

Original Research Article

<https://doi.org/10.20546/ijcmas.2025.1402.016>

Effect of Liquid Formulation of Rhizobium and Phosphate Solubilizing Bacteria on Growth and Yield of Black Gram (*Vigna mungo*)

V. S. Gujar^{1*}, M. A. Gud², A. M. Navale³, A. G. Durgude⁴,
K. C. Gagare⁵ and S. H. Sankpal⁶

¹Department of Plant Pathology and Microbiology, Post Graduate Institute, MPKV, Rahuri, Ahilyanagar, India

²Department of Plant Pathology and Microbiology, PAH College of Agriculture, Halgaon, Mahatma Phule Krishi Vidyapeeth, Rahuri., Ahilyanagar, India

³Department of Plant Pathology and Microbiology, Post Graduate Institute, MPKV, Rahuri, Ahilyanagar, India

⁴Senior Soil Scientist, AICRP on IWM, MPKV Rahuri, Ahilyanagar, India

⁵Assistant Seed Production Officer, Seed Cell Unit, Department of Seed Technology, MPKV Rahuri, Ahilyanagar, India

⁶Department of Plant Pathology and Microbiology, Post Graduate Institute, MPKV, Rahuri, Ahilyanagar, India

*Corresponding author

ABSTRACT

The current investigation entitled “Effect of Liquid Formulation of *Rhizobium* and Phosphate Solubilizing Bacteria on Growth and Yield of Black Gram (*Vigna mungo*.)” was carried out as a field experiment at research farm, Plant Pathology and Microbiology, PGI, MPKV, Rahuri. The soil sample were collected from root rhizospheric soil of black gram. This study involved the isolation of *Rhizobium* and PSB from root nodules and rhizospheric soil. The four isolates of *Rhizobium* i.e RH-I, RH-II, RH-III and RH-IV and three isolates for Phosphorous solubilizing bacteria i.e PS-I, PS-II and PS-III were isolated by serial dilution and pour plate method using YEMA and Pikovaskya’s medium respectively. These isolates undertook laboratory testing to determine their efficacy. The nitrogen fixing *Rhizobium* isolate RH-II fixed the highest amount of nitrogen (14.7 mg of Nitrogen/g of sucrose) than the other isolates tested. Moreover, the phosphate solubilizing bacterial isolate PS-II recorded the highest P-solubilization index (5.66) than the other isolates tested. One isolate of each was chosen as i.e RH-II and PS-II for further studies. Based on biochemical and physiological characterization nitrogen fixing rhizobial isolates were identified as *Rhizobium leguminosarum* and phosphate solubilizing bacterial isolate identified as *Pseudomonas* spp. This isolate were used for further field experimental studies. The field study was undertaken with nine treatments and three replications with randomized block design. Among the different inoculation treatments, seed inoculation with efficient isolated strain of *Rhizobium* and PSB from black gram with 75 % RDF was found to be the most effective as it recorded the highest germination (95.60%), leaf area index(), plant height(), number of root nodules, number of pods dry matter production, nutrient uptake and seed yield of black gram. The treatment reference strain of MPKV liquid formulation *Rhizobium* and PSB with 75% RDF and another treatment 100% RDF application these two treatments are at par with highest treatment. The results from the present investigation it can be concluded that seed inoculation with liquid formulation of *Rhizobium* and PSB with 75% RDF was found the most beneficial for getting higher seed yield of Black gram with 25% saving of nitrogen and phosphorus dose of chemical nutrients to black gram.

Keywords

Black gram,
Rhizobium, PSB,
RDF, Liquid
formulation

Article Info

Received:

18 December 2024

Accepted:

29 January 2025

Available Online:

10 February 2025

Introduction

The Black gram (*Vigna mungo*) belongs to group of the legume family (Fabaceae). Black gram is an erect to sub-erect, self-pollinated, deep rooted, much branched and somewhat hairy annual herb ranging from 45-75 cm. Plants are generally branched and habit can vary from erect to sub-erect. It plays an important role in vegetarian diets in South Asia due to its high nutritive value. Mature dry seeds of black gram possess approximately 24 %–26 % protein, 60 % carbohydrates, 1.3% fats, phosphorus (345 mg/100 g), potassium (993 mg/100g), iron (8.7 mg/100 g), and calcium (185 mg/100 g) along with several essential amino acids (arginine, phenylalanine, leucine, lysine, valine, and isoleucine, etc.), vitamins such as vitamin B3 (niacin; 2 mg/100 g), vitamin A (23 IU/100 g), vitamin B1 (thiamine; 0.42 mg/100 g), and vitamin B2 (riboflavin; 0.37 mg/100 g).

In recent years, biofertilizers have emerged as a promising component of integrating nutrient supply system in agriculture. Biofertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth-promoting microorganisms. Liquid biofertilizers are liquid formulations consisting desired microorganism and their nutrients but also special cell protectants or substances that encourage formation of resting spores or cyst for longer shelf line and tolerance to adverse condition. Unlike the lignite based biofertilizers, liquid biofertilizers have a longer shelf life. Biofertilizers improve nutrient supply, environment friendly, non-bulky and most importantly cost effective. Therefore, there is strong need to have complementary use of available source to plant nutrient including biofertilizer along with mineral fertilizers for maintenance of soil productivity.

The specific objectives of the study include: Isolation of *Rhizobium* and Phosphate solubilizing bacteria by serial dilution and pour plate method. Evaluation effect of liquid inoculation of *Rhizobium* and Phosphate solubilizing bacteria on growth and yield of Black gram.

Materials and Methods

A field experiment was conducted to study the effect of consortium of *Rhizobium* and PSB on growth, nutrient uptake and yield of Black gram during *kharif*, 2019. Field experiment was conducted at Research Farm, Plant Pathology and Microbiology, Post Graduate Institute, MPKV Rahuri. The field experiment was laid out in

Randomized Block Design with 3 replications and 9 treatments. Black gram seed were treated with liquid broth prepared by the efficient strain of RH-II and PS-II. Also, reference strain of *Rhizobium* and PSB of MPKV biofertilizers were used. The control treatment T9 and treatment T8 remained without any microbial treatment.

All types of agronomical practices for field preparation were carried out such as ploughing, harrowing etc. The flatbed layout was prepared for sowing of Black gram and FYM @ 5 tha^{-1} was applied. At harvesting Fully matured pods were harvested.

Results and Discussion

Effect of Liquid Formulation of *Rhizobium* and PSB Inoculation on Seed Germination

The recording of seed germination of black gram was done seven days after sowing showed in table 1. Germination of black gram seeds with three replication was recorded and the mean of three replica was calculated. Among different seed inoculation treatment T₄ i.e. seed inoculation with liquid formulation of *Rhizobium* and PSB@ 25ml per Kg of seed each with 75% RDF recorded highest seed germination (95.60%) over uninoculated treatment T₉. i.e control (83.20%).

This treatment followed by treatment T₇ i.e MPKV liquid formulation of *Rhizobium* and PSB@ 25ml per Kg of seed each+75%RDF which showed 91.55% seed germination. In general, it is observed that, inoculation with *Rhizobium* and PSB along with chemical fertilizers increased germination percentage as compared to chemical fertilizer alone in black gram.

Results of the present investigation are in agreement with results of [Ahmed et al., \(2016\)](#) and [Sahni et al., \(2018\)](#) observed increase in germination percentage of french bean and pea due to inoculation of *Rhizobium*.

Effect of Liquid Formulation of *Rhizobium* and PSB Inoculation on Leaf Area Index.

The leaf area index was recorded at 45 days after sowing of Black gram. The seed inoculation with *Rhizobium* and PSB showed significant influence on leaf area index showed in table 1. Leaf area index was ranged between 3.04 to 4.86. All the seed inoculated treatments showed significant increase in LAI as compared to T₉ i.e

absolute control (3.04). Highest arithmetic value was obtained for T4 i.e seed inoculation with *Rhizobium* and PSB @ 25 ml per Kg of seed each with 75% RDF (4.86), but it was statistically at par with treatment T7 i.e M.P.K.V. *Rhizobium* and PSB @ 25 ml/ kg of seed each with 75% RDF (4.71) and treatment T8 i.e 100% RDF (4.68).

Venkatrao *et al.*, (2017) carried out trial with levels of P and treatments of biofertilizers (PSB, *A. awamori*, PSB + *A. awamori*, control) on mung bean. The co-inoculation showed significant increase in leaf area index. The results for inoculation of PSB concur with present investigation.

Effect of Liquid Formulation of *Rhizobium* and PSB Inoculation on Plant Height

The plant height of plant was measured at flowering and at harvesting time. They showed in table 1. Plant height was ranged during flowering time between 39.00 to 46.75 cm.

The treatment T4 i.e. seed inoculation with *Rhizobium* and PSB @ 25ml per Kg of seed each with 75% R.D.F showed highest plant height (46.75 cm) which was at par with treatment T7 i.e. seed inoculation with MPKV *Rhizobium* and PSB @ 25ml per Kg of seed each with 75% R.D.F (43.52 cm) and treatment T8 i.e. 100% RDF (42.71 cm). The uninoculated treatment T9 i.e. control showed lowest plant height (39.00 cm). Results of the present investigation are in conformity with those of Bansal (2009); Qureshi *et al.*, (2011); Tarafder *et al.*, (2016) and Shete *et al.*, (2019) who reported increased plant height in different legume crops due to seed

inoculation of *Rhizobium*, PGPR and PSB alone or in combination.

Effect of Liquid Formulation of *Rhizobium* and PSB Inoculation on Dry Matter Weight of Plant

Dry matter weight was recorded treatment wise after harvest of the crop showed in table 1. The results as influenced by inoculation of *Rhizobium* and PSB for dry matter weight. All the treatments showed significantly higher dry matter weight in comparison with T9 i.e. absolute control (26.22 g).

The highest numerical value was recorded for treatment T4 i.e. seed inoculation with *Rhizobium* and PSB @ 25 ml per Kg of seed each with 75% R.D.F (34.50 g) which was at par with the treatment T7 i.e. seed inoculation with MPKV *Rhizobium* and PSB @ 25ml per Kg of seed each with 75% R.D.F (33.22 g) and treatment T8 i.e. 100% RDF (32.53 g).

Ravikumar (2012) inoculated *Rhizobium* to *Vigna mungo* and *Vigna radiata* under pot culture conditions. He concluded that *Rhizobium* inoculation had higher dry weight as compared with respective controls, which was in congruence with present study.

Effect of Liquid Formulation of *Rhizobium* and PSB Inoculation on Numbers of pods

The results in respect of number of pods per plant as influenced by seed inoculation with liquid *Rhizobium* and PSB showed in table 1.

Table.1 Treatment details

Tr. No.	Treatments
T1	Only <i>Rhizobium</i> @ 25ml/ Kg of seed
T2	Only PSB @ 25ml/ Kg of seed
T3	<i>Rhizobium</i> and PSB @ 25ml/ Kg of seed each
T4	<i>Rhizobium</i> and PSB @ 25ml/ Kg of seed each + 75% RDF
T5	MPKV liquid formulation of <i>Rhizobium</i> and PSB @ 25ml/ Kg of seed each
T6	MPKV liquid formulation of <i>Rhizobium</i> and PSB @ 25ml/ Kg of seed each+50% RDF
T7	MPKV liquid formulation of <i>Rhizobium</i> and PSB @ 25ml/ Kg of seed each + 75% RDF
T8	100% RDF (Control)
T9	Absolute control

Table.2 Effect of Liquid Formulation of Rhizobium and PSB on Growth and Yield of Black gram Parameter

Sr. No.	Treatments	Seed germination percentage	Leaf area index	Plant height (cm)	Dry matter weight(plant per gram)	Number of pods	Yield (q ha ⁻¹)
1	T1	87.51	3.71	40.73	28.21	21.00	10.71
2	T2	87.12	3.35	40.34	27.72	20.00	10.32
3	T3	89.34	3.90	41.90	29.15	24.67	11.45
4	T4	95.60	4.86	46.75	34.50	32.67	13.56
5	T5	89.13	3.89	41.68	29.37	23.67	11.30
6	T6	90.22	4.08	42.42	30.72	26.33	11.91
7	T7	91.55	4.71	43.52	33.22	31.00	13.22
8	T8	85.73	4.68	42.71	32.53	30.67	12.53
9	T9	83.20	3.04	39.00	26.22	19.67	9.29
	S.E(m)±	2.62	0.12	1.35	0.90	0.79	0.36
	C.D.(0.05)	7.87	0.37	4.06	2.71	2.38	1.07

Among different inoculation treatments, T₄ i.e. seed inoculation with *Rhizobium* and PSB @ 25ml per Kg of seed each + 75% RDF was found to be the most effective as it recorded the highest number of pods (32.67 plant⁻¹) over rest of the treatments, however it was statistically at par with treatments T₇ i.e. MPKV liquid formulation of *Rhizobium* and PSB @ 25ml per Kg of seed each + 75% RDF(31.00 plant⁻¹) and T₈ i.e. 100% RDF(30.67 plant⁻¹).

The lowest number of pods was noticed in the treatment T₉ i.e. uninoculated control plot (19.67 plant⁻¹). Results of the present investigation are in conformity with those of Bansal (2009); Qureshi *et al.*, (2011); Argaw (2012); Tarafder *et al.*, (2016) and Shete *et al.*, (2019) who reported increased number of pods in different legume crops due to seed inoculation of *Rhizobium*, PGPR and PSB alone or in combination. Baronia *et al.*, (2000), observed increase in number of pods per plant by application of *Rhizobium* and PSB on french bean.

Effect of Liquid Formulation of *Rhizobium* and PSB Inoculation on Yield

The results in respect of seed yield of black gram as influenced by seed inoculation with seed inoculation with Liquid *Rhizobium* and PSB showed in table 1.

Among different inoculation treatments, T₄ i.e. seed inoculation with *Rhizobium* and PSB@25ml per Kg of seed each + 75% RDF was found to be the most

effective as it recorded significantly the highest seed yield (13.56 q ha⁻¹) over rest of the treatments, however it was statistically at par with treatments T₇ i.e. seed inoculation MPKV liquid formulation of *Rhizobium* and PSB@ 25ml per Kg of seedeach+75%RDF (13.22 q ha⁻¹) and T₈i.e 100% RDF (12.53 q ha-1) of Black gram.

The lowest seed yield (9.29 q ha⁻¹) was noticed in the T₉ i.e. uninoculated control plot. Bansal (2009) reported that pre-sowing inoculation of mungbean seeds with different inoculants (*Rhizobium*, PGPR and PSB) alone or in combination, significantly increased the seed yield over uninoculated control. The results from the present investigation it can be concluded that seed inoculation with liquid formulation of *Rhizobium* and PSB with 75% RDF was found the most beneficial for getting higher seed yield of Black gram with 25% saving of nitrogen and phosphorus dose of chemical nutrients to black gram.

Acknowledgement

We are very thankful to the Post Graduate Institute Mahatma Phule Krushi Vidyapeeth, Rahurifor providing all facilities and research grants to carry out this experimental work and authors cited in references for providing necessary literature material.

Author Contributions

V. S. Gujar: Investigation, formal analysis, writing—original draft. M. A. Gud: Validation, methodology,

writing—reviewing. A. M. Navale:—Formal analysis, writing—review and editing. A. G. Durgude: Investigation, writing—reviewing. K. C. Gagare: Resources, investigation writing—reviewing. S. H. Sankpal: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Gujar, V. S., M. A. Gud, A. M. Navale, A. G. Durgude, K. C. Gagare and Sankpal, S. H. 2025. “Effect of Liquid Formulation of Rhizobium and Phosphate Solubilizing Bacteria on Growth and Yield of Black Gram (*Vigna mungo*)”. *Int.J.Curr.Microbiol.App.Sci*. 14(02): 178-182. doi: <https://doi.org/10.20546/ijcmas.2025.1402.016>