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Effect of Consortium of *Rhizobium*, PSB and KMB on Growth and Yield of Mungbean (*Vigna radiata* L.)

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A B S T R A C T

A field experiment was conducted for three consecutive years in four different locations to evaluate the effect of consortium of *Rhizobium*, PSB and potash mobilizing bacteria on growth and yield of mungbean. Among different inoculation treatments, seed inoculation with consortium of *Rhizobium*, PSB and potash mobilizing bacteria along with 75% per cent recommended nitrogen and phosphorus was found most effective as it recorded highest number of nodules (73.41 plant^{-1}), number of pods (37.93 plant^{-1}), grain yield (9.16 q ha^{-1}) and thousand grain weight (38.0 g) and found statistically at par with the treatment of consortium + 100% recommended dose of nitrogen and phosphorus for all growth and yield attributing characters. The results indicated saving of 25% chemical nitrogen and phosphorus fertilizer for mungbean.

Introduction

Legume plant possesses a unique ability to establish symbiosis with nitrogen fixing bacteria of the family Rhizobiaceae. Rhizobia have ability to form nodules on their host plants inside of which they fix nitrogen (Javaid 2009). Phosphorus is the major essential nutrient for plant growth. Phosphorus in soil is immobilized or become less soluble either by absorption or chemical precipitation. Only a small fraction of 'P' is available for uptake by plants. The prime mechanism of mineral phosphate solubilization by microorganism is the production of organic acids and acid phosphatases which mineralize and mobilize phosphorus in soil. Potassium is the third major essential nutrient for plant growth. It plays an essential role for enzyme activation, protein synthesis and photosynthesis.

Some microorganisms in the soil are able to solubilize unavailable forms of potash bearing minerals such as micas, illite and orthoclase, by excreting organic acids which either directly dissolves rock phosphate or chelating silicon ions to bring the potash into solution (Bennett *et al.*, 1998; Barker *et al.*, 1998). It is well known that, phosphate solubilizing

bacteria (PSB) and *Rhizobium* have synergistic effect on legume crops (Cao *et al.*, 2016). Development of consortia containing one strain of *Rhizobium*, PSB and PGPR has been attempted (Bansal 2015), whereas potash mobilizing bacteria increased 'K' availability in soils and increased mineral uptake by plants (Sheng and Huang 2002).

Biofertilizers keep the soil environment rich in all kinds of micro and macro nutrients via nitrogen fixation, phosphate solubilization, potash mobilization and release of plant growth regulating substances (Javaid 2009). Shete *et al.*, (2019) formulated MS III culture medium for growth of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria in a consortium.

Keeping this in view, the present research work was undertaken to study effect of consortia of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria on growth and yield of mungbean.

Materials and Methods

Experimental site

A field experiment was conducted at four different locations *viz*., Biological Nitrogen Fixing Scheme, College of Agriculture, Pune; Agriculture Research Station, Kasbe-Digraj, Sangli; Oilseed Research Station, Jalgaon and Pulse Improvement Project, MPKV, Rahuri under the jurisdiction of Mahatma Phule Krishi Vidyapeeth, Rahuri Dist. Ahmednagar, Maharashtra during three consecutive *kharif* seasons *viz*., 2016-17, 2017-18 and 2018-19 to find out the efficacy of consortium of *Rhizobium*, PSB and potash mobilizing bacteria on growth parameters and yield of mungbean. The experiment was laid out in randomized block design with three replications and eight treatments.

Treatment details

T_1: Uninoculated control
T_2: 100% RDF
T_3: Consortium of *Rhizobium*, PSB and KMB
T_4: Consortium + 100% RDF
T_5: 75% RDF
T_6: Consortium + 75% RDF
T_7: *Rhizobium* + 75% RDN + 100% RDP
T_8: PSB + 75% RDP + 100% RDN

Note: RDF = Recommended dose of fertilizers (20:40:00 NPK kg ha^{-1})

RDN = Recommended dose of nitrogen;
RDP = Recommended dose of phosphorus
PSB = Phosphate solubilizing bacteria; KMB = Potash mobilizing bacteria

Preparation of consortium

A consortium of R*hizobium* sp., phosphate solubilizing bacteria (*Bacillus subtilis*) and potash mobilizing bacteria (*Frateuria aurantia*) was prepared in selective medium MS III (Shete *et al.*, 2019).

Lignite powder based formulations duly formulated by using consortium of R*hizobium*, PSB and KMB (2 x 10^7 cfu g^{-1} lignite powder) was applied to mungbean as seed coating at the time of sowing.

The well decomposed farm yard manure @ 5 t ha^{-1} and recommended dose of chemical fertilizers (20:40:00 NPK kg ha^{-1}) were applied for mungbean.

The observations on plant height (cm), number of branches $plant^{-1}$, number of pods $plant^{-1}$, 1000 grain weight (g), grain yield (q ha^{-1}), available N and P in soil at harvest (kg ha^{-1}), N and P uptake by plants at harvest (kg ha^{-1}) and microbial count of *Rhizobium*, PSB and KMB at flowering stage of the mungbean were recorded.

Microbial analysis

Fresh root nodules of mungbean at flowering stage were analyzed for rhizobial population on yeast extract mannitol agar media as described by Rajendran *et al.,* (2008). Moreover, rhizospheric soil samples at flowering stage of mungbean were analyzed for microbial population of phosphate solubilizing bacteria (PSB) and potash mobilizing bacteria (KMB) using serial dilution of soil and agar plating method (Aneja, 2003).

The PSB and KMB population was enumerated on Pikovskaya's media and Alexandrov's agar media, respectively, at 10^6 dilutions. The plates were incubated at 28±2 ^{0}C temperature for 72 hours and colonies were counted. The population was expressed as cfu g^{-1} soil.

Soil analysis

Soil samples were collected from the experimental plot before and after harvesting of the mungbean for analysis of nutrient status of the soil. The collected soil samples were air dried, crushed in wooden Mortar and Pestle. Soil was sieved through 2 mm sieve. Then the soil samples were analyzed for their chemical properties by using standard analytical methods.

The available nitrogen was estimated by alkaline permanganate method (Subbiah and Asija 1956). The available phosphorus was estimated by sodium bicarbonate (0.5 *M* $NaHCO_3$) method (Olsen *et al.*, 1954).

Plant sample analysis

The plant samples for total N, P, and K analysis were collected separately from each treatment plots at harvesting stage. The plant parts were then kept in paper bags and dried in hot air oven at 70ºC for 48 hours. The dried plant parts were finely grounded in a mixer. This fine powder was again dried in oven at 60ºC for a couple of hours and stored in properly till the samples were used for chemical analysis of nutrients.

Nitrogen content of plant was estimated by following Modified Kjeldahl's process (Jackson 1973) and accordingly N uptake (kg ha^{-1}) was estimated as N% x total dry matter yield (kg ha^{-1})/100.

Phosphorus content of plant was determined by colorimetric method (Jackson 1973) employing vandomolybdate phosphoric yellow color colorimetric method. Yellow color intensity was read in spectrophotometer at 470 nm and accordingly P uptake (kg ha^{-1}) was estimated as P% x total dry matter yield (kg ha^{-1})/100.

Statistical analysis

The data recorded on various parameters was subjected to statistical analysis by following standard method of analysis of variance. The level of significance used in 'F' and 't' tests was P = 0.05. Critical difference (CD) values were calculated where the 'F' test was found significant (Panse and Sukhatme 1985).

Results and Discussion

Inoculation effect of consortium on growth parameters and yield of mungbean

The pooled results in respect of growth and yield attributing characters of mungbean are presented in (Table 1 and 2). It was revealed from the pooled analysis that the growth parameters and grain yield differences were significant due to seed inoculation with consortium.

Table.1 Effect of consortium of *Rhizobium*, PSB and KMB on growth parameters of mungbean (Pooled: 2016-17, 2017-18 and 2018-19)

Treatment	Plant height (cm)					Number of branches plant^{-1}					Number of nodules plant^{-1}				
	BNF Pune	ARS K'Digraj	PIP Rahuri	ORS Jalgaon	Pooled mean	BNF Pune	ARS K'Digraj	PIP Rahuri	ORS Jalgaon	Pooled mean	BNF Pune	ARS K'Digraj	PIP Rahuri	ORS Jalgaon	Pooled mean
T_1:Uninoculated control	50.35	53.51	54.28	53.20	52.84	2.83	3.61	3.26	3.24	3.24	25.03	30.71	30.56	33.83	30.03
T_2: 100% RDF	70.85	67.63	61.87	66.08	66.61	6.12	4.97	3.78	4.00	4.72	60.80	61.91	61.77	65.63	62.53
T_3: Consortium	67.92	60.74	56.88	57.72	60.82	6.27	4.55	3.30	3.24	4.34	70.70	63.27	57.53	58.87	62.59
T_4: Consortium + 100% RDF	65.52	65.38	62.66	69.38	65.74	6.21	4.79	3.92	4.31	4.81	72.62	73.55	69.46	71.87	72.24
T_5:75% RDF	60.05	62.90	58.22	64.17	61.33	4.80	4.25	3.35	3.59	4.00	55.45	52.65	52.49	56.00	54.15
T_6:Consortium + 75% RDF	72.73	68.99	64.74	69.82	69.07	6.93	5.37	4.09	4.55	5.24	76.65	74.14	70.36	73.97	73.41
T_7:*Rhizobium* + 75% N+100% P	61.67	61.82	60.06	66.38	62.48	5.49	4.42	3.40	3.96	4.32	60.73	65.28	63.64	70.80	65.11
T_8:PSB + 75% P + 100% N	68.54	67.40	61.24	67.01	66.05	5.15	4.54	3.69	3.97	4.34	68.10	65.92	60.31	66.47	65.20
SEm ±	2.26	2.66	0.90	1.54	1.28	0.35	0.39	0.09	0.12	0.24	4.64	0.85	2.94	3.10	1.57
CD at 5%	6.78	8.06	2.73	4.62	3.75	1.05	1.18	0.27	0.36	0.71	13.92	2.57	8.88	9.30	4.61

RDF- Recommended dose of fertilizers (20:40:00 NPK kg ha^{-1})
Consortium- Consortia of *Rhizobium*, PSB and KMB
PSB- Phosphate solubilizing bacteria
KMB- Potash mobilizing bacteria

Table.2 Effect of consortium of *Rhizobium*, PSB and KMB on growth parameters and yield of mungbean (Pooled:2016-17, 2017-18 and 2018-19)

Treatment	Number of pods plant^{-1}					1000 grain weight (g plant^{-1})					Grain yield (q ha^{-1})				
	BNF Pune	ARS K'Digraj	PIP Rahuri	ORS Jalgaon	Pooled mean	BNF Pune	ARS K'Digraj	PIP Rahuri	ORS Jalgaon	Pooled mean	BNF Pune	ARS K'Digraj	PIP Rahuri	ORS Jalgaon	Pooled mean
T_1:Uninoculated control	23.52	20.88	12.81	24.50	20.43	27.92	31.05	28.19	32.27	29.85	6.10	6.58	5.13	6.49	6.08
T_2: 100% RDF	37.95	34.22	24.88	38.87	33.98	37.53	36.98	29.73	39.92	36.04	9.03	8.75	8.30	8.32	8.60
T_3: Consortium	33.99	28.84	16.86	30.01	27.42	33.77	33.71	28.38	34.45	32.58	7.30	7.14	6.25	6.98	6.92
T_4: Consortium + 100% RDF	39.90	35.82	26.93	40.98	35.91	38.30	37.03	30.10	40.99	36.61	9.74	9.04	8.51	8.90	9.05
T_5:75% RDF	32.10	29.37	19.53	33.97	28.74	33.94	36.06	28.54	37.87	34.10	8.01	8.32	7.32	7.44	7.77
T_6:Consortium + 75% RDF	40.45	37.68	31.40	42.18	37.93	39.58	38.77	31.64	41.99	38.00	9.79	9.13	8.63	9.08	9.16
T_7:*Rhizobium* + 75% N+100% P	36.63	33.71	20.21	36.96	31.88	35.25	36.19	29.26	39.12	34.96	8.64	8.43	7.55	7.97	8.15
T_8:PSB + 75% P + 100% N	36.01	30.02	20.64	36.76	30.86	35.52	35.84	29.37	38.98	34.93	8.66	8.19	8.14	8.05	8.26
SEm ±	3.40	1.53	1.61	1.56	0.70	1.05	0.74	0.31	0.34	0.61	0.33	0.13	0.31	0.16	0.13
CD (P ≤ 0.05)	10.20	4.64	4.83	4.68	2.05	3.15	2.24	0.93	1.83	1.80	0.99	0.41	0.97	0.48	0.38

Table.3 Effect of consortium of *Rhizobium*, PSB and KMB on N and P uptake by mungbean

Treatment	'N' uptake by plants (kg ha^{-1})				'P' uptake by plants (kg ha^{-1})			
	BNF, Pune	PIP, Rahuri	ORS, Jalgaon	Pooled Mean	BNF, Pune	PIP,Rahuri	ORS, Jalgaon	Pooled Mean
T_1:Uninoculated control	11.41	13.08	11.75	12.65	4.21	2.31	3.96	3.53
T_2: 100% RDF	19.41	23.16	15.97	19.69	8.22	5.64	5.49	6.45
T_3: Consortium	12.92	19.94	12.77	15.64	4.75	5.38	4.68	5.05
T_4: Consortium + 100% RDF	20.45	24.25	18.69	21.27	6.62	4.00	10.86	7.15
T_5:75% RDF	18.34	24.38	14.06	19.43	6.33	5.56	4.39	5.52
T_6:Consortium + 75% RDF	22.81	30.12	18.61	24.00	12.04	4.32	9.99	8.61
T_7:*Rhizobium* + 75% N+100% P	20.48	22.50	15.78	19.89	8.55	5.36	5.42	6.44
T_8:PSB + 75% P + 100% N	20.61	25.15	15.30	20.32	10.31	5.54	5.15	6.94
SEm ±	0.07	0.45	0.06	0.18	4.21	2.31	3.96	3.53
CD (P ≤ 0.05)	0.21	NS	0.17	NS	0.04	0.03	0.17	NS

Table.4 Effect of consortium of *Rhizobium*, PSB and KMB on available 'N' and 'P' after harvest in soil

Treatment	Available 'N' (kg ha^{-1}) in soil after harvest				Available 'P' (kg ha^{-1}) in soil after harvest			
	BNF, Pune	PIP,Rahuri	ORS, Jalgaon	Pooled.Mean	BNF, Pune	PIP,Rahuri	ORS, Jalgaon	Pooled Mean
T_1:Uninoculated control	161.67	114.67	157.33	144.55	13.86	13.69	10.53	12.69
T_2: 100% RDF	177.00	94.83	214.33	163.05	21.25	13.29	17.47	17.34
T_3: Consortium	173.00	96.33	209.33	159.55	17.37	11.17	13.00	13.85
T_4: Consortium + 100% RDF	192.00	120.33	212.67	175.00	27.07	14.42	11.47	17.66
T_5:75% RDF	173.00	101.83	208.33	161.05	25.78	11.80	12.23	16.60
T_6:Consortium + 75% RDF	206.67	129.83	209.33	181.94	23.10	16.99	18.97	19.69
T_7:*Rhizobium* + 75% N+100% P	180.33	114.83	217.33	169.83	17.28	15.41	13.47	15.39
T_8:PSB + 75% P + 100% N	177.00	104.17	209.00	163.39	19.68	14.67	15.13	16.49
SEm ±	0.39	0.47	0.43	8.42	2.575	2.05	0.33	1.68
CD (P ≤ 0.05)	1.18	1.54	1.36	NS	7.786	NS	1.00	NS

Table.5 Effect of consortium of *Rhizobium*, PSB and KMB on Microbial population at flowering stage of mungbean

Treatment	Microbial population at flowering (x10^6 cfu g^{-1} soil)		
	Rhizobium	PSB	KMB
T_1:Uninoculated control	12.67	15.00	10.67
T_2: 100% RDF	17.00	21.67	15.67
T_3: Consortium	50.33	46.33	40.00
T_4: Consortium + 100% RDF	56.67	55.00	50.33
T_5:75% RDF	45.00	26.67	25.67
T_6:Consortium + 75% RDF	60.33	58.67	52.33
T_7:*Rhizobium* + 75% N+100% P	50.00	36.67	32.67
T_8:PSB + 75% P + 100% N	41.67	45.33	37.33
SEm ±	3.22	4.07	4.03
CD (P ≤ 0.05)	9.74	12.30	12.18

Table.6 Economics as influenced by seed inoculation of consortium of *Rhizobium*, PSB and KMB in mungbean (Pooled: 2016-17, 2017-18 and 2018-19)

Treatment	Grain yield (q ha^{-1})	Gross Monitory Returns (Rs. ha^{-1})	Cost of Cultivation (Rs. ha^{-1})	Net Monitory Returns (Rs. ha^{-1})	B:C ratio
T_1:Uninoculated control	6.08	31531	29714	1817	1.06
T_2: 100% RDF	8.60	44641	31794	12847	1.40
T_3: Consortium	6.92	35944	30354	5590	1.18
T_4: Consortium + 100% RDF	9.05	47060	32434	14626	1.45
T_5:75% RDF	7.77	40388	31274	9114	1.29
T_6:Consortium + 75% RDF	9.16	47632	31914	15718	1.49
T_7:*Rhizobium* + 75% N+100% P	8.15	42313	31715	10598	1.33
T_8:PSB + 75% P + 100% N	8.26	42928	31352	11576	1.37

Note: Consortium: *Rhizobium*, PSB and KMB consortium,
Selling rate of mungbean: 2016-17: Rs.5000 q^{-1}, 2017-18: Rs.5200 q^{-1} and 2018-19: Rs.5400 q^{-1}
Total cost of cultivation excluding inorganic fertilizers: Rs. 29714 ha^{-1}

Among different inoculation treatments, the treatment T_6 i.e. seed inoculation with consortium of *Rhizobium*, PSB and KMB + 75% per cent recommended nitrogen and phosphorus was found most effective as it recorded significantly highest plant height (69.07 cm), number of branches (5.24 plant^{-1}), number of nodules (73.41 plant^{-1}), number of pods (37.93 plant^{-1}), thousand grain weight (38.0 g) and grain yield (9.16 q ha^{-1}) of mungbean and found statistically at par with the treatment T_4 (consortium + 100% recommended dose of nitrogen and phosphorus) for all growth and yield attributing characters. These results are in agreement with the results of Bansal R.K. (2009) who reported that pre-sowing inoculation of mungbean seeds with different inoculants (*Rhizobium*, PGPR and PSB) alone or in combination, significantly increased the nodulation and grain yield over uninoculated control. Moreover, Cao *et al.*, (2016) reported that the application of rhizobial inoculant and/or PSB inoculant did not have significant difference with chemical fertilizer, they increased grain yield from 15 to 19% in comparison to control. A similar result was reported by Rajendran *et al.*, (2008) and Qureshi *et al.*, (2011).

Nutrient uptake

The pooled results in respect of N and P uptake by mungbean are presented in (Table 3). Although the data was found to be non-significant, the maximum N and P uptake (24.0 and 8.61 kg ha^{-1}, respectively) was recorded in treatments T_6 (Consortium + 75% RDF) followed by T_4 (Consortium+100% RDF) (21.27 and 7.15 kg ha^{-1}, respectively). Qureshi *et al.*, (2011) reported that rizobial inoculation produced highest N-content in grains (3.23 and 3.24%) followed by co-inoculation (3.21 and 3.19%) at half and full dose of P fertilizer, respectively.

Available soil nutrients: After harvest of crop, soil was analyzed for available soil nutrients and the pooled data was analyzed and tabulated in (Table 4). The available soil N and P was found maximum (181.94 and 19.69 kg ha^{-1}, respectively) under the treatment T_6 (consortium+75% RDF) followed by T_4 (Consortium+100% RDF) (175.0 and 17.66

kg ha^{-1}, respectively). Qureshi *et al.,* (2011) reported that the highest soil N was observed with co-inoculation i.e. 0.037 and 0.039% followed by *Rhizobium* i.e. 0.036 and 0.037% at 25 and 50 kg P ha^{-1}, respectively.

Inoculation effect of consortium on microbial population

At flowering stage, soil samples were analyzed for microbial population of phosphate solubilizing bacteria (PSB) and potash mobilizing bacteria (KMB) and fresh root nodules of mungbean were analyzed for rhizobial population and data was analyzed and presented in (Table 5). Among different inoculation treatments, T_6 (consortium+75% RDF) recorded significantly highest population of *Rhizobium*, PSB and KMB (60.33, 58.67 and 52.33 x 10^6 cfu g^{-1} soil, respectively) at flowering stage of mungbean and was found statistically at par with the treatment T_4 (Consortium + 100% RDF) for microbial population of *Rhizobium*, PSB and KMB (56.67, 55.00 and 50.33 x 10^6 cfu g^{-1} soil, respectively). These results are in agreement with the results of Cao *et al.,* (2016) who reported that the application of rhizobial inoculant and/or PSB inoculant to soybean significantly increased the population of *Rhizobium* and PSB at flowering stage of soybean. Moreover, Swedrzynska and Sawicka (2001) reported that inoculation of cereals with *Azospirillum brasilense* bacteria contributed to the increase in their population at different growth stages of crops and the counts were higher in inoculated treatments compared to uninoculated control.

Cost: Benefit analysis

The economics as influenced by seed inoculation of consortium of *Rhizobium*, PSB and KMB in mungbean is presented in (Table 6). The highest net monetary returns (Rs.15718/- ha^{-1}) and B:C ratio (1.49) was observed in treatment T_6 (Consortium + 75% RDF) followed by treatment T_4 (Consortium + 100% RDF) (Rs.14626/- ha^{-1} and 1.45, respectively). Jilani *et al.,* (2007) reported that integration of half dose of NP fertilizers with biofertilizers gave crop yield as with full rate of fertilizer and through reduced use of fertilizers, the production cost was minimized and the net return maximized.

In conclusion the seed inoculation with consortium of *Rhizobium*, PSB and KMB+ 75% per cent recommended nitrogen and phosphorus was found at par with the treatment of consortium + 100% recommended dose of nitrogen and phosphorus for all growth and yield attributing characters of mungbean. The results indicated saving of 25% chemical nitrogen and phosphorus fertilizer for mungbean.

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