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Evaluation of *Rhizobium* along with Multifunctional Rhizobacteria for Improving the Growth and Yield of Black Gram [*Vigna mungo* (L.) Hepper]

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ABSTRACT

Background: Indigenous rhizobial populations are unable to achieve fruitful symbiosis under field conditions due to scanty population and adverse soil environment. Externally introduced plant growth promoting rhizobacteria (PGPR) reported to have synergetic effect on indigenous *Rhizobium* resulting into faster growth and development and yield of the plants. Therefore, co-inoculating legumes with *Rhizobium* and PGPR proved more effective in improving nodulation and consequently higher seed yields of legumes.

Methods: The experiment was conducted at research farm of TCA, Dholi Muzaffarpur a campus of Dr. Rajendra Prasad Central Agriculture University, Pusa, Bihar during *kharif* season of 2021 to investigate the "Evaluation of *Rhizobium* along with multifunctional Rhizobacteria for improving the growth and yield of black gram [*Vigna mungo* (L.) Hepper]". There were eight treatments replicated thrice in a randomized block design. The treatment comprised of T_1-control, T_2-*Rhizobium* (*Bradyrhizobium spp.*), T_3-*Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*), T_4- *Rhizobium* + NE 10 (*Bacillus cereus*), T_5-*Rhizobium* + *Pseudomonas spp.*, T_6-*Rhizobium* + LMSR 45 (*Pantoea agglomerans*), T_7-*Rhizobium* + *Methylobacterium*, T_8- application of nitrogen as per RDF.

Result: The results revealed that co-inoculation of *Rhizobium*+ PUK-171 (*Stenotrophomonas rhizophila*) significantly increased plant growth parameters and nodulation. The yield attributing characters did not differ among treatments except co-inoculation of *Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*), which recorded significantly number of pods plant^{-1} than the remaining treatments. Stover yield was recorded higher in treatment co-inoculation of *Rhizobium* along with NE 10 (*Bacillus cereus*) as compared to T_1 and T_8. Harvest index was not influenced by different treatments.

Key words: Black gram, Growth, Inoculation, *Rhizobium*, Yield.

INTRODUCTION

Pulses are important in Indian agriculture because they provide a substantial quantity of vitamins, protein, minerals and calories to vegetarians (Pingoliya *et al*., 2014). Black gram, a prominent member of family Fabaceae known as *Vigna mungo* L. Hepper with the chromosomal number 2n=22. It is believed to have originated in India, where it has been cultivated since ancient times and is one of the country's most coveted pulse crops. It contains about 24 per cent protein, 60 per cent carbohydrate and 1.3 per cent fat. Black gram seeds are good source of minerals and energy as well as contains ample amount of fibre content and good source of phosphorus. It helps to maintain soil fertility by enhancing soil physical, chemical, biological properties and fixes the atmospheric nitrogen through the mechanism of biological nitrogen fixation (BNF). The crop can be grown as sole and or intercrop and it can tolerant drought and therefore, suitable for dryland areas.

Indiscriminate use of inorganic fertilizer, particularly nitrogenous and phosphorous resulted into widespread pollution of soil, air and water. These fertilizers are not only costly but also deplete non-renewable resources such oil, minerals and natural gas *etc*. (Joshi *et al*., 2006). Excessive and long-term use of these chemicals harm soil microorganism, lowers soil fertility and hence diminishes productivity as well as polluting environment (Youssef and Eissa, 2014). Biofertilizer are ecologically acceptable, non-bulky, low-cost inputs that could augment mineral nutrition and play an energetic role in plant nutrition. They are organic

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substance which contains specific living microorganism, having unique ability to fix atmospheric nitrogen, either non-symbiotically or symbiotically and some microorganism have potential to convert the unavailable (immobile) form of nutrient to available (mobile) form through various biological processes. Keeping this in view, the current investigation was conducted.

MATERIALS AND METHODS

This experimental study was conducted at "Tirhut College of Agriculture, Dholi Muzaffarpur" a campus of "Dr. Rajendra Prasad Central Agriculture University, Pusa, Bihar" during *kharif* season of 2021. The soil of experimental plot was alluvial and having high calcium carbonate ranges from 20-40%. The soil was low in organic carbon (0.36%) and contained 191.8, 22.3 and 139.4 kg ha^{-1} available nitrogen, phosphorus and potassium, respectively and pH 8.6 and EC 0.32 dSm^{-1}. It was estimated that mean maximum temperature during crop season was ranged between 27.9°C to 33.4°C and the minimum temperature ranged between 14.1°C to 27.0°C. The relative humidity fluctuated from 98.0-100% at 7 AM and 73.7%-94.7% at 2 PM. The total rainfall of 675.63 mm was received during the crop season (August-October 2021). There were eight treatments allocated randomly within replications. The variety Pant U-31 was sown in mid of August with a spacing of 30×10 cm using 20 kg ha^{-1} seed. Seed was treated with *Rhizobium* and different rhizobacteria @ 20 gm per 1 kg of seed. The treated seeds was shade dried before sowing. In order to achieve the recommended dose of N, P and K, the amount of Urea, SSP and MOP was 43.7 kg/ha, 225 kg/ha, 33.33 kg/ha respectively. During sowing, the total quantity of all the fertilizers was side dressed in furrows opened at a distance of 5 cm away from the seed row. For crop studies, five selected plants were tagged for taking observations of growth and yield parameters. The results were analysed as per statistically standardized principle of ANOVA technique described by Gomez and Gomez (1984) at 5% level of significance (Fig 1, 2).

RESULTS AND DISCUSSION

Growth attributes

The different treatments did not influence growth parameters except plant height of black gram (Table 1, 2). Co-inoculation of *Rhizobium*+ PUK-171 (*Stenotrophomonas rhizophila*) recorded significantly higher plant height (50.95 cm at 50 DAS), number of leaves $plant^{-1}$ (27.35, 33.89), number of branches $plant^{-1}$(6.15, 7.15), plant dry weight (12.23, 16.22 g), crop growth rate (14.15, 3.80 g m^{-2} day) over T_1-Control, T_2-*Rhizobium* (*Bradyrhizobium spp.*), T_8 was found at par with rest of treatments. The significant growth attributes due to increasing in the availability of nutrients, particularly nitrogen and phosphorous seems to have played an important role in enhancing cell division and metabolic activities resulting into higher production of photosynthates and their translocation. Similar results were reported by Nalawde *et al*. (2015).

Nodulation

At 25 DAS, the number of nodule $plant^{-1}$ (16.23) recorded significantly higher in co-inoculation of *Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*) as compare to T_1-Control, T_2-*Rhizobium* (*Bradyrhizobium spp.*) and T_8-application of N as per RDF and found at par with rest of treatments (Table 2). Whereas at 50 DAS co-inoculation of *Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*) recorded significantly higher nodulation over T_1 and T_8. It might be because *Rhizobium* and multifunctional rhizobacteria have the capacity to promote bacterial development to produce substances that encourage growth, leading to rise in population and ultimately nodule number and their dry weight. Multifunctional rhizobacteria have potential to induce the development of large number of epidermal cell capable of differentiating infectable root hairs. The significantly higher number of nodules with seed pelleting with *Rhizobium* @ 0.2 kg kg^{-1} of seed was also recorded by Vennila *et al*. (2018).

Yield attributes

Table 3 shows that the number of pods $plant^{-1}$ were maximum (22.5) in T_3 and minimum in control treatment (16.5). The treatments T_3, T_4, T_5, T_6 and T_7 did not show significant

Fig 1: Treated seed with various multifunctional rhizobacteria.

Fig 2: Soil analysis of P with spectrophotometer.

Table 1: Effect of *Rhizobium* along with multifunctional rhizobacteria on yield attributing characters of black gram.

Tr. no.	Treatment details	Plant height (cm)			Number of leaves plant^{-1}			Number of branches plant^{-1}		
		25 DAS	50 DAS	At harvest	25 DAS	50 DAS	At harvest	25 DAS	50 DAS	At harvest
T_1	Control	17.02	25.15	36.25	6.31	19.71	25.35	1.01	3.95	4.38
T_2	*Rhizobium* (*Bradyrhizobium spp.*)	21.00	35.50	43.58	7.15	23.23	28.23	1.19	4.98	5.50
T_3	*Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*)	22.80	41.25	51.25	8.17	28.45	34.21	1.26	6.15	7.15
T_4	*Rhizobium* + NE 10 (*Bacillus cereus*)	22.60	40.80	50.95	8.15	27.35	33.89	1.24	6.00	6.95
T_5	*Rhizobium* + *Pseudomonas spp.*	21.90	37.25	47.75	7.86	25.65	31.95	1.22	5.55	6.35
T_6	*Rhizobium* + LMSR 45 (*Pantoea agglomerans*)	21.50	36.75	46.25	7.63	23.91	31.10	1.20	5.35	6.25
T_7	*Rhizobium* + *Methylobacterium*	22.20	39.50	49.85	7.97	26.90	33.15	1.23	5.85	6.65
T_8	N as per RDF	20.80	35.43	42.37	7.02	22.70	27.85	1.10	4.41	4.95
	SEm (±)	0.39	1.15	1.84	0.39	1.31	1.62	0.06	0.28	0.31
	LSD (*p=0.05*)	NS	NS	5.58	NS	3.98	4.92	NS	0.86	0.93

Table 2: Effect of *Rhizobium* along with multifunctional rhizobacteria on yield attributing characters, number of nodules and dry weight of nodules per plant of black gram.

Tr. no	Treatment details	Plant dry weight (g plant^{-1})			CGR (g m^{-2} day)			Number of nodules plant^{-1}		Dry weight of nodules (mg plant^{-1})
		25 DAS	50 DAS	At harvest	25 DAS	50 DAS	At harvest	25 DAS	50 DAS	50 DAS
T_1	Control	1.47	9.26	11.11	1.96	10.39	1.76	11.23	30.50	30.50
T_2	*Rhizobium* (*Bradyrhizobium spp.*)	1.54	10.53	13.30	2.05	11.99	2.64	12.89	34.23	34.23
T_3	*Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*)	1.62	12.23	16.22	2.16	14.15	3.80	16.23	40.10	40.10
T_4	*Rhizobium* + NE 10 (*Bacillus cereus*)	1.61	12.06	15.87	2.15	13.93	3.63	15.87	39.25	39.25
T_5	*Rhizobium* + *Pseudomonas spp.*	1.58	11.50	14.30	2.11	13.23	2.67	14.97	37.55	37.55
T_6	*Rhizobium* + LMSR 45 (*Pantoea agglomerans*)	1.56	11.17	13.95	2.08	12.81	2.65	14.66	37.15	37.15
T_7	*Rhizobium* + *Methylobacterium*	1.60	11.86	15.12	2.13	13.68	3.10	15.67	38.05	38.05
T_8	N as per RDF	1.55	10.46	12.80	2.07	11.88	2.23	11.95	33.25	33.25
	SEm (±)	0.08	0.55	0.71	0.11	0.74	0.15	0.77	1.97	1.97
	LSD (*p=0.05*)	NS	1.68	2.15	NS	2.24	0.45	2.34	5.96	5.96

differences. The yield attributing character like number of seeds pod^{-1}, seed index and length of pod did not differ significantly among the different treatments. The differences in pod plant^{-1}, count of seed pod^{-1}, pod length and seed index with the inoculation of seed with biofertilizer has been recorded by Siddikee *et al*. (2018).

Seed and stover yield

The differences in seed yield were significant among the treatments (Table 4). Highest seed yield (1006 kg) was recorded by co-inoculation of *Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*) (T_3), which was significantly superior over control (T_1), *Rhizobium* (*Bradyrhizobium spp.*)

Table 3: Effect of *Rhizobium* along with multifunctional rhizobacteria on yield attributes of black gram.

Tr. no.	Treatment details	Yield attributes			
		No. of seeds/pod	No. of pods/plant	Seed index (g)	Length of pod (cm)
T_1	Control	4.25	16.50	3.95	3.98
T_2	*Rhizobium* (*Bradyrhizobium spp.*)	4.68	19.39	4.36	4.32
T_3	*Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*)	5.50	22.50	4.48	5.10
T_4	*Rhizobium* + NE 10 (*Bacillus cereus*)	5.43	22.20	4.42	4.95
T_5	*Rhizobium* + *Pseudomonas spp.*	5.34	21.45	4.45	4.75
T_6	*Rhizobium* + LMSR 45 (*Pantoea agglomerans*)	5.24	21.27	4.39	4.62
T_7	*Rhizobium* + *Methylobacterium*	5.39	21.93	4.52	4.85
T_8	N as per RDF	4.65	19.20	4.25	4.12
	SEm (±)	0.39	0.26	1.02	0.23
	LSD ($p=0.05$)	NS	0.80	3.09	NS

Table 4: Effect of *Rhizobium* along with multifunctional rhizobacteria on seed yield, stover yield and harvest index of black gram.

Tr. no.	Treatment details	Seed yield (kg ha^{-1})	Stover yield (Kg ha^{-1})	H.I (%)
T_1	Control	680	1769	27.91
T_2	*Rhizobium* (*Bradyrhizobium spp.*)	877	2158	28.93
T_3	*Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*)	1006	2291	30.47
T_4	*Rhizobium* + NE 10 (*Bacillus cereus*)	998	2300	30.30
T_5	*Rhizobium* + *Pseudomonas spp.*	968	2265	29.96
T_6	*Rhizobium* + LMSR 45 (*Pantoea agglomerans*)	961	2271	29.78
T_7	*Rhizobium* + *Methylobacterium*	981	2270	30.17
T_8	N as per RDF	868	2140	28.81
	SEm (±)	42	90	1.31
	LSD ($p=0.05$)	127	273	NS

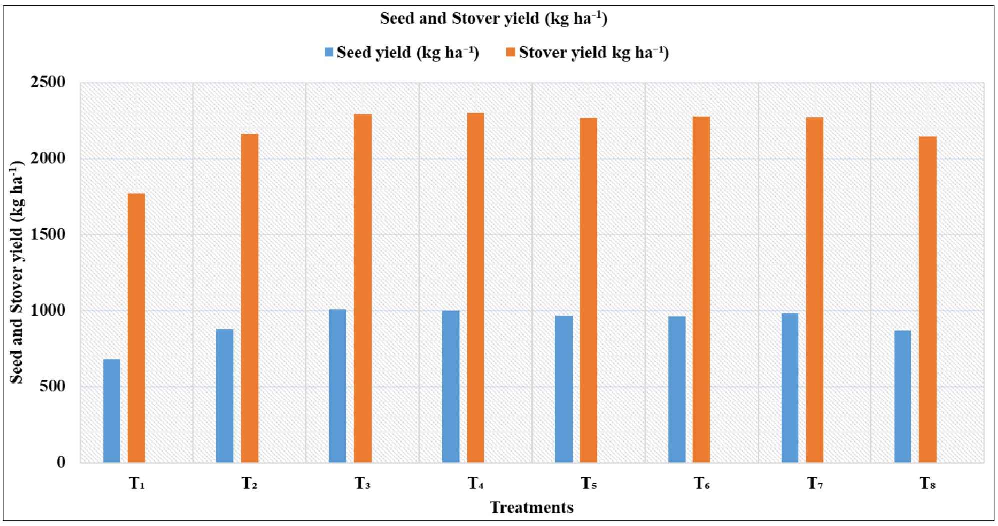

Fig 3: Effect of *Rhizobium* along with multifunctional rhizobacteria on seed and stover yield of black gram.

(T_2) and N as per RDF (T_8) and found statistically at par with *Rhizobium*+ NE 10 (*Bacillus cereus*) (T_4), *Rhizobium*+ Pseudomonas spp. (T_5), *Rhizobium* + LMSR 45 (*Pantoea agglomerans*) (T_6), *Rhizobium* + *Methylobacterium* (T_7). The treatment T-4 was found significantly superior over control (T_1) and found at par with the remaining treatments. The minimum stover yield (1769 kg ha^{-1}) was produced under control (T_1). The harvest index varied from 27.91 to 30.47%. The maximum harvest index (30.47%) was recorded under T_3-*Rhizobium* + PUK-171 (*Stenotrophomonas rhizophila*) whereas minimum (27.91%) was recorded under control plots. Similar results related to seed yield were observed by Hosseini *et al*. (2014) by co-inoculation of *Rhizobium* along with *Pseudomonas* + *Azospirillum*. The data regarding seed and stover yield has been given in Table 4 and graphically depicted in Fig 3.

CONCLUSION

Based on the results, it is concluded that treatment *Rhizobium*+ PUK 171(*Stenotrophomonas rhizophila*) was the most promising since it recorded 26.57% and 53.68% higher CGR during 25-50 DAS and 50 DAS to harvest, respectively. Also 30.80% and 23.90% more number of nodules over control at 25 and 50 DAS, respectively. As a result, highest seed yield (1006 kg), maximum harvest index (30.47%) and B: C ratio (1.54) were also recorded under the same treatment.

Conflict of interest: None.

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