International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 5, Issue 2, Apr 2015, 183-192
TJPRC Pvt. Ltd.

EVALUATION OF PGPR AND ORGANIC MANURE APPLICATIONS ON SOIL FERTILITY, GROWTH, NODULATION AND YIELD OF BLACK GRAM (*Vigna mungo* L. HEPPER)

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ABSTRACT

The study was conducted in Dept. of Botany, Acharya Nagrjuna University, Guntur on effect of different bionoculants (plant growth promoting rhizobacteria viz., *Bacillus*, *Pseudomonas* and *Rhizobium*) and farm yard manure, on soil fertility, growth and yield of black gram. This study was conducted by raising the black gram plants in earthen pots filled with filled soil amended with bioinoculants and Farm Yard Manure, individually and also in combination as 9 different treatments (T1 to T9) along with control (T10) till the end of the crop. The treatment in which FYM and all the three bioinoculants added (T9), was proved to be the best than any other treatments (combinations) studied. In this treated soil maximum increase in N, P and K content of 137.10 kg h-1, 82.50 kg h-1 and 193.05 kg h-1 respectively, was observed after harvesting, which was almost double than it was recorded before the sowings. Similarly this treatment (FYM + all the three bioinoculants, T9) was also proved to be effective for growth and yield of black gram. In this treatment maximum plant height recorded was 71.025 cm which is nearly 50% increase over the control plants. Similarly, number of nodules per plant was recorded with 3 fold increase than that of control plants with 72.25 per plant. Maximum legheamoglobin content in the nodule recorded was 0.305mg/g, which is 400% increase over the control. The organic manure and bioinoculants plays a significant role in improving soil fertility and plus productivity.

KEYWORDS: PGPR and Organic Manure Applications, Soil Fertility, Growth, Nodulation and Yield of Black, (T1 to T9), (Plant Growth Promoting rhizobacteria viz., *Bacillus*, *Pseudomonas* and *Rhizobium*)

INTRODUCTION

Legume *Rhizobium* interactions are unique because they supply 80-90% total nitrogen requirement of legumes. It involves a complex interaction among host, microbial symbiont and environment. Among nitrogen fixing systems, legume *Rhizobium* symbiosis is one of the most promising and the bacterial species of *Rhizobium* complex are very important (Sprent, 2001).

In the recent years *Rhizobium* complex received much attention as bio fertilizer, because of their ability to form nodules on root system and occasionally on stem of many leguminous plants. Nodules are the sites, where the *Rhizobium* reduces (fix) molecular nitrogen (N_2) in to ammonia (NH_3), which the host plant can utilize as a nutrient. The resulting ability of the plant to grow out with large amounts of soil nitrogen makes this an important process agriculturally and this probably contributed to the ecological and evolutionary success of the family leguminoceae.

In addition to nitrogen fixation, the symbiotic nodule bacteria are also involved in various biochemical activities in free living state, which can directly or indirectly promote plant growth (Tilak *et al.,* 2005). The activities like solubilization of insoluble phosphates, production of plant growth regulators (hormones) and siderophores, production of antagonistic substances etc, play an important role in plant growth.

Use of chemical fertilizers contributes largely to the deterioration of the environment through depletion of fossil fuels, generation of carbon dioxide and contamination of water resources. Imbalanced use of fertilizers leads to loss of soil fertility that has adversely impacted agricultural productivity and caused soil degradation. Organic manures act not only as a source of nutrients and organic matter, but also increase size, biodiversity and activity of the microbial population in soil (Albiach et al., 2000). However, main drawback in organic manures is the slow release of nutrients at initial stages which may cause significant reduction in crop yield.

Use of organic manures alone or in combination of chemical fertilizers will help to improve physic-chemical properties of the soils, Organic manures provide a good substrate for the growth of microorganisms and maintain a favourable nutritional balance and soil physical properties.

Black gram is one of the important leguminous crop, with unique characteristics of maintaining and restoring soil fertility through biological nitrogen fixation. However, many agro-ecological, biological and management related constraints are responsible for the lower productivity of black gram. Availability of a proper biofertilizer is the need of the day to achieve high yields at low cost. The present study was aimed at identifying a suitable bioinoculant or soil amendment that promotes maximum growth and high yields in black gram with sustainable increase in soil fertility.

MATERIALS AND METHODS

Clay loamy soil in the botanic garden of the botany department, ANU was used for the present investigation. The certified seeds of black gram (*Vigna mungo*) were purchased from the National seeds corporation, Guntur.

The bacterial cultures of *Bacillus, Pseudomonas* were maintained in nutrient agar and *Rhizobium* in Yeast extract mannitol agar slants. The cultures maintained in the University for research, were used in this study.

The earthen pots filled with soil in the botanical garden were used for sowings. A total of 10 treatments with three replicates were designed in complete randomized block design for this study. In all the treatments except control, soil was mixed with farm yard manure (5g/100g). The first treatment was FYM alone (T1) second being *Pseudomonas* (T2) only, third *Bacillus* (T3) only, forth *Rhizobium* only (T4), Fifth is combination of all bacteria inoculants T5 (T2 + T3 + T4), Six, Seventh and Eighth are FYM with individual bacterial inoculants T6 (FYM + T2), T7 (FYM + T3), T8 (FYM + T4), Ninth one is combination of all bacteria with FYM T9 (FYM + T5), and T10 (Control, without any amendments).

The earthen pots were filled with 5 Kg soil in all the treatments and small pits were made in each pot for seeding purpose. Into each pit 1ml of bacterial suspension with 10^9 cells/ml concentration was poured. The bacterial suspension was prepared in sterilized distilled water from the pure cultures and the concentration of bacterial cells per ml was measured using a haemocytometer and the concentration was appropriately adjusted. After adding the bacterial suspension to the pits, 3-6 surface sterilized black gram seeds were sown in each pot at the rate of one seed per each pit. The potted plants were maintained properly throughout the period of study.

Leghaemoglobin Content

Leghemoglobin content of the nodule was estimated according to the method described by Tu *et al* (1970).

In all the treatments, 5 plants at flowering stage were uprooted gently, washed thoroughly under tap water and nodules were collected. The nodule samples were frozen before leg hemoglobin extraction. For the estimation, 500 to 1 gm nodules were homogenized in 5ml of 0.1N KOH and centrifuged for 10min at 12000 g. To the 1.5ml of supernatant, 1ml of water, 0.5ml of 5N KOH and 0.1 g of $Na_2S_2O_4$ were added for reduction and OD of leg hemoglobin was determined at 537, 557 and 577nm wavelengths after through mixing. The amount of leg hemoglobin was calculated using the formula.

µg of leg haemoglobin = OD_{557} -- ½ (OD_{537} + OD_{577})

Morphological Studies

Morphological parameters like plant height, no. of leaves, no. of nodules per plant, no. of pods per plant, no. of seeds per pod were studied from the randomly selected three plants from each pot in all the treatments at three different stages of growth i.e., 30, 60 and 90 days after sowing. Average values of three plants were represented in the results.

Soil Analysis

The soil was analysed for physico chemical properties like pH, EC, organic carbon, Nitrogen, Phosphorous, Potassium, and soil texture, before the crop season in all the treatments, at the soil testing laboratory in Guntur. Similarly, at the end of study also the soil analysis was conducted to understand the effect of treatments on soil fertility.

Statistical Analysis

One way analysis of variance (ANOVA) was calculated for data collected using Genstat Discovery Edition 3. All the parameters studied were statistically significant (at 5%) with $p<0.001$. No. of nodules / plant was significant as given by F- value of 4.936. No. of pods / plant was significant as given by F- value of 2.237. No. of seeds/ pod was significant as given by F- value of 0.745. No. of leaves / plant was significant as given by F- value of 3.175. Plant height was significant as given by F- value of 2.251.

RESULTS AND DISCUSSIONS

Effect of Treatments on Soil Fertility

Effect of different treatments on soil properties before and after sowing of *Vigna mungo* plants shown in table (1)

Table 1

Treatments	pH	EC (ds /m)	Organic Carbon (%)	Available N (Kg ha^{-1})	Available P (Kg ha^{-1})	Available K (Kg ha^{-1})
T1: FYM	8.30	0.15	0.61	106.07	78.61	165.20
T2: *Pseudomonas*	8.33	0.13	0.42	76.14	16.57	123.70
T3: *Bacillus*	8.28	0.11	0.43	74.12	12.08	128.80
T4: *Rhizobium*	8.17	0.11	0.48	77.07	12.70	120.23
T5: T2 + T3 + T4	8.09	0.13	0.51	86.03	18.20	129.71
T6: FYM + T2	8.18	0.15	0.72	131.15	80.60	150.30
T7: FYM + T3	8.13	0.16	0.74	136.06	80.53	180.40
T8: FYM + T4	8.05	0.16	0.71	106.80	80.80	185.70
T9: FYM + T5	8.02	0.14	0.75	137.10	82.50	193.05
T10: Control	8.51	0.17	0.37	71.17	9.81	118.02
Soil before sowing	7.20	0.35	0.29	72.17	2.07	100.50

*Each value in the table is an average of three replicates.

All the parameters studied were statistically significant.

pH and EC

All the treatments reduced the pH than the control. A minimum reduction in pH from 8.5 to 8.3 was observed in soil amended with FYM. Among the treatments PGPR soils with *Pseudomonas* reduced the pH and recorded as 8.33 followed by *Bacillus* and *Rhizobium* as 8.28 and 8.17 for (T2), (T3) and (T4) respectively. The combination of all bioinoculant treatments (T5) showed further decrease in pH to 8.09. Similar pattern has observed after the addition of FYM in T6, T7 and T8. The maximum decrease of pH 8.02 was observed in T9 with FYM and treatments PGPR. In this study pH of the all treatments decreased from the initial 8.51 in control to 8.02 in T9.

Application of FYM alone (T1), decreased the EC value from 0.17 ds/m in control to 0.15 ds/m. Among the bioinoculants, *Rhizobium* and *Bacillus* (T3) and (T4) inoculated soil recorded with lowest EC value of 0.11 ds/m. Combination of FYM with bioinoculants showed a maximum increase in EC value with 0.15 ds/m in T6 and 0.16 ds/m in T7 and T8 than bioinoculant treatments. However, treatments on the whole decreased the soil EC value from 0.17 that of control, indicating a highly negative effect.

FYM treated soils showed lower pH and EC followed by soils with only PGPR. This decrease might be due to the production of organic acid during decomposition of FYM in soil (Das and Sing, 2013). Similarly in the present study also FYM + PGPR treatments showed lowest pH and EC value due to secretion of organic acid during decomposition of FYM. Sajal Roy and Abul Kashem (2014) also reported that soil pH gradually decreased with the increase of incubation time in amended soils.

Organic Carbon Content

Organic carbon content of the soil in FYM treatment (T1) increased from 0.37% in control to 0.61% after the end of the crop. Among the treatments, *Rhizobium* inoculant showed 0.48% of organic carbon (T4) followed by *Bacillus* with 0.43% (T3) and *Pseudomonas* with 0.42% (T2). The combination of all the inoculants (T5) showed higher organic carbon content of 0.51%. FYM with PGPR showed still higher organic carbon content in (T6), (T7) and (T8) with 0.72%, 0.74% and 0.71% respectively. The maximum increased was observed in (T9) with FYM + PGPR 0.75%. All the treatments shows significant in organic carbon over the control.

Hatim (2013) reported that each of organic manure treatments alone or with *Rhizobium* seed inoculants significantly ($P<0.05$) produced more soil organic carbon. However, *Rhizobium* seed inoculation alone had no significant ($P \leq 0.05$) increased in soil organic carbon and soil C/N ratio. Significant improvement was noticed in organic matter content after application of biofertilizers (Archana, 2014). Bouajila and Sanaa, (2011) reported that of the various organic amendments, compost treatment showed significant increase of organic and total nitrogen contents.

Available N, P, K in Soil

Soil N, P and K levels were tremendously increased after sowing the crop than before sowing indicates the direct effect of organic manure and bioinoculants. Nutrient content of the soil showed increase in all the treatments with FYM combination. The role of FYM was more promosing than the bioinoculants. The amount of soil available N. P and K showed an increase from (71. 17 kg ha-1) to (106.07 kg ha-1), (9.81 kgha-1) to (78.61 kg ha-1) and (118.02 kg ha-1) to (185.20 kg ha-1) respectively in FYM alone (T1). The lowest values available N, P and K were recorded in case of

bioinoculants without FYM (T2), (T3) and (T4) as available N (74.12 kg ha-1) and available P (12.08 kg ha-1) with *Bacillus* and available K (120.23 kg ha-1) with *Rhizobium.*

The combination of all the inoculants (T5) showed further increase in available N, P and K (86.03 kg ha-1), (18.20 kg ha-1) and (129.71 kg ha-1) respectively. The maximum available N, P and K were observed with mixed treatment FYM + PGPR in (T6), (T7) and (T8) as (131.15, 80.60 and 150.30 kg ha-1), (136.06, 80.53 and 180.40 kg ha-1) and (106.80, 80.80, and 185.70 kg ha-1) respectively. The highest value of available N, P and K (137.10 kg ha-1), (82.50 kg ha-1) and (193.05 kg ha-1) respectively was recorded in (T9) after harvest of black gram. Organic manure with bioinoculants was found much superior as compare to FYM alone or individual inoculation in improving the availability of N, P and K in soil.

Both biofertilizer and organic manure treatment led significant increase in the stocks of N, P and K (Rajasekaran, 2011). Organic manure treatments alone or with *Rhizobium* inoculation significantly increased soil available Phosphorus (Hatim, 2013). The complete organic and biofertilizer increase soil N, P and K (Stephen, 2003). Significant improvement in N, P and K after application of biofertilizers was also reported by Archana (2014). Glending and Powlson (1993), Jedidi *et al*., (2004) have reported that the application of manure and waste household compost concentrations (40 and 120 t/ha) resulted in significant increase of organic nitrogen.

EFFECT OF TREATMENTS ON GROWTH AND YIELD

Effect of different treatments on growth and yield parameters of black gram plants, recorded at 90 days after sowing was given in the table (2).

Treatment	Plant Height* (cm)	No. of Leaves /plant*	No. of Nodules/ Plant*	Leg Haemoglobin Content (mg/g)	No. of Pods/Plant*	No. of Seeds/Pod*
T1:FYM	60.425	30.250	38.250	0.255	30.250	7.250
T2: *Pseudomonas*	47.350	22.750	23.250	0.105	20.750	6.250
T3: *Bacillus*	53.575	22.750	21.750	0.110	21.250	6.250
T4: *Rhizobium*	52.425	22.250	51.750	0.120	24.250	7.250
T5: T2 + T3 + T4	54.450	22.500	47.500	0.170	27.250	6.500
T6:FYM + T2	65.750	33.250	40.500	0.200	30.250	7.250
T7: FYM +T3	68.050	33.750	63.250	0.210	32.750	7.250
T8: FYM + T4	69.425	36.750	71.500	0.210	35.500	7.500
T9: FYM + T5	**71.025**	**38.250**	**72.250**	**0.305**	**37.750**	**7.750**
T10:Control	42.650	19.750	21.750	0.075	21.500	6.250

*Each value is an average of three replicates.

All the parameters studied were statistically significant.

Plant Height and Leaves Number

Plant height and average number of leaves was studied from randomly selected plants from each pot in all the treatments at three different stages of growth, 30, 60 and 90 days after sowing tables (5, 6). Plant height and number of leaves were significantly increased with the application of bioinoculants and organic manure than in control. Plant height and number of leaves in FYM alone (T1) increased from 42.650 cm to 60.425 cm and 19.750 to 30.250 respectively (photo 1). The lowest values of plant height and number of leaves was recorded in the each and combination of the bioinoculants without FYM of T2 (47.350 cm and 22.750), T3 (53.575 cm and 22.750), T4 (52.425 cm and 22.50) and T5 (54.450 cm and 22.500) respectively.

The maximum values of plant height and average number of leaves recorded in mixed treatments of FYM with bioinoculants in T6, T7 and T8 as (65.750 cm and 31.250), (68.050 cm and 33.750) and (69.425 cm and 36.750) respectively. The highest values of plant height and number of leaves were observed in T9 (71.025 cm) and (38.250) respectively due to the application of organic manure and bioinoculants. These finding are in accordance with the results in soybean plant by Konthoujam *et al.*, (2013). Similar findings were also reported by Uma and Elakkiya, (2014) who reported that liquid biofertilizers in combination with inoculation of treatments increased plant height and number of leaves.

Nodulation

The average number of nodules, in FYM treatment alone (T1) increased from (10.750) per plant at 30 days to (38.250) per plant after sowing. The bioinoculants with *Pseudomonas* (T2) and *Bacillus* (T3) doesn't showed any improvement of number of nodules per plant than control (23.250 and 21.750) per plant respectively may be due to of unsterilized sample in the study. The *Rhizobium* inoculation alone in (T4) supported maximum number of nodules (51.750) per plant had dominated the other inoculants in nodule production. All the three bioinoculants together in (T5) produced improvement in nodules number (47.500) per plant when compared their to individual application. The FYM when combined with bioinoculants showed maximum number of nodules in (T6), (T7) and (T8) as (40.500), (63.250) and (71.500) per plant respectively. When FYM was mixed with all the three bioinoculants (T9) maximum increase in nodules number has observed. In the present study, nodules number increased with increase in age from 30 to 90 days after sowing. The organic manure showed positive effect on nodules production.

These results are in conformity with the findings of Javaid (2009), who reported that both the organic amendments result in a marked suppression in nodules number and Biswas et al,. (2003) reported that seed inoculation increase the number of nodules per plant. Haqu and Khan, (2012) who indicated that inoculation of plant seeds by fertilizer enhanced that growth and nodulation of plants than uninoculated treatments and Khosro (2010) reported that biofertilizers and organic manure had a significant effect on nodules number and nodule activity.

Leghaemoglobin Content

Leghaemoglobin content of the nodules from healthy nodules was estimated at 70 days after sowings. A rapid increase in Leghaemoglobin content was observed in all the treatment than the control. All the treatments showed positive effect on Leghaemoglobin content of the nodules. Treatment with FYM alone (T1) increase the Leghaemoglobin content to a maximum of (0.255) mg/g, while in combination of FYM with all the treatment bioinoculants (T9) proved to the best with highest content of (0.305) mg/g. *Rhizobium*, among the three bioinoculants produced maximum Leghaemoglobin content of (0.120) mg/g than *Bacillus* and *Pseudomonas* which reduced a Leghaemoglobin content of (0.110) and (0.105) mg/g respectively. All there three bioinoculants increase the Leghaemoglobin content of the nodules when they are amended along with FYM for (T6), (T7) and (T8) as (0.200), (0.210) and (0.210) mg/g, respectively. The FYM with all the treatments (T9) produced maximum Leghaemoglobin content of (0.305) mg/g which in as 100 fold increase over that of a bioinoculants combination in (T5). Tagore et al., (2013) found the Leghaemoglobin content in chickpea root nodules increased with the advancement of crop age in *Rhizobium* + PSB was mainly due to better root and nodules development.

EFFECT OF ORGANIC AND BIOFERTILIZERS ON YIELD

The yield (pods per plant) in all the treatments was positively affected with an increase over the control. FYM alone (T1) or in combination with any one of the bioinoculants of (T6), (T7), (T8) and (T9) showed maximum increase in pod number (30.250), (32.750), (35.500) and (37.750) per plant, respectively over their individual bioinoculants without FYM (T2), (T3), (T4) and (T5). Treatments (T2) with 20.750 and (T3) with 21.250 per plant, didn't show much influence on number of pods than the control, but when they are in combination with FYM produced high pod number. Finally highest number of pods per plant was observed in (T9) treatment with FYM and all the three bioinoculants.

However, *Pseudomonas* (T2) and *Bacillus* (T3) doesn't show any improvement of number of seeds per pod over the control. *Rhizobium* bioinoculant showed promising improvement in seed yield when compared to other two bioinoculants. Grain yield was positively affected mainly by FYM treatment (T3). All the three bioinoculants showed almost similar improvement in number of seeds per pod when they are combined with FYM than their individual bioinoculants. The FYM alone and in combination with bioinoculants individually or all three bioinoculants together produced maximum number of seeds per pod. The highest seed number recorded in (T9) due to the application of organic manure and PGPR.

These results were in agreement with those of Rajasakaran *et al*., (2011) who reported that the application of organic manures significantly increased number of seeds and pods, and Selva Kumar *et al*., (2011) who showed that the utilization of biological fertilizer increased number of pods per plant and seed yield that it could be due to increasing other nutrient absorption, also biological phosphate fertilizer can be used as solution for increasing phosphate and micronutrient sorption in the alkaline soil. Zodape (2001) also reported that, the increase in yield productivity with biofertilizer application is due to micro- element and plant growth regulator contained in the fertilizer. Similar results were also reported by Balasubramanian, (1994) who showed that the use of farm yard manure significantly increased plant production. Similar Sajid, et al., (2010) also reported that the use of organic manures increased the growth and yield of plant.

CONCLUSIONS

Biofertilizers along with farm yard manure giving higher of nutrient of N, P, and K as well as the increase in growth and yield of black gram compared with control treatment. I recommend focusing on the use of the biofertilizers with organic manures which increases the nitrogen content and available phosphorus and potassium helping to reduce damage from the use of chemical fertilizers that pollute the environment.

ACKNOWLEDGEMENTS

I would like to express my appreciation to my supervisor, Dr. M. Raghu Ram, Assistant Professor, Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur for his valuable guidance to work on this subject. I am also grateful to Acharya Nagarjuna University for the facilities provided during my work.

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