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Growth and development of blackgram (*Vigna mungo*) under co-inoculation of different bacterial cultures with *Rhizobium phaseoli*

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Abstract
The present study was aimed to improve the growth and development of Blackgram (*Vigna mungo*) under co-inoculation of different bacterial cultures with *Rhizobium phaseoli*. Experiment consist of ten treatments in which eight pre-evaluated bacterial cultures in laboratory (*Rhizobium phaseoli, Bacillus megaterium, Bacillus subtilis, Bacillus polymyxa, Pseudomonas striata, Pseudomonas flurescens, Azotobacter chroococcum* and *Azospirilllum lipoferum*) and were used with recommended dose of fertilizer (RDF) in randomized block design (RBD). Seed treatment of black gram was done with bacterial cultures along with application RDF at the time of sowing. Results emerged out indicated that the height, number of branches, number of nodules and its fresh and dry weight, weight of shoot and root (both fresh and dry), root density, shoot: root ratio, grain yield, dry matter yield, test weight, protein content and protein yield were significantly improved by co-inoculation over non-inoculation and single inoculation of *Rhizobium phaseoli*. The co-inoculation of *Rhizobium phaseoli + Bacillus megaterium, Rhizobium phaseoli + Pseudomonas striata* and *Rhizobium phaseoli + Pseudomonas flurescense* found to be at par with each other and having more potential than the other combinations.

Keywords: Growth, development, biometric, co-inoculation, bacterial cultures

Introduction
Before emergence of Soybean as main field crop of Marathwada region (Maharashtra state, India) blackgram is one of the important legume crop which sustain the economy of farmers. But due to lack of researchers attention blackgram suffered from lots of problems like outbreaks of diseases, increased attack of pests and misbalancing of fertilizers, the area under it gradually reduced and ultimately its production lowered. Blackgram is annual pulse crop and native to Central Asia. This crop is endowed with many desirable characters like, short in duration, restorative (soil fertility building crops), low water requiring and highly suitable to be grown in mixed or intercropping systems and also as a catch crop to scavenge the residual soil moisture and fertility. This is an excellent source of high-quality protein. Black gram is the main source of deity protein (24%), carbohydrate (67%), Fibre (3.5%), fat (1.74%) and major portion of lysine in a vegetarian diet (Elangaimannan *et al*. 2008) [6]. Keeping these points in view the present study were carried out by using different bacterial cultures. Different bacterial genera are vital components of soils. They are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and sustainable for crop production. In the rhizosphere the synergism between various bacterial genera such as *Bacillus*, *Pseudomonas* and *Rhizobium* has been demonstrated to promote plant growth and development. Compared to single inoculation, co-inoculation has improved the absorption of nitrogen, phosphorus and mineral nutrients by plants (Dashadi *et al*. 2011) [5].

Materials and Methods
A field experiment was conducted during *kharif* season of 2018 at Research farm, Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani to assess the co-inoculation effect of different bacterial cultures with *Rhizobium phaseoli* on nutrient dynamics. Experiment consist of ten treatments in which eight pre-evaluated bacterial cultures in laboratory (*Rhizobium phaseoli, Bacillus megaterium, Bacillus subtilis, Bacillus polymyxa, Pseudomonas striata, Pseudomonas flurescens, Azotobacter chroococcum and Azospirilllum lipoferum*) and were used with recommended dose of fertilizer (25:50:00:10 N:P:K and S kg ha^{-1} respectively) in randomized block design. Seed treatment of

black gram was done with bacterial cultures along with application RDF at the time of sowing. The observations on plant height, number of branches plant^{-1}, number of nodules plant^{-1}, fresh and dry weight of nodule, root and shoot, root density and root: shoot ratio were recorded manually on five randomly selected representative plants from each plot of each replication separately at two stages i.e. at flowering and pod filling stage. The test weight, seed yield, straw yield and protein content were also recorded as per the standard methods. The data obtained were statistically analyzed and appropriately interpreted as per the methods described by Panse and Sukhatme (1985). Appropriate standard error (S.E.) and critical differences (C.D.) at 5 per cent levels were worked out for interpretation of result.

Result and Discussion

Effect on plant height and number of branches

The data pertaining in Table 1 indicates significant impact of different bacterial cultures with *Rhizobium phaseoli* on plant height and number of branches of black gram both at flowering and pod filling stage.

Plant height (cm)

Height of plant was recorded maximum in treatment T_7 (RDF + *Rhizobium phaseoli* + *Pseudomonas striata*) both at flowering and pod filling stage (28.43 cm and 30.08 cm respectively) over other treatments. Results revealed that the co-inoculated plants showed significant increase in plant height over un-inoculated T_1 (absolute control), T_2 (RDF) and T_3 (RDF + *Rhizobium phaseoli*).

Number of branches

Regarding to number of branches per plant the same number of branches observed at both flowering and pod filling stage. The co-inoculated treatments showed non-significant positive impact over control and mono-inoculated. Among all treatments treatment T_7 (6.87) showed maximum number of branches than the other.

Table 1: Effect of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on height and number of branches in blackgram

Sr. No.	Treatments	Plant height (cm)		Number of brnches (Found same at flowering and pod filling)
		At flowering	At pod filling	
T_1	Absolute control	19.56	21.30	5.40
T_2	Only RDF	22.01	23.42	5.60
T_3	RDF + *Rhizobium phaseoli*	23.43	24.93	5.73
T_4	T_3 + *Bacillus megaterium*	27.64	29.11	6.40
T_5	T_3 + *Bacillus subtilis*	24.47	26.17	6.27
T_6	T_3 + *Bacillus polymyxa*	24.13	25.47	5.93
T_7	T_3 + *Pseudomonas striata*	28.43	30.08	6.87
T_8	T_3 + *Pseudomonas flurescens*	27.85	29.20	6.47
T_9	T_3 + *Azotobacter chroococcum*	26.45	27.63	6.07
T_{10}	T_3 + *Azospirillum lipoferum*	23.58	24.81	5.60
	S.Em.±	1.66	1.60	0.48
	C.D. at 5%	4.94	4.75	NS

Our results collaborated with the findings of Mishra *et al.* (2011) [12] and Singh *et al.* (2016) [18]. The availability of nutrient accumulates more dry matter which reflected through various plant growth parameters like plant height, number of branches and number of leaves and nutrient made available more efficiently by dual inoculation than the single. Similarly, Nalawde *et al.* (2015) [13] found that black gram seed treated with bio-fertilizers showed significant improvement in the parameters like, number of leaves, length of leaves and breadth of leaves. Similar results were found by Patel *et al.* (2017) [15] Similarly, Mir *et al.* (2013) [11].

Effect on number and weight of nodules

The data presented in Table 2 shows that the influence of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on number of nodules per plant and its fresh and dry weight.

Number of nodules per plant

In case of number of nodules per plant at both flowering and pod filling stage treatment T_7 (55.50) receiving co-inoculation of *Rhizobium phaseoli* and *Pseudomonas striata* along with RDF found significantly superior over other treatments at flowering stage and treatment T_4 (51.60) receiving co-inoculation of *Rhizobium phaseoli* with *Bacillus megaterium* and T_8 (48.64) receiving co-inoculation of *Rhizobium phaseoli* with *Pseudomonas flurescens* found at par with T_7. Least number of nodules was observed in treatment T_1 (absolute control) both at flowering (34.24) and pod filling stage (15.30). The enhancement in nodule number, nodular mass and consequently yield components due to combined inoculation might be the expansion in root length and mass, thus more number of active sites for nodulation by the rhizobial strains. The curling of root hairs and infection thread are the key steps during nodule development that was boosted with co-inoculation due to the production of growth hormones (Qureshi *et al.* 2011) [16]. Earlier workers, Biswas and Bhowmick (2008) [4] found that co-inoculation of *Rhizobium* with PGPR in black gram recorded the highest mean nodule number over control and mono-inoculation of *Rhizobium* or PGPR. Similarly, Selvakumar *et al.* (2009) reported number of nodules were maximum in combined effect of *Rhizobium* with phosphobacteria than that of single inoculation. Similar results were found by Dashadi *et al.* (2011) [5], Mishra *et al.* (2011) [12] and Stajkovic *et al.* (2011) [19].

Table 2: Effect of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on number of nodule and fresh and dry weight of nodule in black gram

Sr. No.	Treatments	No. of nodules per plant		Weight of nodule (mg)			
		At flowering	At pod filling	At flowering		At pod filling	
				Fresh	Dry	Fresh	Dry
T_1	Absolute control	34.24	15.30	27.87	17.37	51.08	35.16
T_2	Only RDF	38.91	22.40	31.94	21.68	60.34	47.33
T_3	RDF + *Rhizobium phaseoli*	37.14	22.32	34.69	27.99	69.65	47.90
T_4	T_3 + *Bacillus megaterium*	51.60	30.53	48.08	34.10	80.63	55.41
T_5	T_3 + *Bacillus subtilis*	32.34	13.83	47.92	33.24	53.46	34.87
T_6	T_3 + *Bacillus polymyxa*	34.61	19.45	40.85	30.52	66.99	45.52
T_7	T_3 + *Pseudomonas striata*	55.50	40.02	52.11	36.24	103.48	76.56
T_8	T_3 + *Pseudomonas flurescens*	48.64	30.62	49.34	37.04	92.97	66.71
T_9	T_3 + *Azotobacter chroococcum*	39.09	19.20	41.77	24.33	56.45	37.64
T_{10}	T_3 + *Azospirillum lipoferum*	38.15	22.15	45.46	30.58	90.15	64.22
	S.Em.±	2.60	1.76	0.95	1.01	4.92	3.38
	C.D. at 5%	7.74	5.23	2.83	3.00	14.61	10.06

Weight of nodule

In case of fresh weight of nodules treatment T_7 receiving co-inoculation of *Rhizobium phaseoli* with *Pseudomonas striata*, showed maximum value at both flowering (52.11 mg) and pod filling stage (103.48 mg) over the other treatments and treatment T_8 having co-inoculation of *Rhizobium phaseoli* with *Pseudomonas flurescens* found at par at flowering stage (49.34 mg). Similarly, treatments T_8 (92.97 mg) and T_{10} having co-inoculation of *Rhizobium phaseoli* with *Azospirillum lipoferum* (90.15 mg) found at par at pod filling stage with T_7. In case of dry weight of nodules treatment T_8 (37.04 mg) found significant over other treatments at flowering stage and found at par with T_4 receiving co-inoculation of *Rhizobium phaseoli* with *Bacillus megaterium* (34.10 mg) and T_7 (36.24 mg) and at pod filling stage treatment T_7 (76.56 mg) found maximum value over other treatments and treatment T_8 (66.71 mg) found at par with T_7. The least fresh and dry weight of nodules at both flowering (27.84 and 17.37 mg) and pod filling stage (51.08 and 35.16 mg) was recorded in T_1 which is absolute control. The nodulation effect is a result of rhizobia inoculation and boosted by other bacterial cultures and due to microbial co-inoculation number of halo nodules reduced and hence weight of nodule both fresh and dry found maximum in co-inoculated plots.

In line with our work, Biswas and Bhowmick (2008) [4] recorded co-inoculation with PGPR at all the treatments increased the mean nodule weight at 45 DAS in comparison to the inoculation with *Rhizobium* alone. Furthermore, Mishra *et al.* (2011) [12] found significant increase in nodule dry weight (1.08-1.39 times) by co-inoculation of different strains of *Pseudomonas sp*. and *Rhizobium sp.* over the control and mono-inoculation. Similarly, Stajkovic *et al.* (2011) [19], Bhattacharjya and Chandra (2013) [3], Mariangela *et al.* (2013) [10], Korir *et al.* (2017) [8] and Tiwari *et al.* (2017) [20] found that the co-inoculation of bacterial cultures with native *Rhizobium sp.* enhance the fresh and dry weight of nodule.

Effect on weight of shoot and root, root density and shoot: root ratio

The data recorded in Table 3 expressed the fresh and dry weight of shoot and root, root density and shoot: root ratio at flowering and pod filling stage.

Weight of shoot

Regarding to fresh weight of shoot treatment T_8 receiving co-inoculation of *Rhizobium phaseoli* with *Pseudomonas flurescens* found significantly superior at flowering (12.18 g) over rest of treatments and T_4 (10.69 g) and T_7 (10.95 g) found at par with T_8 but at pod filling treatment T_7 receiving co-inoculation of *Rhizobium phaseoli* with *Pseudomonas striata* showed significant value (50.16 g) over other treatments and treatments T_4 (41.98 g) and T_8 (44.29 g) followed the T_7 at pod filling stage. The lowest fresh shoot weight was found in treatment T_1 at both flowering (8.70 g) and pod filling stage (32.16 g). In case of dry weight of shoot treatment T_8 at flowering (4.06 g) and treatment T_7 at pod filling (27.16 g) showed significant value over other treatments and treatments T_4 (3.56 g) and T_7 (3.75 g) found at par with T_8 at flowering stage and treatments T_8 (25.69 g) found at par with T_7 at pod filling stage. The lowest dry shoot weight was found in treatment T_1 at both flowering (2.90 g) and pod filling stage (17.54 g). The PGPR effect of co-inoculation might have reflected on weight of shoot.

Our results collaborates with, Mishra *et al.* (2011) [12] found that the shoot dry-weight gains due to co-inoculation of *Pseudomonas sp.* with *R. Leguminosarum* varied from 1.33 to 1.89 times as compared to *R. leguminosarum* (0.011 g) alone. Further, Stajkovic *et al.* (2011) [19] found that co-inoculation of *Rhizobium* with *Bacillus* strains in bean influenced positively on shoot dry weight (3.07 mg plant^{-1}) compared to inoculation with *Rhizobium* alone (2.75 mg plant^{-1}). Moreover, Bhattacharjya and Chandra (2013) [3] stated that *Mesorhizobium* sp. and PGPR alone inoculation significantly increased plant dry weight, by 33.6 and 42.4%, over uninoculated control but their co-inoculation gave significant increases in plant dry weight by 11.5% over PGPR and Rhizobium alone.

Similarly, Tiwari *et al.* (2017) [20] reported fresh and dry shoot biomass (12.99 and 3.21 g) by co-inoculation of *Rhizobium* and *Azotobacter sp*. over control and mono-inoculation. Like, Patel *et al.* (2017) [15] stated that application of PSB with *Rhizobium* significantly increased the weight of dry matter plant^{-1} (8.12 g) over the un-inoculated control.

Weight of root

The data presented in Table 7 showed the fresh and dry weight of root at flowering and pod filling stage. In case of fresh weight of root, treatment T_8 having dual inoculation of *Rhizobium phaseoli* and *Pseudomonas flurescens* at flowering (690.00 mg) and pod filling (2106.67 mg) showed significant value over other treatments and treatments T_4 (683.33 mg), T_5 (610.00 mg) and T_7 (636.67 mg) found at par with T_8 at flowering stage and treatment T_7 (1980.00 mg) found at par with T_8 at pod filling stage. The lowest fresh root weight was found in treatment T_1 at both flowering (490.00 mg) and pod filling stage (1000.33 mg). Regarding to dry weight of root treatment T_8 at flowering (210.00 mg) and pod filling (756.67 mg) showed significant value over other treatments and treatments T_4 (200 mg) and T_7 (206.67 mg) found at par with T_8 at flowering stage and treatments T_7 (693.33 mg) found at par with T_8 at pod filling stage. The lowest dry root weight was found in treatment T_1 at both flowering (110.00 mg) and pod filling stage (353.33 mg).

Our findings matched with, Stajkovic *et al.* (2011) [19] found that co-inoculation of *Rhizobium* with Bacillus strains in bean influenced positively on root dry weight (0.973 mg plant^{-1}) compared to inoculation with *Rhizobium* alone. Further, Mishra *et al.* (2011) [12] also found that the root dry-weight gains due to co-inoculation of *Pseudomonas sp.* with *R. leguminosarum* varied from 1.16 to 3.08 times as compared to *R. leguminosarum*- PR1 inoculation. Similarly, Tiwari *et al.* (2017) [20] reported fresh and dry root biomass (3.54 and 0.99 g) by co-inoculation of *Rhizobium* and *Azotobacter sp.* over control and mono-inoculation.

Root density (g cc^{-1})

Regarding to root density the treatment T_4 receiving co-inoculation of *Rhizobium phaseoli* and *Bacillus megaterium* (1.08 g cc^{-1}) showed maximum value over other treatments and treatments T_7 (1.06 g cc^{-1}) and T_8 (1.03 g cc^{-1}) found at par with T_4 at flowering but at pod filling treatment T_8 receiving co-inoculation of *Rhizobium phaseoli* and *Pseudomonas flurescens* (1.98 g cc^{-1}) showed significant value over other treatments and treatment T_4 (1.95 g cc^{-1}), T_7 (1.92 g cc^{-1}), T_9 (1.83 g cc^{-1}) and T_{10} (1.89 g cc^{-1}) found at par with T_8. The least value found in treatment T_1 at both flowering (0.78 g cc^{-1}) and pod filling stage (1.36 g cc^{-1}).

Shoot: Root ratio

In case of shoot: root ratio all treatments found non-significant at flowering stage. Although all co-inoculated treatments shows higher value over the control and single inoculated. But at pod filling shoot: root ratio found significant at treatment T_1 (51.78) showed maximum value over all other treatments and followed by T_4 (39.47) and T_8 (36.04).

Our results were collaborated with findings of Tiwari *et al.* (2017) reported that the bacteria from groups like *Azotobacter, Bacillus, Pseudomonas, Azospirillum* etc. have friendly associations with *Rhizobium* and they have different physiology and habitat hence they help plant growth promotion by them own system. Therefore, such combinations can be recommended for field application for sustainable agriculture. Due to availability of nutrient at critical growth stages of black gram crop dry matter accumulation increased which reflects in weight of shoot and root and weight of root and shoot reflects in root density and shoot: root ratio.

Table 3: Effect of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on fresh and dry weight of shoot and root in black gram

Sr. No.	Treatments	Weight of shoot per plant (g)				Weight of root per plant (mg)				Root density (g cc^{-1})		Shoot: Root Ratio	
		At flowering		At pod filling		At flowering		At pod filling					
		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	At flowering	At pod filling	At flowering	At pod filling
T_1	Absolute control	8.70	2.90	32.16	17.54	490.00	110.00	1000.33	353.33	0.78	1.36	26.34	51.78
T_2	Only RDF	9.28	3.29	37.59	19.73	513.33	146.67	1425.00	523.33	0.81	1.65	23.20	37.87
T_3	RDF + *Rhizobium phaseoli*	9.33	3.11	37.97	19.89	586.67	150.00	1490.00	563.33	0.84	1.66	20.79	35.78
T_4	T_3 + *Bacillus megaterium*	10.69	3.56	41.98	24.49	683.33	200.00	1756.67	650.00	1.08	1.95	18.73	39.47
T_5	T_3 + *Bacillus subtilis*	9.12	3.03	38.71	20.07	610.00	180.00	1610.00	623.33	0.87	1.67	16.83	32.23
T_6	T_3 + *Bacillus polymyxa*	8.39	2.79	38.43	20.19	593.33	143.33	1336.67	523.33	0.94	1.67	20.36	38.63
T_7	T_3 + *Pseudomonas striata*	10.95	3.75	50.16	27.16	636.67	206.67	1980.00	693.33	1.06	1.92	17.33	35.38
T_8	T_3 + *Pseudomonas flurescens*	12.18	4.06	44.29	25.69	690.00	210.00	2106.67	756.67	1.03	1.98	19.30	36.04
T_9	T_3 + *Azotobacter chroococcum*	9.78	3.16	38.98	20.57	593.33	160.00	1583.33	630.00	0.85	1.83	19.92	32.68
T_{10}	T_3 + *Azospirillum lipoferum*	9.46	3.21	38.65	20.14	606.67	153.33	1573.33	630.00	0.91	1.89	21.50	32.01
	S.Em.±	0.50	0.23	0.86	0.79	26.45	11.55	46.10	22.97	0.04	0.05	2.11	2.92
	C.D. at 5%	1.48	0.69	2.55	2.35	78.59	34.32	136.97	68.26	0.13	0.16	NS	8.68

Effect on yield attributing characters and quality parameters of blackgram

The data presented in Table 4 indicates yield attributing characters (number of pods per plant, seed and straw yield) and quality parameters (protein content and test weight) of blackgram that significantly influenced by co-inoculation of different bacterial cultures with *Rhizobium phaseoli*.

Effect on number of pods

In case of number of pods per plant treatment T_7 receiving co-inoculation of *Rhizobium phaseoli* with *Pseudomonas striata* showed significantly higher (21.30) than other treatments followed by treatment T_4 (18.35) with RDF + *Rhizobium phaseoli* + *Bacillus megaterium* and T_8 (18.32) with RDF + *Rhizobium phaseoli* + *Pseudomonas fluorescens* and lowest value was found in T_1 (6.55) with absolute control.

Effect on Seed and Straw yield

Significantly highest seed yield and straw yield of black gram was noted in T_7 (628.31 and 1407.50 kg ha^{-1}) over other treatments and treatment T_4 (614.14 and 1384.28 kg ha^{-1}) and T_8 (602.47 and 1309.60 kg ha^{-1}) were found statistically at par with T_7 and in case of both seed and straw yield lowest value was recorded in T1 (487.78 and 940.92 kg ha^{-1}) in absolute control. The increase in the seed and straw yield with co-inoculation might be results of growth promotion by making more nutrients available at critical growth stages of the crop.

Table 4: Effect of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on seed and straw yield in black gram

Sr. No.	Treatment	No. of pods per plant	Seed yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Test weight (g)	Protein content (%)
T_1	Absolute control	6.55	487.78	940.92	49.25	17.90
T_2	Only RDF	10.82	517.29	1045.08	49.44	19.29
T_3	RDF + *Rhizobium phaseoli*	11.25	571.60	1233.16	51.23	20.19
T_4	T_3 + *Bacillus megaterium*	18.35	614.14	1384.28	53.57	22.33
T_5	T_3 + *Bacillus subtilis*	15.65	575.31	1151.82	51.58	21.79
T_6	T_3 + *Bacillus polymyxa*	10.75	495.06	1095.75	51.41	20.65
T_7	T_3 + *Pseudomonas striata*	21.30	628.31	1407.50	55.19	23.44
T_8	T_3 + *Pseudomonas flurescens*	18.32	602.47	1309.60	53.81	23.08
T_9	T_3 + *Azotobacter chroococcum*	11.78	498.77	1114.94	53.28	21.46
T_{10}	T_3 + *Azospirillum lipoferum*	11.00	549.38	1136.68	52.77	21.23
	S.Em.±	0.95	14.43	43.24	0.78	0.37
	C.D. at 5%	2.79	42.88	128.47	2.30	1.10

Similarly Kumar *et al*. (2016) [9] showed the consortia of bio-inoculants were more efficient at growth promotion and disease suppression than the strains applied individually and this might resulted in higher yield. Earlier, Biswas and Bhowmick (2008) [4] reported that co-inoculation of *Rhizobium* and PGPR recorded the highest mean values of dry matter accumulation, at harvest, as compared with only *Rhizobium* inoculation and control. In case of grain yield *Rhizobium* inoculum alone increased the mean seed yield by 5.93% over uninoculated control but dual inoculation with *Rhizobium* and PGPR strains recorded more seed yield over single inoculation with *Rhizobium*. Similar results found by Jain and Trivedi (2005) [7] reported that the application of *Rhizobium* co-inoculated with PSB registered highest seed yield and straw yield over control in soybean.

Moreover, Selvakumar *et al.* (2009) also noted that the biofertilizers alone and in co-inoculation and results revealed *Rhizobium* + *Phosphobacteria* showed maximum number of pods $plant^{-1}$ and grain yield and all other co-inoculated treatments showed maximum number of pods $plant^{-1}$ and grain yield over the single inoculation and control. Further, Verma *et al.* (2010) [21] found the grain yield and straw yield was increased significantly in co-inoculation of *Rhizobium* sp. with *Pseudomonas sp*. followed by *B. megaterium* and *A. chroococcum* over uninoculated control.

Singh *et al.* (2016) [18] also reported seed inoculation of biofertilizer treatment *Rhizobium* + PSB produced significantly highest number of pods $plant^{-1}$ grain yield and straw yield which was at par with *Rhizobium* and PSB alone. Moreover, Amule *et al.* (2017) [1] found the average impact of mono-inoculation on seed yield against co-inoculation and found to be 7 per cent more yield due to co-inoculation practice over mono-inoculation, while it was 8 per cent higher with straw yield. Similarly, Korir *et al*. (2017) [8] noted that co-inoculation of bacterial cultures and *Rhizobium* has a synergistic effect on black gram. Use of different bacterial cultures along with *Rhizobium* may improve effectiveness of *Rhizobium* biofertilizers. Selvakumar *et al.* (2009) also stated that single inoculation biofertilizers could not increase the yield as compared to dual inoculation. The dual inoculation of phosphobacteria and *Rhizobium* gave highest yield. It is evident that the increases in plant height, leaf number and leaf area have contributed to increased yield.

Effect on test weight and protein content

Test weight and protein content of blackgram significantly influenced by co-inoculation of different bacterial cultures with *Rhizobium phaseoli*. Treatment T_7 (RDF + *Rhizobium phaseoli* + *Pseudomonas striata*) was noted significantly superior over other treatments in case of increasing test weight (55.19 g) and protein content (23.44 per cent) and treatment T_4 having co-inoculation of *Rhizobium phaseoli* + *Bacillus megaterium* (53.57 g and 22.33 per cent, respectively), T_8 having co-inoculation of *Rhizobium phaseoli* + *Pseudomonas flurescens* (53.81 g and 23.08 per cent, respectively) and T_9 having co-inoculation of *Rhizobium phaseoli* + *Azotobacter chroococcum* (53.28 g and 21.46 per cent, respectively) found at par with treatment T_8. This might be a result of steady supply on nutrients for proper assimilation in reproductive plant parts.

Earlier, Badawi *et al.* (2011) [2] reported that the application of *Bradyrhizobium* + *Serratia marcescens* (S.) in peanut gave maximum test weight (86.89 g) over control (77.59 g). Similarly, Nalawde *et al.* (2015) [13] observed that when the biofertilizer *Rhizobium japonicum* was applied to *Vigna mungo* (L.hepper), the plant showed highest protein content (3 mg g^{-1}) as compared to control (2.8 mg g^{-1}). Further, Singh *et al.* (2016) [18] revealed that the protein content and protein yield of black gram was significantly influenced by seed inoculation of biofertilizer. Treatment *Rhizobium* + PSB recorded maximum protein content (23.10%) and protein yield (305.83 kg ha^{-1}) over rest of the treatment. Similar treatment gave maximum test weight (39.02 g). Similarly, Selvakumar *et al*. (2009) stated that the trend of variation in protein content observed due to absorption of nitrogen and phosphorus by plants.

Conclusions

Significant impact of co-inoculation of different microorganisms was noted; particularly the height of black gram were significantly increased with co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* and RDF over rest of the treatments, while in case of number of branches the same treatment shows non-significant positive effect. Similarly the maximum number of nodules per plant, fresh nodules weight and dry nodules weight in black gram found in treatment receiving co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli*. Significantly highest seed and straw yield of black gram was noted with RDF + *Rhizobium phaseoli* +*Pseudomonas striata*. The seed quality attributes of black gram showed significant increase in test weight and protein content with co-inoculation of RDF + *Rhizobium phaseoli* + *Pseudomonas striata*.

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