b	255	1.20	0.15
T8: 2 t FYM/ha + *R. pusense* AAU P16	18.1 c	32.7 b	56.0 b	35 bc	28.4 b	257	N/A*	N/A*
S. Em.± (T)	0.66	0.91	1.66	1.30	1.06	4.03	0.03	0.01
C.D. (P=0.05) (T)	Sig.	Sig.	Sig.	Sig.	Sig.	NS	NS	NS
C.V. %	9.31	7.78	8.66	11.63	9.97	5.34	6.64	12.11

Note: Data followed by similar lowercase letters within a column do not differ significantly at a 5% level of significanc

these approaches. Treatments T7 and T8, which relied on less effective *Rhizobium* strains, recorded significantly lower yields. The ability of T6 to maintain competitive yields without any synthetic nitrogen highlights the efficacy of *R. selenitireducens* AAU M1 (Table 2). Recent agronomic evaluations by Menaria *et al.,* (2024) confirm that robust bio-inoculants, when supported by a carbon source like FYM, can completely substitute chemical nitrogen requirements in pulse crops by maintaining an optimal source-sink relationship during the reproductive phase.

Economic Feasibility

While integrated and organic nutrient management systems demonstrate immense biological benefits, their adoption by the farming community is largely dictated by economic viability. Treatment T1 (100% RDF) required the lowest cost of cultivation (₹15,758/ha) and subsequently generated the highest gross return (₹78,670/ha), net return (₹62,912/ha), and Benefit-Cost Ratio (BCR) of 4.99. This highlights the immediate, short-term financial efficiency of highly subsidized synthetic urea. Treatment T3 proved to be a highly profitable alternative, securing a net return of ₹55,807/ha and a strong BCR of 4.00, despite a slightly higher cultivation cost of ₹18,593/ha. Remarkably, the purely organic-biological approach in T6 yielded a net return of ₹51,606/ha with a BCR of 3.80. Conversely, applying 4 t FYM/ha alone (T2) resulted in the highest cost of cultivation (₹21,410/ha) and a lower BCR of 3.56. These economic outcomes demonstrate that applying massive quantities of organic manure alone (T2) is less economically efficient than halving the manure dose and supplementing it with a low-cost biofertilizer (T6) (Table 2). Sartaj et al. (2025) and Sasmal *et al.,* (2024) both recently concluded that while 100% chemical fertilization yields the highest immediate net returns, integrated practices (like T3 and T6) offer highly competitive BCRs. They represent a superior, sustainable trade-off that secures farmer profitability while preventing the long-term soil degradation associated with continuous chemical farming.

Soil Microbial Population Dynamics

The long-term sustainability of agricultural soils is heavily dependent on maintaining a robust and active microbial community. The introduction of synthetic chemicals versus organic amendments profoundly alters the rhizosphere microbiome, as evidenced by the population dynamics of *Rhizobium* and Phosphate Solubilizing Bacteria (PSB) over the three-year study period.

Rhizobium Population in the Rhizosphere

The data illustrated in the lower graph 1 reveals a dramatic shift in the soil *Rhizobium* population based on the nutrient management strategy applied. The baseline (initial) average *Rhizobium* population was recorded at 2.80E+02 CFU/g of soil. Under the continuous application of 100% synthetic nitrogen (T1), the population marginally increased to an average of only 3.27E+03 CFU/g of soil. This highlights a well-documented ecological drawback: high concentrations of exogenous, readily available inorganic nitrogen actively suppress the proliferation of symbiotic nitrogen-fixing bacteria. In stark contrast, treatment T6 (2 t FYM/ha + *R. selenitireducens* AAU M1) recorded the absolute highest *Rhizobium* population across all three individual years, culminating in a three-year average peak of 9.40E+06 CFU/g of soil. This is a staggering 100-fold increase over the sole organic treatment (T2: 5.00E+04 CFU/g of soil) and a nearly 3,000-fold increase over the synthetic treatment (T1) (Graph 1). The explosive proliferation of *Rhizobium* in T6 is attributed to the synergistic microenvironment created by the Farm Yard Manure (FYM). FYM acts as a vital carbon substrate and a protective organic matrix, shielding the inoculated *R. selenitireducens* from desiccation and nutrient starvation, thereby enhancing its survival and multiplication in the rhizosphere. Recent microbiological assessments by Sasmal *et al.,* (2024) confirm that organic amendments buffer soil pH and provide the necessary microbial exudates to sustain high populations of bio-inoculants. In contrast, purely synthetic regimes actively degrade the native symbiotic flora.

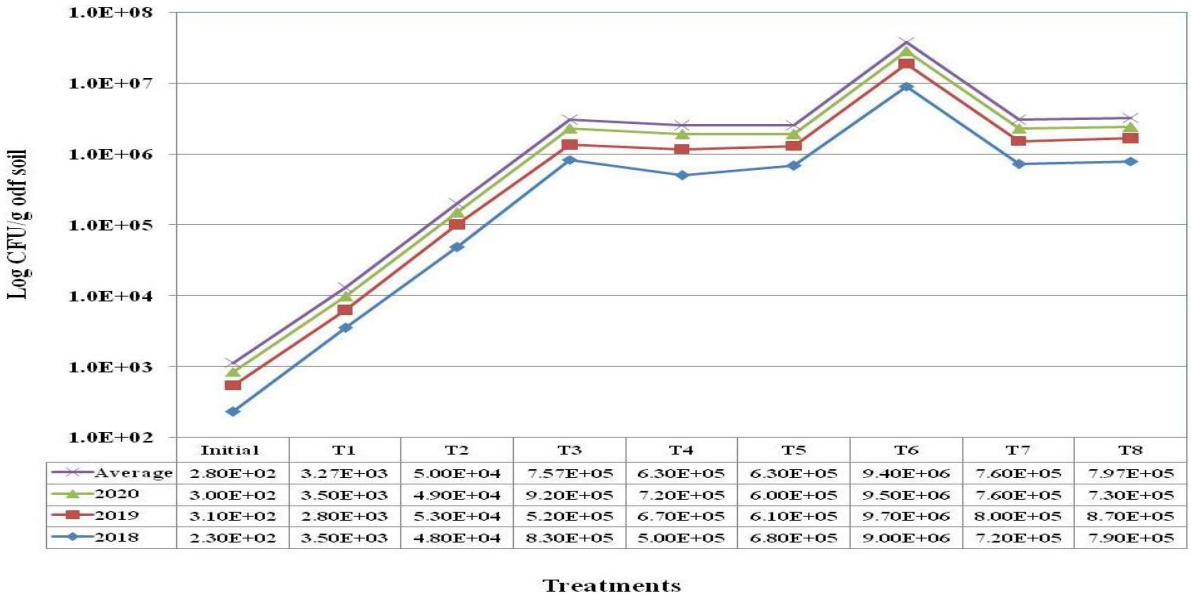
Phosphate Solubilizing Bacteria (PSB) Population

Phosphorus is notoriously immobile in soils, making the presence of native Phosphate Solubilizing Bacteria (PSB) crucial for converting insoluble phosphates into plant-available forms. The upper graph 2 outlines the dynamic response of PSB to the various treatments. Starting from an initial baseline of 7.57E+04 CFU/g of soil, the PSB population surged most significantly under integrated and organically amended treatments. Treatment T3 (2 t FYM/ha + 50% RDF (N) + *R. selenitireducens* AAU M1) recorded the highest three-year average PSB population at 8.67E+06

Table 2: Effect of Nutrient Management on Crop Yield and Economics

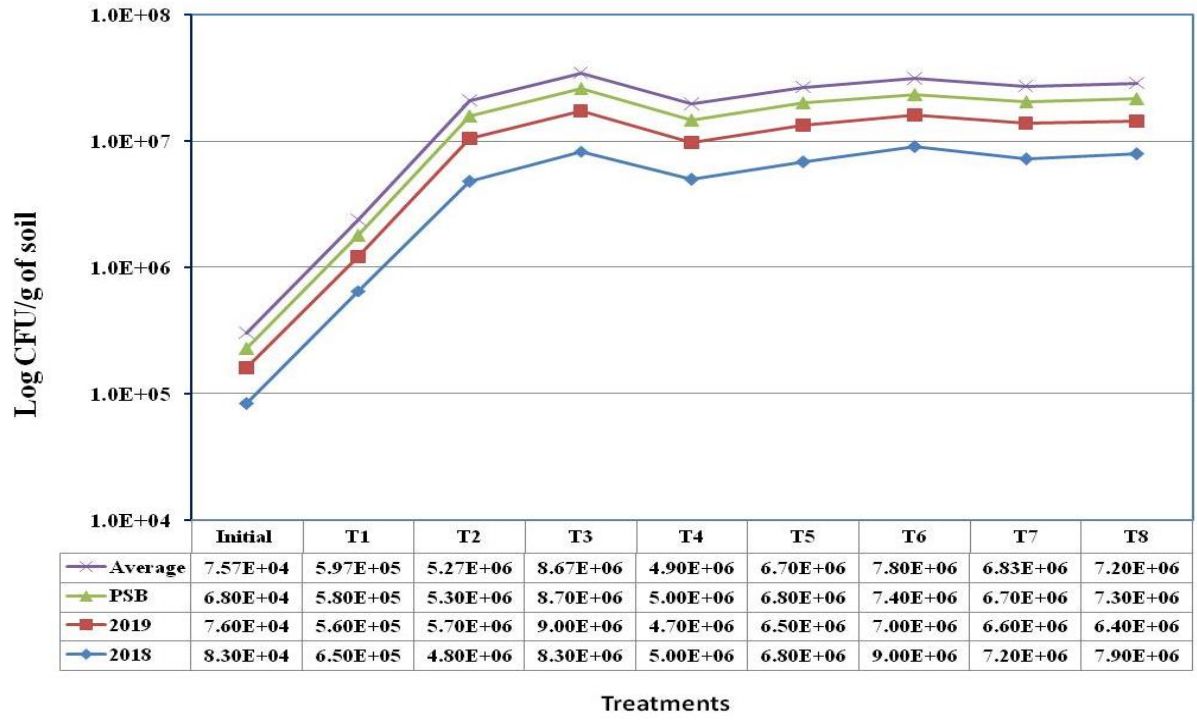
Treatment Details	Seed Yield (kg/ha)	Haulm Yield (kg/ha)	Gross Return (₹/ha)	Cost of Cultivation (₹/ha)	Net Return (₹/ha)	B: C Ratio
T1: 100% RDF (N)	1103 a	1460 a	78,670	15,758	62,912	4.99
T2: 4 t FYM/ha	1068 ab	1496 a	76,256	21,410	54,846	3.56
T3: 2 t FYM/ha + 50% RDF (N) + *R. selenitireducens* AAU M1	1042 ab	1460 a	74,400	18,593	55,807	4.00
T4: 2 t FYM/ha + 50% RDF (N) + *R. cellulosilyticum* AAU PP16	990 ab	1448 a	70,748	18,593	52,155	3.81
T5: 2 t FYM/ha + 50% RDF (N) + *R. pusense* AAU P16	976 b	1425 a	69,745	18,593	51,152	3.75
T6: 2 t FYM/ha + *R. selenitireducens* AAU M1	980 ab	1425 a	70,025	18,419	51,606	3.80
T7: 2 t FYM/ha + *R. cellulosilyticum* AAU PP16	779 c	1175 b	55,705	18,419	37,286	3.02
T8: 2 t FYM/ha + *R. pusense* AAU P16	728 c	1116 b	52,076	18,419	33,657	2.83
S. Em.± (T)	33.53	42.15	-	-	-	-
C.D. (P=0.05) (T)	Sig.	Sig.	-	-	-	-
C.V. %	11.58	10.42	-	-	-	-

*Note: N/A** indicates data not documented for Treatment 8 in the original nutrient analysis source text. Non-significant differences were observed for pooled haulm N and P content across the tested treatments.



	Initial	T1	T2	T3	T4	T5	T6	T7	T8
Average	2.80E+02	3.27E+03	5.00E+04	7.57E+05	6.30E+05	6.30E+05	9.40E+06	7.60E+05	7.97E+05
2020	3.00E+02	3.50E+03	4.90E+04	9.20E+05	7.20E+05	6.00E+05	9.50E+06	7.60E+05	7.30E+05
2019	3.10E+02	2.80E+03	5.30E+04	5.20E+05	6.70E+05	6.10E+05	9.70E+06	8.00E+05	8.70E+05
2018	2.30E+02	3.50E+03	4.80E+04	8.30E+05	5.00E+05	6.80E+05	9.00E+06	7.20E+05	7.90E+05

Graph 1: Effect on soil Rhizobium population



	Initial	T1	T2	T3	T4	T5	T6	T7	T8
Average	7.57E+04	5.97E+05	5.27E+06	8.67E+06	4.90E+06	6.70E+06	7.80E+06	6.83E+06	7.20E+06
PSB	6.80E+04	5.80E+05	5.30E+06	8.70E+06	5.00E+06	6.80E+06	7.40E+06	6.70E+06	7.30E+06
2019	7.60E+04	5.60E+05	5.70E+06	9.00E+06	4.70E+06	6.50E+06	7.00E+06	6.60E+06	6.40E+06
2018	8.30E+04	6.50E+05	4.80E+06	8.30E+06	5.00E+06	6.80E+06	9.00E+06	7.20E+06	7.90E+06

Graph 2: Effect on soil PSB population

CFU/g of soil. This was closely followed by the purely organic-biological treatment T6, which maintained a robust average of 7.80E+06 CFU/g of soil. Conversely, the lowest post-initial PSB average was found in T1 (100% RDF) at 5.97E+05 CFU/g of soil. The superior PSB counts in T3 and T6 indicate that the addition of FYM not only supports introduced *Rhizobium* but simultaneously acts as a bio-stimulant for native, beneficial soil bacteria like PSB. The organic acids produced during the decomposition of FYM serve as a primary energy source for PSB (Graph 2). Furthermore, Sartaj *et al.,* (2025) note that the co-existence of robust *Rhizobium* populations and PSB often results in a mutualistic rhizosphere effect; *Rhizobium* fixes nitrogen that PSB utilizes for growth, while PSB mineralizes phosphorus essential for *Rhizobium* nodulation and energy transfer (ATP synthesis). The

suppression seen in T1 further cements the argument that relying entirely on synthetic fertilizers deteriorates soil biological health, ultimately threatening long-term crop productivity.

CONCLUSION

The continuous and exclusive reliance on synthetic chemical fertilizers poses a significant threat to soil ecological balance and long-term agricultural sustainability. This three-year field investigation provides compelling evidence that Integrated Nutrient Management (INM) and organic-biological approaches can successfully substitute high-dose chemical inputs in green gram cultivation without compromising crop productivity or economic viability. Based on the multi-year pooled analysis, the application of 100% Recommended Dose of Fertilizer (RDF) generated the highest immediate seed yield and financial return. However, it concurrently suppressed vital symbiotic root nodulation and drastically degraded native soil microbial populations, specifically *Rhizobium* and Phosphate Solubilizing Bacteria (PSB). In contrast, the integrated application of 2 t FYM/ha + 50% RDF (N) + *Rhizobium selenitireducens* AAU M1 (T3) and the purely organic-biological treatment of 2 t FYM/ha + *R. selenitireducens* AAU M1 (T6) emerged as highly sustainable alternatives. These treatments sustained vegetative growth, optimized assimilate partitioning (test weight), and produced seed and haulm yields that were statistically at par with the 100% synthetic treatment. More importantly, the inclusion of FYM alongside the highly efficient *R. selenitireducens* AAU M1 strain significantly enhanced root nodulation and fostered a vibrant rhizosphere, multiplying the beneficial *Rhizobium* and PSB populations by up to 1,000-fold compared to the chemical regimen. Economically, both T3 and T6 proved to be highly competitive, delivering robust net returns and Benefit-Cost Ratios (4.00 and 3.80, respectively) that justify their adoption by the farming community.

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Ethics Statement:

As this study exclusively involved agronomic field experiments on green gram (*Vigna radiata* L.) plants and soil microorganisms, no formal ethical approval was required.

Originality and Plagiarism:

The authors confirm that this manuscript is entirely original, has not been previously published or submitted elsewhere, and properly cites all external sources to ensure the absolute absence of plagiarism.

Consent for Publication:

All contributing authors have thoroughly reviewed the final content of this manuscript and unanimously agree to its submission and subsequent publication in this journal.

Competing Interests:

The authors explicitly declare that they have no known competing financial interests or personal relationships that could have appeared to influence the research or interpretations presented in this paper.

Data Availability:

All data supporting the findings and conclusions of this study are included directly within this published article.

Author Contributions:

Dr. Y. K. Jhala and Mrs. H. N. Shelat conceptualized the study, performed the microbiological evaluations and conducted the field experiment, and drafted the manuscript.

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