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Mahendra SinghBihar Agricultural University,
Sabour, Bhagalpur, Bihar, India**Kasturikasen Beura**Bihar Agricultural University,
Sabour, Bhagalpur, Bihar, India**Amit Kumar Pradhan**Bihar Agricultural University,
Sabour, Bhagalpur, Bihar, India

Co-inoculation of *Rhizobium* with a phosphorus solubilizer and its effect on the growth of green gram (*Vigna mungo* L.)

Mahendra Singh, Kasturikasen Beura and Amit Kumar Pradhan

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Abstract

We investigated the effect of co-inoculation of *Rhizobium* with Phosphate Solubilizing Bacteria (PSB) in green gram under pot experiment in alluvial soil during *Zaid* season of 2015-16. Five treatments along with one un-inoculated control treatment were examined with three replications under Completely Randomized Block Design experiment. It has been concluded from the study that the seed inoculated with *Rhizobium sp.* @ 20 g kg⁻¹ seed + PSB @ 20 g kg⁻¹ seed + 75 % RDF significantly increased nodule number by 79.13% and 33.41 % over un-inoculated control treatment and application of PSB alone + 100% RDF. The similar results were also found for shoot dry weight for the rest four treatments by 40.02%, 22.04%, 12.88% and 13.0% when compared to control one. As evident from the results, the co-inoculation of *Rhizobium sp.* and PSB also increased the soil microbial biomass carbon than that of absolute control.

Keywords: *Rhizobium*, PSB, Green gram, Nodulation, grain yield

Introduction

Bihar ranks 9th in terms of pulses production with a contribution of 0.52 million tonnes to the national pulse pool. In the Indo-Gangetic plains nearly 60% of mung bean is grown in Bihar (largely the northern part). As the crop fits well in small window after harvest of rabi crop (such as wheat and rabi maize) mungbean cultivation will help in sustaining productivity of the cereal based cropping systems in Bihar (Singh et.al. 2017) [11]. Mungbean (*Vigna radiata* L.) is an important legume for human nutrition and a major protein (Khan and Mallik, 2001) [6]. Seeds contain 60-65% carbohydrates, fat (1-1.5%) and 3.5-4.5% fiber. Most pulses growing areas have low to medium population of native *Rhizobium* and seed inoculation with biofertilizer (*Rhizobium*) can increase pulses productivity by 10-12%. In traditional pulses grown area *Rhizobium* spp present in soil results in effective nodulation, but when introduced in new areas the host specific *Rhizobium* also needs to be introduced through inoculation. S. Pande, 1996 [8] observed that in Bihar, Khesari (lathyrus) nodulates prolifically, in meeting its own nitrogen (N) requirements as well as also fix N₂ to the cropping system. This has already been proved that the inoculation with *Rhizobium* spp. had enhanced nodulation and nitrogen fixation, plant biomass and grain yield in various leguminous species including mungbean, chickpea, bean and soybean (Hadi and Elsheikh, 1999; Yadegari and Rahmani, 2010; Kashem et al, 2000; Mukhtar, 2015) [3, 10, 12, 5]. Daramola et al., 1994 [1] reported that increased nodule number, nodule weight, nitrogen fixation, dry matter and nitrogen yield of soybean when inoculated with *Bradyrhizobium japonicum* strain. Thus, we investigated the effect of co-inoculation of *Rhizobium* with PSB on the yield attributes of green gram and soil microbial biomass.

Material and Methods

The experiment was laid out in the green house of the Department of Soil Science and agricultural Chemistry, Bihar Agricultural University of Sabour, Bhagalpur (Bihar) in alluvial soil during *Zaid* season of 2015-16. Five treatments along with one un-inoculated control treatment were examined with three replications under completely Randomized Design.

Corresponding Author:**Kasturikasen Beura**Bihar Agricultural University,
Sabour, Bhagalpur, Bihar, India

Table 1: Treatment details of the pot experiment

T1	Uninoculated control +100% RDF
T2	<i>Rhizobium sp.</i> +100% RDF
T3	PSB +100% RDF
T4	<i>Rhizobium sp.</i> + PSB + 100% RDF
T5	<i>Rhizobium sp.</i> + PSB + 75 % RDF

RDF: Recommended dose of fertilizers; PSB: Phosphorus solubilizing bacteria-*Burkholderia cepacia* (@ 20.0 g kg⁻¹ seed); *Rhizobium*: *Bradyrhizobium japonicum* (@10 20.0 g kg⁻¹ seed)

The seeds of mungbean were sown in pots having a depth of 2-3 cm. The matured pods were collected by hand picking at the time of harvesting. The harvested samples were used to study the number of nodules plant⁻¹, nodule dry weight, shoot dry weight and yield in gram per pot maturity. The routine soil parameters were analyzed following the standard soil testing procedures by Jackson, 1973 [4]. For estimation of microbiological parameters, freshly collected soil samples from rhizosphere were used and Microbial Biomass Carbon (MBC) of the fresh rhizospheric soil was determined by the chloroform fumigation method as described by Jenkinson and Powlson (1976) [9] and calculation was made by Vance *et al.*, (1987) [14].

Results

The soil was silty loam in texture with pH of 7.13 and EC of 1.30 dS/m. The values for mineralizable N, Olsen's P and Available K were 172. 91, 18.26 and 225.31 kg ha⁻¹ respectively. A significant variation was observed in the number of nodules plant⁻¹, nodule dry weight, shoot dry weight and yield in gram per pot in mungbean due to the inoculation and fertilization.

Yield and yield attributes

Data from Table 2 reveal that the nodule dry weight significantly increased by the application of *Rhizobium sp.* + PSB + 100% RDF when compared with over uninoculated control treatment, *Rhizobium sp.* + 100% RDF and application of PSB + 100% RDF, respectively. Seed inoculation with PSB @20 g kg⁻¹ seed + 100% RDF significantly increased shoot dry weight by 33.45% over uninoculated control. The application of *Rhizobium sp.* + PSB + 100% RDF numerically increased shoot dry weight by 40.02%, 22.04%, 12.88% and 13.0% when compared with the

application of treatment un inoculated control, *Rhizobium sp.* + 100% RDF, PSB + 100% RDF and *Rhizobium sp.* + PSB + 75% RDF, respectively. The results were in accordance with the results obtained from the study conducted by Singh and Kumar (2007). Co-inoculated seeds of green gram showed increased root nodule (103.34 plant⁻¹), nodule dry weight (0.203 g plant⁻¹), shoot dry weight (32.78 g plant⁻¹), microbial biomass carbon (418.630 µg g⁻¹ Soil) and grain yield (18.6 pot⁻¹) among all the applied treatments. However, different treatments had varied effect on the crop growth. Treatment of the individual strains of rhizobium and P solubilizer has also showed significant increase in growth parameters over the non-inoculated seeds. The application of *Rhizobium sp.* + PSB + 100% RDF significantly increased nodule number by 79.13% and 33.41 % over uninoculated control treatment and application of PSB alone + 100% RDF. Egamberdieva *et al.*, 2017 [2] also concluded that seed inoculation with *Rhizobium* bacteria increase the height of plant, length of root, dry weight of root and dry weight of shoot of legume crops.

The grain yield was significantly increased in all the treatments over uninoculated control. It was also observed that the grain yield was significantly increased in all the treatments over uninoculated control. The grain yield was also significantly increased the application of *Rhizobium sp.* + PSB + 100% RDF by 48.73%, 31.88%, 30.00% and 14.37% when compared with application of treatments un inoculated control, *Rhizobium sp.* + 100% RDF, PSB + 100% RDF and *Rhizobium* + PSB + 75% RDF, respectively. But there was no significant difference between the treatments receiving 75% RDF and 100% RDF for different yield attributes of moong bean after harvest. Thus, we can also reduce the 25 % of the fertilizer dose with the application of co-inoculation of *Rhizobium sp.* and PSB without a significant decrease in the yield of the crop. It could be due to more availability of nitrogen through biological nitrogen fixation by *Rhizobia* from atmosphere and increased the plant growth and plant vigor. Results obtained from the study conducted by Youseif *et al.*, 2017 [13] revealed that, under field conditions, growth faba bean increased significantly in response to inoculation with the most effective rhizobial strains. Similar results were reported by Das *et al.* (2012) who reported that the inoculation of *Rhizobium species* significantly increased grain yield and straw yield by 23.75 and 29.71 % in chickpea.

Table 2: Co-inoculation effect of *Rhizobium* species with PSB on yield attributes of green gram

Treatments	Nodule number (plant ⁻¹)	Nodule dry wt. (g plant ⁻¹)	Shoot dry wt. (g plant ⁻¹)	Yield (g pot ⁻¹)
Uninoculated control +100% RDF	19.333	0.036	19.000	9.533
<i>Rhizobium sp.</i> +100% RDF	92.667	0.091	25.553	12.667
PSB +100% RDF	61.667	0.096	28.553	12.833
<i>Rhizobium sp.</i> + PSB + 100% RDF	93.333	0.126	28.333	15.923
<i>Rhizobium sp.</i> + PSB + 75 % RDF	103.333	0.203	32.777	18.597
C.D.	15.255	0.019	5.385	2.611
SE(m)	4.780	0.006	1.687	0.818
C.V.	11.177	9.231	10.885	10.184

The microbial biomass carbon was also significantly increased by 37.71%, 26.81% and 20.37% by the application of *Rhizobium sp.* + PSB + 100% RDF when compared with application of treatments un inoculated control, *Rhizobium sp.* + 100% RDF and PSB + 100% RDF (Fig.1). There was no statistically significant difference between the values of MBC for the treatments receiving 75% and 100% of the RDF. It

might be due to the synergistic effect between *rhizobia* and PSB for which more availability of phosphorous as energy source to the *rhizobia* and more nitrogen fixation occurs, phosphate solubilizing bacteria make more availability of phosphorous through its activity. The similar results were obtained by Singh *et al.* (2015) [7]. Similar findings were also obtained by Gupta (2006) and Singh and Kumar (2008).

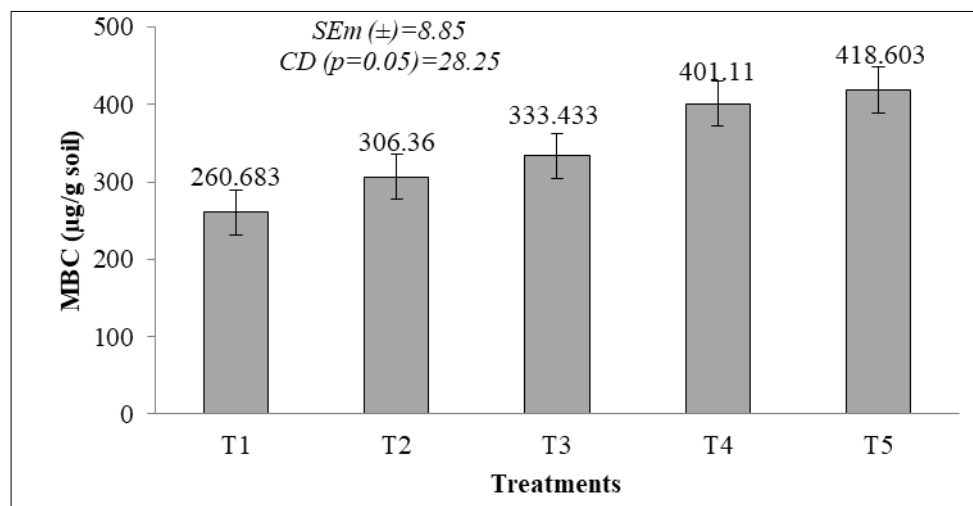


Fig. 1: Changes in Microbial Biomass Carbon due to the treatments

Conclusion

Most inoculants often rely on application of a single strain which might partially account for the recorded inconsistencies in field. A way to overcome this problem is to include different species or strains of beneficial microbes in the same microbial formulation. Co-inoculation helps to increase plant performance, and enhance the efficacy and reliability of constituent inoculants. Thus, we conclude that, the *Rhizobium* sp. + PSB + 75 % RDF treatment performed better in comparison to the other treatments taken under this study with respect to the yield and yield attributes of green gram.

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