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RESEARCH ARTICLE

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The Response of *Vigna radiata* to Various Sources of Fertilizers in Vellore District

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ABSTRACT

In the current global scenario, farmers face a considerable challenge in maintaining a balance between sustainable agriculture and good yield. A field experiment was undertaken at Vellore Institute of Technology (VIT), Vellore district to examine the performance of various sources fertilizers on *Vigna radiata*. A new combination of plant growth stimulating bacterial consortium was compared with exsting organic and inorganic fertilizer. The outcome of the study showed that the grain yield and root nodules production was significantly increased by the effect of microbial consortia with maintaining the soil pH. In the present study, we report the enhanced soil organic carbon (SOC) and flowering initiation by the application of farmyard manure (FYM), bio-fertilizer (*Rhizobium*) and panchagavya. Moreover inorganic fertilizer did not show any positive improvements for the present soil condition. The outcome of the study suggested the imperative role of organic and biofertilizer which improved the crop yield production than the other inorganic fertilizers.

Key words: Bio-fertilizers, Inorganic fertilizer, Organic fertilizer, Plant growth promoting bacteria.

INTRODUCTION

Agriculture in India contributes a large share in the economy and employment. Out of 329 million hectares, 114 million hectares of the land have been reported to be used for agricultural purposes (Raghuwanshi 2012). In the system of agriculture, fertilizers facilitate to increase the yield of crops along with other production inputs. Mainly three different forms of fertilizers such as organic, chemical and bio-fertilizers are prominently used for crop cultivation by Indian farmers. Chemical fertilizers play a significant role in improving crop production, crop yield, and it also helps in meeting the food need. Noticeable problem of chemical fertilizers leads to changes in the soil pH causing water pollution through leaching of such chemicals, and hence reducing the beneficial soil bacterial load. Indian farmers are highly dependent on chemical fertilizers which have caused environmental pollution and soil infertility Ahmadian *et al*. (2011).

In the category of fertilizers, organic fertilizers are one of the essential fertilizers including farmyard manure (FYM), vermicompost, bio-fertilizers, neem cake and green manure which help to increase crop yield by providing balanced nutrients Dahiphale *et al*. (2003). However, many bacterial species like *Azospirillum*, *Azotobacter*, *Azorhizobium*, *Pseudomonas fluorescens*, *Bacillus subtilis* have been used mainly as bio-fertilizers in the agricultural fields, and these bacteria were used either as soil application or seed treatment with the mode of action as biocontrol agents (Okon and Labandera Gonzalez, 1994). The plant growth promoting rhizobacteria (PGPR) influence the growth of plants directly with mechanisms either increasing nutrient accessibility through several metabolic cycles such as nitrogen fixation, solubilization or mobilization of mineral forms of nutrients and mineralization of organic products. The rhizobacteria also influence the plant growth indirectly by the action and production of HCN (hydrogen cyanide), antibiotics, cellulose and siderophore (Mathiyazhagan *et al*. 2004). Green gram is the third most leguminous plant consumed in India. Approximately 9,00,000 hectares are used for pulses cultivation annually with a production of 4,00,000 tones (Sumathi 2012). In usual practices green gram is cultivated annually due to more seed consumption (Parry *et al*. 2018). Green gram was cultivated in 30,084 hectares of land in India and its corresponding production was 10,532 tones during 2005-06 (Sarma *et al*. 2012). In 2015-16, the total production of pulses was around 5, 84,969 tones from 8, 87,650 hectares of land, indicating the yield of 659 kg per hectare land (Anonymous, 2016). Green gram (*V. radiata)* plays a significant role in sustaining agriculture soil (Subba Rao 2011). Insufficient and overuse of fertilizers affects the growth and yield of green gram. Therefore, the study was undertaken to explore the effects of various fertilizers such as panchagavya, FYM, plant growth promoting bacteria (PGPB), commercially available inorganic and bio-fertilizer.

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MATERIALS AND METHODS

Experimental design

A field experiment was carried out at the horticulture department of VIT, Vellore, Tamil Nadu, India during February

2018. The study area was arranged in a completely randomized block design with triplicates. The experimental area of 9m × 9m was selected which includes a canal of 1m in the middle. The study contains six different treatments: Treatment 1: Panchagavya (0.1% for seed treatment and 1% for foliar application at 10, 30 and 60 days after sowing-DAS). Treatment 2: Inorganic fertilizers (NPK 12.5:25:12.5 kg $hectare^{-1}$ were applied at basally, 45 and 60 DAS). Treatment 3: PGPB in the form of microbial consortia used for seed treatment and foliar application (1.5 liters). Treatment 4: Commercially collected *Rhizobium* was utilized for this experimental study. Treatment 5: FYM (20t/ha) was treated with soil 20 days before sowing. Treatment 6: Control group.

Characterization and preparation of PGPB

Bacterial strain like *Pseudomonas fluorescens* (MTCC 9768), *Bacillus subtilis* (MTCC 8141) and *Lactobacillus plantarum* (soil isolates) were tested for plant growth promoting properties including phosphate solubilization activity Vazquez *et al*. (2000), hydrogen cyanide (Lorck and Veterinary, 1948), ammonia Simha *et al*. (2017) and indole acetic acid production (IAA) Goswami *et al*. (2013). These three bacterial strains were cultivated in LB (Luria-Bertani) medium, of which *P. fluorescens* and *B. subtilis* were cultured at 37°C for 12h and *L. Plantarum* was grown at 28°C for 24h. After the growth, the PGPB were washed in 10mM $MgSO_4$. The final concentrations of cell biomass (10^8cfu) were used for the field application. For studying the antagonistic activity of fungal pathogens (*Rhizoctonia solani* and *Alternaria solani*) were tested against bacterial strains Hye *et al*.(2014).

Field experiment

The green gram (VRM (Gg) 1) seeds were collected from Krishi Vigyan Kendra, Virinjipuram, Vellore, Tamil Nadu, India and 250g of seeds were surface sterilized with 80% ethanol for 20 min. Further, the seeds were kept overnight under sterile conditions at 28°C for easy germination. The sprouted seeds were equally used for all the treatments. The seed treatment was given only for *Rhizobium* (liquid bio-fertilizer), panchagavya (0.1%) and PGPB consortia for 30 min. For NPK, manure, and control the germinated seeds were sowed directly without any seed treatment. The experiment was conducted in triplicates with control. After five days, the percentage of seed germination was measured. Ten plants were randomly picked from the center of the plot. Plant height, number of branches, flowers, nodules and grains yield were monitored and calculated. Quantification of chlorophyll was done by UV-Visible spectrophotometer (Anuradha *et al*. 2015). Relative water content (RWC) of fresh leaves were calculated by Dutta *et al*. (2016). After harvesting, field soil analysis was carried out at Shri AMM Murugappa Chettiyar Research Centre, Taramani, Chennai, Tamil Nadu, India. The collected data were analyzed by one-way-analysis of variance (ANOVA) and predicted Dunnett's multiple comparison test.

RESULTS AND DISCUSSION

Characterization of PGPB strain

Out of the three strains, *L. plantarum* was noted for the maximum level of phosphate solubilization (322.11±5.17 µg/ml) and IAA production (17.43±5.51 mg/ml). Further, *P. fluorescence* and *B.subtilis* exhibited the production of HCN. Moreover all three strains were positively identified for ammonia production and *P. fluorescence* for siderophore synthesis (Table 1). Researchers reported that the strain *Pseudomonas* GRP3A stimulated the growth of *Vigna radiata* by producing siderophore, IAA and solubilization of phosphate under limited iron conditions (Sharma and Johri, 2003). *Pseudomonas*, *Bacillus*, *Serratia*, *Streptomyces* and *Azospirillum* are considered to be both profitable and beneficial for plant growth, simultaneously biocontrol activity expression adds to the economic market value Tabassum *et al*. (2017). Moreover the phosphate solubilizing bacteria improved the nutient utility (N,S and P) of the seeds (Patel *et al*. 2018). In our study, dual culture assay plate showed that only *B. subtilis* inhibited the growth of *R. solani* and *A. solani* mycelial, which are similar to the previous reports of phytopathogenic acivity by the antifungal compounds in *B. subtilis* NSRS 89-24 culture filtrate, from which β-1,3-glucanase and antibiotics were proved to be responsible for the fungicidal activity Leelasuphakul *et al*. (2006).

Growth and yield attributes

The efficacy of organic and inorganic fertilizers on the growth and yield of *V. radiata* was recorded. Various percentages of seed germination (using the formula % germination = (number of germinated seeds × 100) / total number of seeds) were recorded (78, 76, 73, 61, 56, and 62%) among the treatments of panchagavya, PGPB, NPK, *Rhizobium*, FYM and control, respectively. Parameters such as the plant height, relative water content, number of branches, flowers and nodules were recorded. There were no considerable variations among the treatments regarding the plant height, pod length and number of branches. Significant results were noticed in the formation of nodules, flowerings, pod fresh

Table 1: Biochemical characterization of plant growth promoting rhizobacteria.

Strain name	Phosphate solubilization (µg/ml)	IAA (µg/ml)	Siderophore	HCN	Ammonia (NH_3) production
P. fluorescens	107.544±3.85	10.62±0.54	+	++	+
B. subtilis	126.18±4.49	9.80±1.39	-	+++	+
L. plantarum	322.11±5.17	17.43±5.51	-	+	+

Values in mean ± Standard deviation (SD), (-) Indicate absent, (+) Present, (++) Moderate, (+++) Strong producer.

Table 2: Crop growth and yield parameters.

Treatments	Number of opened flowers per plant	Flowering duration (Recorded Days)	Number of nodules	Grain yield Pod^{-1} (g)	Pod fresh weight $^{-1}$ (g)
Panchagavya	7.75 ± 0.48[a]	26 - 37	4.25 ± 0.85	1.01 ± 0.1[ab]	2.1 ± 0.09[a]
NPK	6.00 ± 0.4[ab]	31 - 36	3.5 ± 0.64	0.96 ± 0.09[b]	1.58 ± 0.16[b]
Microbes	7.25 ± 0.25[ab]	28 - 38	7.0 ± 0.4*	1.42 ± 0.16[a]	2.03 ± 0.11[ab]
Rhizobium	6.00 ± 0.4[ab]	30 - 36	4.75 ± 0.85	1.19 ± 0.09[ab]	1.91 ± 0.09[ab]
Manure	6.75 ± 0.48[ab]	30 - 37	4. 0± 0.91	0.99 ± 0.08[ab]	1.73 ± 0.17[ab]
Control	5.75 ± 0.48[b]	31 - 36	4.0 ± 0.91	1.03 ± 0.08[ab]	1.9 ± 0.05[ab]

One way analysis of variance values indicated in mean ± SD significant difference at $p<0.05$. Same letter represents non significant values; different letters are significantly different within rows. * indicate significant differences in the number of nodules.

Table 3: Physico-chemical soil characterization.

Treatment/ nutrients	NPK	Microbes	Manure	Panchagavya	*Rhizobium*	Control	Initial Stage
pH	Neutral	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline
Organic carbon	Low	Low	Medium	Low	Medium	Low	Low
N (kg/acre)	Low	Medium	Medium	Medium	Medium	Medium	Medium
P(kg/acre)	High	High	High	High	High	High	Low
K (kg/acre)	High	High	High	Medium	Medium	High	High

weight and grain yield. Each of the organic, inorganic and bio-fertilizer influences the plant growth and yield in a subjective manner and panchagavya treated plants produced the highest pod fresh weight. Bacterial consortium treated plants recorded the highest grain yield (Table 2). Moreover, panchagavya plays a role in flower formation, as well as decreased the duration of flowering (26^{th} day) and FYM involved in higher pod yield. There are previous reports that the 6% panchagavya showed greater nodules formation and seed yield in the *Vigna unguiculata* (Desai *et al*. 2014). Foliar application of panchagavya (3%) was reported to have significant differences in the growth and grain yield of *Vigna mungo* (Kumar *et al*. 2011). Abd El-Wahed *et al* (2017) revealed that FYM produced the highest level of bean yield (Singh *et al*. 2019) and identified that grain yield of green gram was significantly increased by the application of phosphorus. The maximum percentage of relative water content (85.1%) was observed in manure treated plants, in contrast the lowest percentage (61%) was recorded in the control leaves. In the present study, the chlorophyll content was increased significantly in the microbial treated plants (Fig 1).

The application of various kinds of fertilizer treatments induces the nodule formation. The result of nodulation data revealed that the application of microbial consortia was effective in the formation of nodules. In the current study, microbial consortia enhanced to produce maximum nodules as compared to *Rhizobium* bio-fertilizer and other treatments (Fig 2). There are reports that opportunistic bacterial strains like *Pseudomonas* spp., *Klebsiella* spp. and *Enterobacter* spp. were identified from the sterilized root nodules which colonizes and stimulate root nodules in the presence of soil rhizobia Ibáñez *et al*. (2009). The positive attribute of co-inoculation of *P. polymyxa* and *B. megaterium* showed increased nodule weight and nodules count compared to *Rhizobium* alone in common bean (Korir *et al*. 2017). Rosendahl and Jochimsen (1995) noted that IAA helps to initiate the H^+-ATPase enzyme, which is essential for the nodules formation. Hence, the microbial consortia used in this present study, have promoted growth of opportunistic bacteria which could have influenced the nodule formation as well as *P. fluorescens*, *B. subtilis* and *L. plantarum* are identified as IAA producers.

Soil characteristics

The field soil was tested during the initial and the final stages of this study. At the end of this study, the soil was collected from the rhizosphere region from each treatment including control plants and soil nutrients were analyzed. Parameters such as pH, organic carbon, nitrogen, phosphorus and potassium were noticed to be differentially regulated. A low level of nitrogen was noticed in the NPK treatment when compared to other treatments. The medium level of organic carbon was spotted in the manure and *Rhizobium* than the other soil samples (Table 3). The results of soil physico-chemical characteristics depicted soil organic carbon (SOC)

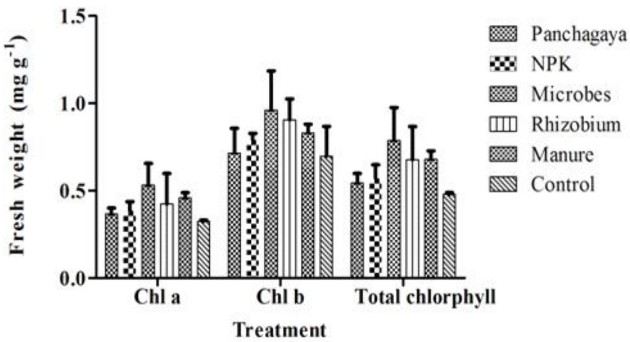

Fig 1:Various fertilization practices significantly affect the production of chlorophyll.

Fig 2: Effect of various treatments in the nodules development (A-F). (A) Panchagavya (B) Microbes (C) Manure (D) NPK (E) *Rhizobium* (F) Control. Formation of root nodules and evidence for nitrogen fixation. Number nodules significantly increased in the treatment of microbial consortium.

was increased by the treatment of FYM and *Rhizobium* bio-fertilizer, and alkaline nature of the soil was converted into neutral with the recommended level of NPK fertilizer treatment.

In this present study, it was noticed that the amount of nitrogen was maintained by all the treatments except inorganic fertilizer. However, researchers reported that the use of urea and ammonium sulfate fertilizer reduced the soil pH, and comparatively, ammonium sulfate reduces the soil pH to a greater extent and this fertilizer significantly increased the rice grain yield (Fageria *et al*. 2010). Protons reduce the soil pH at minute level around the granules of fertilizer (Bolan *et al*. 1999). Hence, our report strongly indicates that the application of inorganic fertilizer like NPK affects the soil pH, on the other hand application of organic manure exhibited positive action of microbial biomass carbon (MBC), enzyme activity and nitrogen mineralization. Simililarly, Mangalassery *et al*. (2019) reported that FYM in the combination of organic cakes, cashew biomass and bio-fertilizer consortia significantly increased the SOC.

CONCLUSION

Based on the above findings, we firmly conclude that panchagavya improves flowering initiation and microbial consortium plays a vital role in nitrogen fixation by developing root nodules, which indirectly helps to protect the crop from pathogens. FYM and *Rhizobium* inoculation maintained good soil carbon. The bacteria consortium increased the crop yield without affecting the soil pH unlike the other fertilizers.

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