



ISSN Print: 2618-060X
 ISSN Online: 2618-0618
 NAAS Rating (2026): 5.20
 IJRA 2026; 9(9): 1231-1234
www.agronomyjournals.com
 Received: 29-06-2026
 Accepted: 27-08-2026
 Published: 28-09-2026

B P Lingaraj

PG Student, Department of Microbiology, College of Agriculture (CoA), University of Agricultural Sciences (UAS), Raichur, Karnataka, India.

Pampangouda

Assistant Professor, Department of Microbiology, College of Agriculture (CoA), B. Gudi, University of Agricultural Sciences (UAS), Raichur, Karnataka, India.

Ningappa

Farm Superintendent, Agricultural Research Station (ARS), Bidar, Karnataka, India.

M Yallappa

Assistant Professor, Department of Microbiology, College of Agriculture (CoA), B. Gudi, University of Agricultural Sciences (UAS), Raichur, Karnataka, India.

C Anupama

Associate Professor, Department of Microbiology, University of Agricultural Sciences (UAS), Raichur, Karnataka, India.

Corresponding Author:

B P Lingaraj

PG Student, Department of Microbiology, College of Agriculture (CoA), University of Agricultural Sciences (UAS), Raichur, Karnataka, India.

Evaluation of efficient *Rhizobium* spp. and *Pseudomonas* spp. on growth and biomass of green gram under pot culture conditions

B P Lingaraj¹, Pampangouda², Ningappa³, M Yallappa⁴, C Anupama⁵

DOI: <https://www.doi.org/10.33545/2618060X.2026.v9.i9q.6675>

ABSTRACT

Aims: The present study was conducted to evaluate the effect of efficient *Rhizobium* spp. and *Pseudomonas* spp. isolates on the growth and biomass of green gram under pot culture conditions.

Study design: The experiment was conducted using different combinations of recommended dose of fertilizer (RDF), reference strains and efficient microbial isolates.

Place and Duration of Study: The pot culture experiment was conducted at the College of Agriculture, University of Agricultural Sciences, Raichur.

Methodology: Green gram variety BGS-9 was used for the experiment. The treatments included 100% RDF, 75% RDF and 75% RDF supplemented with reference strains or efficient isolates of *Rhizobium* spp. and *Pseudomonas* spp. Based on earlier in vitro screening, GRL-11 was selected as the efficient *Rhizobium* isolate and GPL-7 as the efficient *Pseudomonas* isolate.

Results: Among the treatments, 75% RDF + GRL-11 + GPL-7 (T7) recorded the highest plant height at harvest (51.86 cm), compared with 39.28 cm under 75% RDF alone (T2). T7 also recorded the highest root length (18.64 cm), number of branches (11.20 plant⁻¹) and total dry weight (12.17 g plant⁻¹) at harvest. The highest number of nodules (32.74 plant⁻¹), nodule dry weight (42.37 mg plant⁻¹), pods per plant (17.87 plant⁻¹), seeds per pod (10.62) and chlorophyll content (46.74 SPAD) were also recorded in T7. The total microbial population was highest in T7 at 30 DAS (32.34×10^7 CFU g⁻¹ soil), 60 DAS (43.76×10^7 CFU g⁻¹ soil) and harvest (31.48×10^7 CFU g⁻¹ soil).

Conclusion: The combined application of efficient GRL-11 (*Rhizobium* spp.) and GPL-7 (*Pseudomonas* spp.) along with 75% RDF enhanced the growth and biomass of green gram under pot culture conditions.

Keywords: *Rhizobium*, *Pseudomonas*, Pot culture, Biofertilizer

1. Introduction

Green gram (*Vigna radiata* L.) is an important short-duration pulse crop widely cultivated for its nutritional value and adaptability to diverse agro-climatic conditions. Its relatively short growth duration makes it suitable for intensive and multiple cropping systems. However, maintaining crop productivity with reduced dependence on chemical fertilizers is an important aspect of sustainable crop production. Beneficial micro-organisms can contribute to improved nutrient availability and plant growth and may support efficient fertilizer use.

Rhizobium forms symbiotic associations with leguminous plants and plays an important role in biological nitrogen fixation. They colonize compatible roots and induce the formation of root nodules, where atmospheric nitrogen is converted into a form that can be utilized by the plant. Efficient rhizobial inoculation can consequently improve nitrogen nutrition, nodulation and plant biomass. The effectiveness of inoculation may vary among bacterial strains, making the selection of efficient isolates important for crop production.

Pseudomonas are important rhizosphere-associated plant growth-promoting bacteria that can contribute to plant development through several mechanisms, including phosphate solubilization, production of growth-promoting substances and improvement of nutrient availability. Their application in combination with *Rhizobium* may provide complementary benefits by enhancing nutrient uptake, root development and overall plant growth.

Although efficient microbial isolates may exhibit promising plant growth-promoting characteristics *in vitro*, their effectiveness must be evaluated under actual plant-growing conditions. Therefore, the present study was undertaken to evaluate the potential of efficient *Rhizobium* spp. and *Pseudomonas* spp. isolates, individually and in combination with 75% RDF, for enhancing growth and biomass of green gram under pot culture conditions.

2. Material and Methods

2.1 Pot culture experiment

A pot culture experiment was conducted at the College of Agriculture, UAS, Raichur, to evaluate the effect of efficient *Rhizobium* and *Pseudomonas* isolates on the growth and biomass of green gram. The BGS-9 variety of green gram was used for the experiment. Pots containing 5 kg of normal black soil were used. Seeds were treated with respective microbial inoculants before sowing. The experiment was laid out in a Completely Randomized Design (CRD).

2.2 Selection of efficient isolates

Based on the earlier in vitro screening, GRL-11 was selected as the efficient *Rhizobium* isolate and GPL-7 as the *Pseudomonas* isolate for evaluation under pot culture conditions.

2.3 Recording of observations

Plant height, root length, number of branches, total dry weight, number of pods per plant and number of seeds per pod were recorded at harvest. Number of nodules and nodule dry weight were recorded at 45 DAS. Chlorophyll content was recorded as SPAD values at 30 DAS, 60 DAS and at harvest. The total microbial population in the rhizosphere soil was recorded at 30 DAS, 60 DAS and at harvest and expressed as $\times 10^7$ CFU g^{-1} soil.

2.4 Treatments

Treatment	Treatment details
T1	100% RDF
T2	75% RDF
T3	75% RDF + reference strain (<i>Rhizobium</i> spp.)
T4	75% RDF + reference strain (<i>Pseudomonas</i> spp.)
T5	75% RDF + GRL-11(<i>Rhizobium</i> spp.)
T6	75% RDF + GPL-7 (<i>Pseudomonas</i> spp.)
T7	75% RDF + GRL-11 + GPL-7

2.5 Statistical analysis

The experimental data were statistically analyzed and treatment differences were assessed using S.E.m. $\pm$ and CD at 1% level as presented in the respective tables.

3. Results and Discussion

3.1 Plant height and root length

Significant differences in plant height were observed among the treatments at harvest (Table 1). Treatment T7 (75% RDF + GRL-11 + GPL-7) recorded the highest plant height (51.86 cm), whereas the lowest value (39.28 cm) was recorded in T2 (75% RDF). Root length also differed significantly among treatments (Table 1). T7 recorded the maximum root length (18.64 cm) at harvest, while T2 recorded the minimum value (13.56 cm).

The greater plant height under combined inoculation may be attributed to the complementary activities of *Rhizobium* and *Pseudomonas*, which can improve nitrogen availability, nutrient acquisition and production of growth-promoting substances. The present findings are supported by Ahmad *et al.* [1], who reported that co-inoculation of *Rhizobium* and *Pseudomonas* increased plant height of mung bean by up to 21 % compared with the uninoculated control. Enhanced root development may be associated with improved nutrient availability and growth-promoting activities of the inoculated microorganisms, thereby enhancing water and nutrient uptake and shoot growth. Similar enhancement in root length following *Rhizobium*–*Pseudomonas* co-inoculation was reported by Verma *et al.* [2].

3.2 Number of branches and total dry weight

The number of branches per plant varied significantly among treatments (Table 1). T7 recorded the highest number of branches at harvest (11.20), whereas T2 recorded the lowest (8.37). A significant variation in total dry weight was also observed among the treatments (Table 1). T7 recorded the highest dry weight (12.17 g plant⁻¹) at harvest, while T2 recorded the lowest (8.74 g plant⁻¹).

The increased branching under combined inoculation may be related to improved nitrogen nutrition and the plant growth-promoting activities of the inoculated bacteria. Comparable enhancement in the number of branches following *Rhizobium*–*Pseudomonas* co-inoculation was reported by Netwal *et al.* [3]. The higher biomass accumulation under combined inoculation may be associated with improved nodulation, nitrogen fixation, nutrient availability and root development. Ahmad *et al.* [1] also reported increased total dry matter of mung bean following co-inoculation.

3.3 Nodulation and nodule dry weight

Significant differences in nodulation were observed among the treatments (Table 2). At 45 DAS, T7 recorded the highest number of nodules (32.74 plant⁻¹), whereas T1 (100% RDF) recorded the lowest (16.14 plant⁻¹). Nodule dry weight followed a similar trend. T7 recorded the highest nodule dry weight (42.37 mg plant⁻¹), whereas the lowest value was recorded in T1 (24.76 mg plant⁻¹).

The higher nodulation in T7 may be attributed primarily to the efficient *Rhizobium* isolate GRL-11, which established an effective symbiotic association with green gram roots. The additional contribution of *Pseudomonas* may have supported rhizobial establishment and root development through its plant growth-promoting activities. Similar increase in nodulation through *Rhizobium*–*Pseudomonas* co-inoculation has been reported by Ahmad *et al.* [1]. The increase in nodule dry weight can be attributed to *Rhizobium* inoculation, which establishes a symbiotic association with green gram roots. These findings are similar to the reports of Selvakumar *et al.* [4] and (Khan and Prakash) [5].

3.4 Number of pods and seeds per pod

The number of pods per plant differed among treatments (Table 2). T7 recorded the highest number of pods (17.87 plant⁻¹), while T2 recorded the lowest (10.33 plant⁻¹). The number of seeds per pod also varied among treatments. T7 recorded the highest value (10.62 seeds pod⁻¹), whereas T2 recorded the lowest (7.33 seeds pod⁻¹).

The increase in pod formation under combined inoculation may be associated with improved vegetative growth, nodulation, nitrogen fixation and nutrient availability. Yadegari *et al.* [6] reported a similar improvement in the number of pods per plant following *Rhizobium* + *Pseudomonas* co-inoculation in common bean. Improved nitrogen and phosphorus availability through microbial activity may have supported flower retention, pod development and seed setting. Similar yield components following combined inoculation with *Rhizobium* and *Pseudomonas* have been reported by Ahmad *et al.* [1].

3.5 Chlorophyll content and rhizosphere microbial population

Significant differences in chlorophyll content were observed among treatments (Table 3). T7 recorded the highest chlorophyll content at 30 DAS (44.72 SPAD), 60 DAS (50.16 SPAD) and harvest (46.74 SPAD), whereas T2 recorded the lowest values of 34.60, 38.12 and 36.41 SPAD, respectively.

Significant differences in the rhizosphere microbial population were also observed among treatments (Table 3). T7 recorded the highest microbial population at 30 DAS (32.34×10^7 CFU g^{-1} soil), 60 DAS (43.76×10^7 CFU g^{-1} soil) and harvest (31.48×10^7 CFU g^{-1} soil). T2 recorded the lowest populations of 13.83, 19.35 and 12.68×10^7 CFU g^{-1} soil, respectively.

The higher chlorophyll content under combined inoculation may be associated with improved nitrogen availability through biological nitrogen fixation and enhanced nutrient acquisition. Nitrogen is an important component of chlorophyll and therefore improved nitrogen

nutrition may contribute to greater chlorophyll formation and photosynthetic activity. Ahmad *et al.* [1] reported that co-inoculation of *Rhizobium phaseoli* and *Pseudomonas* strains significantly improved chlorophyll content, photosynthetic activity and physiological performance of mung bean under salt-affected conditions.

The higher microbial population under T7 indicates better establishment and proliferation of the inoculated microorganisms in the green gram rhizosphere. Increased root growth and availability of root-derived organic compounds may have supported microbial colonization and multiplication. Shravani *et al.* [7] also reported higher bacterial populations in the rhizosphere of mungbean following biofertilizer application.

4. Conclusion

The pot culture experiment demonstrated that inoculation with efficient *Rhizobium* spp. and *Pseudomonas* spp. isolates positively influenced the growth and performance of green gram. Among the treat-

ments, T7 (75% RDF + GRL-11 + GPL-7) consistently recorded superior values for plant height, root length, number of branches, total dry weight, nodulation, nodule dry weight, number of pods, seeds per pod, chlorophyll content and rhizosphere microbial population. The combined application of GRL-11 and GPL-7 with 75% RDF therefore showed better performance than 75% RDF alone and individual microbial inoculation treatments. These findings indicate that the efficient *Rhizobium* spp. and *Pseudomonas* spp. isolates, particularly their combined application, have potential to improve green gram growth and productivity under pot culture conditions.

5. Acknowledgements

The authors acknowledge the College of Agriculture, University of Agricultural Sciences, Raichur and the co-workers involved in the study.

Table 1. Effect of *Rhizobium* spp. and *Pseudomonas* spp. isolates on plant height, root length, Number of branches and total dry weight at harvest of green gram.

Sl. No.	Treatments	Plant height (cm)	Root length (cm)	Number of branches	Dry weight (g/plant)
		At harvest	At harvest	At harvest	At harvest
1	T1- RDF 100%	49.41	16.82	9.54	10.46
2	T2- RDF 75%	39.28	13.56	8.37	8.74
3	T3- RDF 75% + Reference strain (<i>Rhizobium</i> spp.)	47.92	16.34	9.92	11.08
4	T4- RDF 75% + Reference strain (<i>Pseudomonas</i> spp.)	45.65	15.27	9.52	10.34
5	T5- RDF 75% + GRL-11 (<i>Rhizobium</i> spp.)	48.18	17.14	10.14	11.38
6	T6- RDF 75% + GPL-7 (<i>Pseudomonas</i> spp.)	46.23	15.88	9.83	10.89
7	T7- RDF 75% + GRL-11 + GPL-7	51.86	18.64	11.2	12.17
	S. Em ±	1.002	0.688	0.312	0.248
	CD @1%	3.529	2.473	1.148	0.843

DAS: Days after sowing

RDF: Recommended dose of fertilizer

Table 2. Effect of efficient *Rhizobium* spp. and *Pseudomonas* spp. isolates on number of nodules, nodule dry weight, number of pods and number of seeds per pod of green gram

Sl. No.	Treatments	No. of Nodules/plant (45 DAS)	Nodule dry weight (mg/plant)	No. of pods / plant	Number of seeds/pod
1	T1- RDF 100%	16.14	24.76	16.48	9.71
2	T2- RDF 75%	16.32	25.14	10.33	7.33
3	T3- RDF 75% + Reference strain (<i>Rhizobium</i> spp.)	25.66	32.87	14.02	8.64
4	T4- RDF 75% + Reference strain (<i>Pseudomonas</i> spp.)	21.23	30.68	12.66	8.02
5	T5- RDF 75% + GRL-11 (<i>Rhizobium</i> spp.)	26.45	34.50	15.16	9.33
6	T6- RDF 75% + GPL-7 (<i>Pseudomonas</i> spp.)	22.33	31.64	13.82	8.32
7	T7- RDF 75% + GRL-11 + GPL-7	32.74	42.37	17.87	10.62
	S. Em ±	0.642	0.844	0.348	0.215
	CD @1%	2.274	2.752	1.226	0.842

DAS: Days after sowing

RDF: Recommended dose of fertilizer

(continued on next page)

Table 3. Effect of efficient *Rhizobium* spp. and *Pseudomonas* spp. isolates on chlorophyll content and microbial count of rhizosphere at different intervals of green gram

Sl. No.	Treatments	Chlorophyll content (SPAD values)			Microbial count (x10 ⁷ CFU/g soil)		
		30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest
1	T1- RDF 100%	39.41	44.64	40.38	17.15	26.81	16.74
2	T2- RDF 75%	34.60	38.12	36.41	13.83	19.35	12.68
3	T3- RDF 75% + Reference strain (<i>Rhizobium</i> spp.)	41.02	45.84	41.87	18.94	29.47	17.39
4	T4- RDF 75% + Reference strain (<i>Pseudomonas</i> spp.)	39.02	43.16	40.14	18.52	28.86	16.88
5	T5- RDF 75% + GRL -11 (<i>Rhizobium</i> spp.)	42.64	47.02	43.31	21.47	34.40	19.63
6	T6- RDF 75% + GPL-7 (<i>Pseudomonas</i> spp.)	40.86	45.14	40.67	20.11	31.52	18.96
7	T7- RDF 75% + GRL -11 + GPL-7	44.72	50.16	46.74	32.34	43.76	31.48
	S. Em ±	0.553	0.698	0.486	0.542	0.613	0.571
	CD @1%	1.739	2.382	1.953	1.626	1.854	1.713

DAS: Days after sowing

RDF: Recommended dose of fertilizer

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How to cite this article: Lingaraj BP, Pampangouda, Ningappa, Yallappa M, Anupama C. Evaluation of efficient *Rhizobium* spp. and *Pseudomonas* spp. on growth and biomass of green gram under pot culture conditions. *International Journal of Research in Agronomy.* 2026;9(9):1231-1234. doi: <https://www.doi.org/10.33545/2618060X.2026.v9.i9q.6675>