Original Research Article

Effect of *Rhizobium* Inoculation Methods on Growth, Nodulation and Yield of Black Gram (*Vigna mungo* L.)

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A B S T R A C T

A field experiment was conducted to assess the effect of *Rhizobium* inoculation on nutrient uptake and productivity at Crop Research Centre of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.), during kharif season of 2019. The experiment was arranged in randomized block design with nine treatments in three replications. Treatment consisted of T1 (control), T2 (RDF), T3 (RDF + FYM), T4 (RDF + Vermicompost), T5 (RDF + *Rhizobium* seed inoculation), T6 (RDF + *Rhizobium* soil treatment), T7 (RDF + *Rhizobium* inoculation with Vermicompost), T8 (RDF + *Rhizobium* seed inoculation + *Rhizobium* soil inoculation), T9 (RDF + *Rhizobium* seed inoculation + *Rhizobium* inoculation with Vermicompost. From the data, it was observed that RDF + *Rhizobium* seed inoculation + *Rhizobium* inoculation with vermicompost treatment exhibited significant influence on the growth, nodulation, yield attributes and yields of black gram as compared to control during the year of experimentation with higher values for all the above mentioned parameters and also 90.82 and 41.68 % increase in grains and straw yield.

**Keywords**

Black gram, *Rhizobium*

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**Introduction**

After cereals and oilseeds, pulses occupy an important place in Indian agriculture. The total world acreage under pulses is about 85.40 Mha with production of 87.40 Mt at productivity 1023 kg ha\(^{-1}\) production with 34 and 26 % respectively with average productivity of 835 kg ha\(^{-1}\) (Agricultural Statistics Division, Directorate of Economics and Statistics, 2019). 70 % of the total world’s black gram and green gram production comes from India, of which black gram constitutes 1.65 Mt with the share of 12.4 % (Elzebroek and Wind, 2008). Pulses are excellent source of high quality protein, essential amino acids, fatty acids, fibers, minerals and vitamins. The protein level of black gram is quite high i.e about 24 % with nutritional value of 10.9 % moisture, 1.4 % fats, 60.3 % carbohydrates and 3.4 % ash (Shroti *et al.*, 2018). It improves soil health by enriching nitrogen status and also maintains sustainability of the cropping systems. Most
of its nitrogen requirement is fulfilled by symbiotic nitrogen fixation from air and substantial amount of residual nitrogen and organic matter are left behind for subsequent crops. These crops do not have much nutritional requirements and these requirements can be fulfilled using various organic manures and bio-fertilizers which prove to be as cheapest and environmentally sound practice of applying nutrition to soil and plant.

Vermicompost have been effective in promoting the development of beneficial organisms in the soil. It enhances the growth, yield and quality of crops (Meena 2013; Mujahid and Gupta, 2010) and also increases bio-fertilizers efficiency. FYM through its positive effects on soil physical, chemical and biological properties and balanced plant nutrition plays an important role in improving the fertility and productivity of soils (Kumar et al., 2011). Although, it cannot be considered as a major source of nutrients but can be regarded as a good complimentary and supplementary source with mineral or chemical fertilizers. Use of organic source of nutrient is majorly appreciated over any chemical fertilizer due to the fact that they are not involved in causing any type of pollution and they cut those higher costs of cultivation that are caused by using mineral fertilizers.

Black gram, being leguminous crop, use symbiotic nitrogen fixation which is performed with the help of bacterium called *Rhizobia* as an important source to fulfil its major part of nitrogen requirement (Pareek, 1978). This symbiotic nitrogen fixation proves to an important source of nitrogen, and around 200 to 300 kg nitrogen ha$^{-1}$ is often fixed by various legume crops and pasture species (Dudeja and Duhan, 2005). These microbes vary in number, effectiveness and nodulation. Although *Rhizobia* is great source of symbiotic nitrogen fixation but sometimes due to less number of *Rhizobia* and ineffective native *Rhizobia*, nodulation and nitrogen fixation does not occur properly. To ensure efficient population of effective *Rhizobia* in soil, these are introduced in soil from outside in the form of biofertilizers. Seed treatment as well as soil application is the way to apply biofertilizer. Another approach may be application of biofertilizer through organic sources.

**Materials and Methods**

The field experiment was conducted in kharif season of 2019 at Crop Research Centre, Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut (U.P.), India, to evaluate the “Effect of *Rhizobium* inoculation methods on root nodulation, nutrient uptake and productivity of Black gram (*Vigna mungo* L. Hepper)”. The experiment was arranged in randomized block design with nine treatments in three replications, each plot size being 3.0m x 4.0m. Treatment consisted of T$_1$ (control), T$_2$ (RDF), T$_3$ (RDF + FYM), T$_4$ (RDF + Vermicompost), T$_5$ (RDF + *Rhizobium* seed inoculation), T$_6$ (RDF + *Rhizobium* soil treatment), T$_7$ (RDF + *Rhizobium* inoculation with Vermicompost), T$_8$ (RDF + *Rhizobium* seed inoculation + *Rhizobium* soil inoculation), T$_9$ (RDF + *Rhizobium* seed inoculation + *Rhizobium* inoculation with Vermicompost). Soil collected from research farm was analysed by standard procedure for various initial physicochemical properties given in parentheses, viz. bulk density (1.38 g cm$^{-3}$), particle density (2.65 g cm$^{-3}$), pH (8.2), EC (0.28 dSm$^{-1}$), organic carbon (0.45%), porosity (46.52%), available N (150.0 kg ha$^{-1}$), available P (11.30 kg ha$^{-1}$) and available K (170.0 kg ha$^{-1}$). For preparing an experimental field 1 deep ploughing and 2 harrowing was done and pre sowing irrigation was given at least 10 days before sowing.
Recommended dose of NPK (20:60:00) was applied through urea, single superphosphate. Also FYM and Vermicompost were applied in the field @ 20-25 tonnes ha\(^{-1}\) and 5 tonnes ha\(^{-1}\) respectively. Urad-10B strain of *Rhizobium leguminosarum var. phaseoli* brought from IARI, New Delhi was used to treat seed, vermicompost and soil. For 10 kg seed 200g of *Rhizobium* culture was used to inoculate the seed. For this, 5% solution of jaggery in water was prepared.

The water was taken in amount which is enough to moisten the seed. In this solution one packet of *Rhizobium* culture was added and mixed well. The coating of seed with prepared slurry was done. Then the seeds were dried in shade and sown immediately. Similarly, manual mixing of *Rhizobium* with Vermicompost, FYM or for its incorporation in soil was done. The black gram variety Shekhar-2 was sown at the rate of 15 kg ha\(^{-1}\). Intercultural operations viz., weeding, irrigation and insecticide spray were done as and when required. The yield, nutrient content and uptake and physico-chemical properties were recorded at pertinent stages.

**Results and Discussion**

**Growth parameters**

In the present study *Rhizobium* inoculation methods exhibited a significant effect on various growth parameters viz. plant height, number of branches and dry matter accumulation plant\(^{-1}\) recorded at different time periods of crop growth (Table-1 and Fig. 1.1, 1.2, 1.3). At 30 and 60 DAS and at harvest stage the treatment T\(_9\) (RDF + *Rhizobium* seed inoculation + *Rhizobium* inoculation with Vermicompost) shows significantly higher plant height (23.6, 54.5, 57.8 cm) than the rest of the treatments. The shortest plant height was obtained in control plot at all the time period of crop growth (18.1, 43.3, 46.0 cm, respectively). Similar results were found for number of branches plant\(^{-1}\), number of trifoliate leaf plant\(^{-1}\) and dry matter accumulation plant\(^{-1}\) with higher values as 3.83, 4.92 and 5.96, 3.9, 9.6, 8.5, and 7.10, 14.06, 14.50 g plant\(^{-1}\) respectively at 30, 60 and harvest stage.

Significant increase in plant height was found due to *Rhizobium* inoculation, which may maintain favourable balance between the applied nutrients in the plant for its optimum growth while elongation and chlorophyll biosynthesis in turn, improve the branches plant\(^{-1}\). The result is supported by Singh and Pareek (2003), Sripriya *et al.*, (2005), Kumar and Elamathi (2007) Bhuiyan *et al.*, (2008) and Giri *et al.*, (2010).

**Nodulation**

The number of nodules plant\(^{-1}\) and their dry weight in black gram was significantly influenced by *Rhizobium* inoculation methods (Table-2 and Fig. 2). The highest number of nodules and their dry weight 41.0 plant\(^{-1}\) and 50.10 mg plant\(^{-1}\), respectively were recorded in T\(_9\) (RDF + *Rhizobium* seed inoculation + *Rhizobium* inoculation with Vermicompost) at 45 DAS. However, lowest number of nodules and their dry weight 24.3 plant\(^{-1}\) and 34.18 mg plant\(^{-1}\) respectively were recorded in control (T\(_1\)).

It might have resulted due to more competitive ability of microbes near roots which is the site for microbial infection. Well-developed root system provides more evidence for infection resulting in greater number of nodules.

These finding are found relevant to Hussain *et al.*, (2015), Dhakal *et al.*, (2016), Meena and Ram (2016), Kant *et al.*, (2016) and Mohammad *et al.*, (2017).
### Table 1: Effect of *Rhizobium* inoculation methods on growth parameters of black gram

| Treatments                                      | Plant height(cm) | No. of branch plant$^{-1}$ | No. of trifoliate leaf plant$^{-1}$ | Dry matter accumulation (g plant$^{-1}$) |
|------------------------------------------------|------------------|-----------------------------|-------------------------------------|------------------------------------------|
|                                                 | 30 DAS | 60 DAS | At harvest | 30 DAS | 60 DAS | At harvest | 30 DAS | 60 DAS | At harvest | 30 DAS | 60 DAS | At harvest |
| T1 – Control                                    | 18.1   | 43.3   | 46.0       | 2.14   | 3.25   | 4.41       | 1.9     | 6.3     | 5.2       | 5.54   | 8.78   | 9.50       |
| T2 – RDF                                        | 20.0   | 46.3   | 51.2       | 2.42   | 3.64   | 4.78       | 2.1     | 7.7     | 6.6       | 5.82   | 9.90   | 11.20      |
| T3 - RDF + FYM                                  | 20.1   | 47.5   | 51.9       | 2.66   | 3.79   | 5.19       | 2.3     | 7.9     | 6.8       | 5.96   | 10.20  | 12.55      |
| T4 - RDF + Vermicompost                         | 21.1   | 49.6   | 53.0       | 2.82   | 3.90   | 5.26       | 2.7     | 8.5     | 7.2       | 6.34   | 11.26  | 12.30      |
| T5 - RDF + *Rhizobium* seed inoculation         | 21.9   | 52.1   | 55.7       | 3.28   | 4.57   | 5.69       | 3.4     | 9.3     | 8.2       | 6.70   | 12.84  | 13.65      |
| T6 - RDF + *Rhizobium* soil treatment           | 21.4   | 49.8   | 53.9       | 2.98   | 4.21   | 5.45       | 2.8     | 8.7     | 7.4       | 6.51   | 11.68  | 12.90      |
| T7 - RDF + *Rhizobium* inoculation with Vermicompost | 21.6 | 51.0   | 54.7       | 3.10   | 4.36   | 5.57       | 3.2     | 9.1     | 7.9       | 6.53   | 12.15  | 13.50      |
| T8 - RDF + *Rhizobium* seed inoculation + *Rhizobium* soil inoculation | 22.5 | 53.3   | 56.3       | 3.58   | 4.78   | 5.82       | 3.6     | 9.4     | 8.3       | 6.88   | 13.58  | 13.68      |
| T9 - RDF + *Rhizobium* seed inoculation + *Rhizobium* inoculation with Vermicompost | 23.6 | 54.5   | 57.8       | 3.83   | 4.92   | 5.96       | 3.9     | 9.6     | 8.5       | 7.10   | 14.06  | 14.50      |

$SEm \pm$ $0.7$ | $1.5$ | $1.8$ | $0.14$ | $0.18$ | $0.26$ | $0.10$ | $0.30$ | $0.30$ | $0.17$ | $0.38$ | $0.49$

$CD (P= 0.05)$ $1.9$ | $4.3$ | $5.2$ | $0.39$ | $0.52$ | $0.75$ | $0.30$ | $0.90$ | $0.88$ | $0.48$ | $1.10$ | $1.44$
**Table 2** Effect of *Rhizobium* inoculation methods on nodulation of black gram

| Treatment                                           | Number of root nodules plant⁻¹ | Dry weight of nodules plant⁻¹ (mg plant⁻¹) |
|-----------------------------------------------------|--------------------------------|-------------------------------------------|
|                                                     | 45 DAS                         | 60 DAS                                    |
|                                                     | 45 DAS                         | 60 DAS                                    |
| T₁ – Control                                        | 24.3                           | 22.2                                      | 34.18 | 28.22 |
| T₂ – RDF                                            | 30.8                           | 26.0                                      | 42.36 | 33.71 |
| T₃ - RDF + FYM                                      | 32.1                           | 29.7                                      | 43.38 | 35.16 |
| T₄ - RDF + Vermicompost                            | 34.0                           | 31.1                                      | 45.12 | 37.62 |
| T₅ - RDF + *Rhizobium* seed inoculation             | 38.6                           | 35.4                                      | 47.56 | 39.41 |
| T₆ - RDF + *Rhizobium* soil treatment              | 36.2                           | 32.0                                      | 45.41 | 37.72 |
| T₇ - RDF + *Rhizobium* inoculation with Vermicompost | 37.3                           | 33.3                                      | 46.34 | 38.28 |
| T₈ - RDF + *Rhizobium* seed inoculation + *Rhizobium* soil inoculation | 39.2                           | 36.2                                      | 48.58 | 40.38 |
| T₉ - RDF + *Rhizobium* seed inoculation + *Rhizobium* soil inoculation with Vermicompost | 41.0                           | 38.6                                      | 50.10 | 42.67 |
| SEm ±                                               | 0.48                           | 0.43                                      | 0.64  | 0.58  |
| CD (P= 0.05)                                        | 1.42                           | 1.30                                      | 1.89  | 1.72  |

**Table 3** Effect of *Rhizobium* inoculation methods on yield attributes of black gram

| Treatment                                           | Pod length (cm) | No. of pod plant⁻¹ | No. of grain pod⁻¹ | Test weight (g) |
|-----------------------------------------------------|-----------------|--------------------|--------------------|-----------------|
|                                                     | 4.0             | 18.30              | 4.09               | 29.25           |
| T₁ – Control                                        | 4.6             | 19.10              | 4.60               | 31.26           |
| T₂ – RDF                                            | 4.8             | 19.70              | 4.82               | 32.28           |
| T₃ - RDF + FYM                                      | 5.1             | 20.65              | 5.17               | 32.68           |
| T₄ - RDF + Vermicompost                            | 6.1             | 23.10              | 5.58               | 34.88           |
| T₅ - RDF + *Rhizobium* seed inoculation             | 5.5             | 21.72              | 5.32               | 33.21           |
| T₆ - RDF + *Rhizobium* soil treatment              | 5.8             | 22.14              | 5.41               | 33.72           |
| T₇ - RDF + *Rhizobium* inoculation with Vermicompost | 6.5             | 24.63              | 5.74               | 35.02           |
| T₈ - RDF + *Rhizobium* seed inoculation + *Rhizobium* soil inoculation | 6.9             | 25.68              | 5.95               | 35.42           |
| SEm ±                                               | 0.32            | 0.51               | 0.25               | 0.44            |
| CD (P= 0.05)                                        | 0.92            | 1.49               | 0.72               | NS              |
Table 4 Effect of *Rhizobium* inoculation methods on grain, straw, biological yield (q ha$^{-1}$) and harvest index (%) of black gram

| Treatment                                           | Grain Yield | Straw Yield | Biological Yield | Harvest Index (%) |
|-----------------------------------------------------|-------------|-------------|-------------------|-------------------|
| T1 – Control                                        | 6.21        | 21.29       | 29.17             | 21.29             |
| T2 – RDF                                            | 8.60        | 21.98       | 39.12             | 21.98             |
| T3 - RDF + FYM                                      | 9.75        | 23.73       | 41.08             | 23.73             |
| T4 - RDF + Vermicompost                             | 9.90        | 23.96       | 41.32             | 23.96             |
| T5 - RDF + *Rhizobium* seed inoculation              | 10.92       | 25.32       | 43.13             | 25.32             |
| T6 - RDF + *Rhizobium* soil treatment                | 10.05       | 23.93       | 42.00             | 23.93             |
| T7 - RDF + *Rhizobium* inoculation with Vermicompost | 10.81       | 25.17       | 42.94             | 25.17             |
| T8 - RDF + *Rhizobium* seed inoculation + *Rhizobium* soil inoculation | 11.43       | 26.07       | 43.84             | 26.07             |
| T9 - RDF + *Rhizobium* seed inoculation + *Rhizobium* inoculation with Vermicompost | 11.85       | 26.70       | 44.38             | 26.70             |

$SEm \pm$  

| $SEm \pm$ | 0.33 | 0.81 | 1.37 | 0.81 |

$CD (P= 0.05)$  

| $CD (P= 0.05)$ | 0.98 | 2.34 | 4.00 | 2.34 |
**Fig. 1.1** Effect of *Rhizobium* inoculation methods on plant height (cm) of black gram plant$^{-1}$ black gram at different growth periods

**Fig. 1.2** Effect of *Rhizobium* inoculation methods on number of branches plant$^{-1}$ at different growth periods

**Fig. 1.3** Effect of *Rhizobium* inoculation methods on number of trifoliate leaf plant$^{-1}$ (left) and dry matter accumulation (g plant$^{-1}$) (right) of black gram at different growth periods
**Fig. 2** Effect of *Rhizobium* inoculation methods on number of nodules plant\(^{-1}\) and dry weight of nodules plant\(^{-1}\) (mg plant\(^{-1}\)) at different growth periods of black gram

![Graph showing number of nodules and dry weight of nodules for different treatments]

**Fig. 3** Effect of *Rhizobium* inoculation methods on yield attributes of black gram

![Graph showing yield attributes for different treatments]

**Fig. 4** Effect of *Rhizobium* inoculation methods on grain, straw, biological yield (q ha\(^{-1}\)) and harvest index (%) of black gram

![Graph showing grain, straw, biological yield, and harvest index for different treatments]
Yield and Yield Attributes

Yield attributes

Yield attributes viz. pod length (cm), number of pods plant\(^{-1}\), number of grains pod\(^{-1}\) and test weight (Table-3 and Fig. 3) differs significantly under the treatment T\(_9\) (RDF + Rhizobium seed inoculation + Rhizobium inoculation with Vermicompost) and sole application of Rhizobium inoculation. The highest pod length (cm), number of pods plant\(^{-1}\), number of grains pod\(^{-1}\) and test weight (g) (6.9, 25.68, 5.95 and 35.42, respectively) were found in T\(_9\) (RDF + Rhizobium seed inoculation + Rhizobium inoculation with Vermicompost) while, lowest in control (T\(_1\)). In the process of tissue differentiation from somatic to reproductive, meristmatic activity and development of floral primordial might have increased with Rhizobium inoculation methods resulting in more flowers and pods and ultimately the higher grain yield. The result is supported by Singh and Pareek (2003), Sripriya et al., (2005), Kumar and Elamathi (2007) and Ghosh and Joseph (2008).

Yield

The maximum grain yield of 11.85 q ha\(^{-1}\) (Table-4 and Fig. 4), statistically at par with T\(_8\) (RDF + Rhizobium seed inoculation + Rhizobium soil treatment) and significantly higher than remaining treatments was found in T\(_9\) (RDF + Rhizobium seed inoculation + Rhizobium inoculation with Vermicompost), which were higher over control by 90.82 and 84.05% in T\(_8\) and T\(_9\), respectively. Similar result was found for harvest index with values highest in T\(_9\) i.e. 26.70 % and lowest in control i.e. 21.29 %. The highest straw and biological yield (32.53 and 44.38 q ha\(^{-1}\)) were found in T\(_9\) (RDF + Rhizobium seed inoculation + Rhizobium inoculation with Vermicompost) followed by 32.41 q ha\(^{-1}\) and 43.84 q ha\(^{-1}\) in T\(_8\) (RDF + Rhizobium seed inoculation + Rhizobium soil treatment) while, lowest 22.96 and 29.17 q ha\(^{-1}\) was found in control (T\(_1\)), respectively. The improvement in yield and yield attributing character might be attributed to the fact that combined and balanced application of Rhizobium inoculation increases photosynthetic activity of leaves, translocation of photosynthates from source to sink with consequent improvement, nutrients uptake and better metabolism and increase efficiency of other nutrients. The results of our study are in line with the findings of Dudeja and Duhan (2005), Sahu and Singh (2009), Kachhave et al., (2009) and Sardar et al., (2016).

From the above study it is concluded that application of Rhizobium treated seed with Rhizobium treated vermicompost and recommended fertilizers was found superior than rest of the treatments in terms of growth, nodulation and yield parameters of black gram. Its application resulted in higher values for all the different aspects studied above. Thus for obtaining higher productivity of black gram and sustainable soil health the Rhizobium treated seed should be sown with Rhizobium treated vermicompost and recommended fertilizers.

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