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Nutrient Dynamics in Blackgram (*Vigna mungo*) Grown Vertisols as Influenced by Co-inoculation of Different Bacterial Cultures with *Rhizobium phaseoli*

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A B S T R A C T

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 (RDF) in randomized block design. 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 both macronutrient and micronutrient mobilization 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* were found to be at par with each other and having more potential than the other combinations in influencing soil nutrient availability.

Introduction

Bio-fertilizers play a crucial role in soil health and plant growth as it has efficient strains of nitrogen fixing, phosphate solubilising, potash and micronutrient mobilizers (Verma *et al.*, 2010). 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). Microbial consortia behave synergistically by considerably increasing the amount of solubilized nutrients, growth hormone production, and ultimately promote plant growth (Mishra *et al.*, 2011). Taking these facts under consideration, it was hypothesized that co-inoculation or composite inoculum of rhizobia and endophytic bacteria has greater potential for plant growth improvement compared to the single strain inoculation.

Blackgram (*Urad*) is annual pulse crop and native to Central Asia. At national level it is the 3rd important crop, was cultivated over an area of 5.44 Mha (kharif + rabi) and recorded a production of 3.56 Mt at a productivity level of 655 kg ha^{-1}. Major contributing states have been Madhya Pradesh, Rajasthan, Andhra Pradesh, Uttar Pradesh, Tamil Nadu, Maharashtra, Jharkhand and Gujarat. Maharashtra contribute 9.62 per cent (4.84 lakh ha) to the total area under blackgram and 5.39 per cent (1.77 lakh tones) to the total production (Anonymous 2018). 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. It is rich 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). Black gram is rich in potassium, phosphorous, calcium, sodium and vitamins (retinoic acid, thiamine, riboflavin). It has several therapeutic properties like curing diabetes, sexual dysfunction, and nervous hair and digestive system disorders.

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 initial soil pH was 7.89, EC 0.27 dSm^{-1}, organic carbon 3.68 g kg^{-1}, calcium carbonate 21.6 g kg^{-1}, available N 164.68 kg ha^{-1}, available P_2O_5 13.89 kg ha^{-1}, available K_2O 544.56 kg ha^{-1}, available zinc 0.40 mg kg^{-1}, available iron 4.39 mg kg^{-1}, available manganese 5.69 mg $kg^{-1}$and available copper was 3.20 mg kg^{-1}. The soil was clayey in texture, moderately alkaline in reaction, medium in available nitrogen, phosphorus and sufficient in available potassium and low in iron and zinc. The soil samples we also analysed after harvest of crop and analysed for various soil properties using standard procedures. The results 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.

Results and Discussion

Effect on physico-chemical properties

pH, EC and $CaCO_3$

The result presented in Table 1 regarding changes in soil pH, EC and $CaCO_3$ after harvest of black gram indicates non-significant impact. In the study, treatments receiving dual inoculation of bacterial cultures with *Rhizobium phaseoli* showed the physico-chemical properties i.e. pH, EC and $CaCO_3$ of soil decreased positively. The pH is an intrinsic property of soil which usually does not change easily. Qureshi *et al.,* (2011) supported our result that lowered the soil pH due to the production of organic acids. Similarly, Maseko and Dakora (2013) noted that the rhizosphere pH of different bacterial species was generally lower than that of the corresponding bulk soil, although these were statistically non-significant. Similarly, Kranthikumar *et al.,* (2017a) revealed non-significant positive effect in case of pH, EC and $CaCO_3$ due to application of bio-fertilizers with *Rhizobium*.

Organic carbon

The organic carbon (O.C) content of soil after harvest of black gram ranged between 3.62 to 5.10 g kg^{-1} (Table 1). Soil organic carbon content increased significantly and attained a maximum value of 5.10 g kg^{-1} in the treatment T_7 that received co-inoculation of *Pseudomonas striata* and *Rhizobium phaseoli* along with 100 % RDF over other treatments and found at par with treatments T_4 (4.83 g kg^{-1}) having co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli* and T_8 (5.05 g ha^{-1}) receiving co-inoculation of *Pseudomons flurescens* with *Rhizobium phaseoli*. This could be ascribed to the dual inoculation of bacterial cultures in combination with fertilizers that increased residual biomass through root, leaves, stubles and rhizodeposition which helped in increasing organic matter and alternately soil organic carbon content (Singh *et al.,* 2016). This finding was supported by Goutami *et al.,* (2015) that organic carbon positively increased with level of nitrogen and application of biofertilizers. Furthermore, Qureshi *et al.,* (2011) found that due to co-inoculation of microbial cultures the rate of degradation of soil organic matter increased which ultimately helps in increasing the soil organic carbon.

Effect on macronutrient availability

The data presented in Table 2 showed that significant increase in nutrient availability in soil after harvest of black gram crop under the influence of co-inoculation of different bacterial cultures with *Rhizobium phaseoli*.

Available Nitrogen

Distinct differences in available N with values lying between 164.53 to 197.29 kg ha^{-1} were observed among the treatments. The available N was the highest in treatment T_7 (197.29 kg ha^{-1}) received co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* which found significantly superior over rest of treatments at 5 per cent significance level and treatment T_4 (192.34 kg ha^{-1}) received co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli* found at par with treatment T_7. The increase in available N may be due to dual inoculation of bacterial cultures helping in multiplication of soil microbes and ultimately enhancing the conversion of organically bound N to mineral form (Singh *et al.,* 2016).

Available phosphorous

In case of available phosphorous the values were varied from 13.96 to 16.87 kg ha^{-1} under the influence of joint effect of *Rhizobium phaseoli* and bacterial cultures. The maximum P availability was found in treatment T_8 (16.87 kg ha^{-1}) receiving co-inoculation of *Pseudomonas flurescens* with *Rhizobium phaseoli* which significantly superior over rest of treatments and found closely at par with T_7 (15.68 kg ha^{-1}) received co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli*. This build up P in soil is attributed to solubilization of native P through greater release of organic acids under co-inoculation of different bacterial cultures with *Rhizobium sp.* (Shashidhar *et al.,* 2009).

Qureshi *et al.,* (2011) also noted the highest available P with *Bacillus sp.* was observed after 30, 60 and 90 days of sowing at highest P fertilizer level i.e. 90 kg P ha^{-1}.

Available potassium

Regarding to available K, all co-inoculated treatments shows superior values over mono-inoculation and un-inoculated control.

The highest available K was found under the influence of co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* (T_7- 622.71 kg ha^{-1}) which found significantly superior over other treatments and found at par with treatment T_4 (602.13 kg ha^{-1}) receiving co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli* and T_8 (612.58 kg ha^{-1}) receiving co-inoculation of *Pseudomonas flurescens* with *Rhizobium phaseoli*.

Earlier, Nirmal *et al.,* (2006) noted that the dual inoculation of *Rhizobium* and PSB resulted into more availability of nitrogen and phosphorous because of their associative effect of solubilization from non-exchangeable to liable form, which leads to significant increase in growth and yield attributes as compared to single or un-inoculated plot.

Similarly, Kumar *et al.,* (2004) proved to have a favourable effect on the availability of N, P and K, thereby indicating the vital role of these organisms in the transformation reaction of these three nutrients in the soil.

Moreover, Dhage *et al.,* (2008) noted that availability of N, P and K recorded in a treatment with 100% RDF + *Rhizobium* + PSB over control in soybean. Further, Amule *et al.,* (2013) reported that available N, P and K reflected 2 % increase due to co-inoculation over mono-inoculation. Similarly, Goutami *et al.,* (2015) found that there was significant increase in available N, P and K was observed in the presence of bio-fertilizer consortium and /or FYM over control. Singh *et al.,* (2016) also noted that maximum available nitrogen, phosphorous and potassium was observed in *Rhizobium* + PSB.

Similarly, Vidhyashree *et al.,* (2017) reported that PSB + *Aspergillus awamori* inoculation increase availability N, P and K in soil.

Effect on DTPA extractable micronutrients

The scrutiny of result presented in Table 3 reveals that the available micronutrients (DTPA extractable) in soil after harvest of black gram crop were influenced significantly by co-inoculation of different bacterial cultures with *Rhizobium phaseoli*.

DTPA extractable zinc

The availability of Zn significantly influenced by treatment T_7 (0.59 mg kg^{-1}) receiving co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* and found at par with treatment T_8 (0.57 mg kg^{-1}) receiving co-inoculation of *Pseudomonas flurescens* with *Rhizobium phaseoli* and T_4 (0.58 mg kg^{-1}) receiving co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli*.

DTPA extractable iron

In case of Fe availability the values varied from 4.41 to 5.12 mg kg^{-1} due to influence of co-inoculation of bacterial cultures with *Rhizobium phaseoli*.

Table.1 Effect of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on physico-chemical properties of soil after harvest of black gram

Sr. No.	Treatments	pH (1:2.5)	EC (dSm^{-1})	$CaCO_3$ ($g\ kg^{-1}$)	Organic carbon ($g\ kg^{-1}$)
T_1	Absolute control	7.89	0.27	21.43	3.73
T_2	Only RDF	7.90	0.27	20.97	3.90
T_3	RDF + *Rhizobium phaseoli*	7.88	0.25	20.50	4.00
T_4	T_3 + *Bacillus megaterium*	7.86	0.25	20.87	4.83
T_5	T_3 + *Bacillus subtilis*	7.86	0.26	21.03	4.60
T_6	T_3 + *Bacillus polymyxa*	7.88	0.26	21.53	4.07
T_7	T_3 + *Pseudomonas striata*	7.87	0.25	21.43	5.10
T_8	T_3 + *Pseudomonas flurescens*	7.87	0.25	21.53	5.05
T_9	T_3 + *Azotobacter chroococcum*	7.88	0.27	21.37	4.47
T_{10}	T_3 + *Azospirillum lipoferum*	7.89	0.27	21.57	4.27
	S.E.±	0.013	0.006	0.32	0.15
	C.D. at 5 %	NS	NS	NS	0.45

Table.2 Effect of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on macronutrient availability in soil after harvest of black gram

Sr. No.	Treatments	Available N ($kg\ ha^{-1}$)	Available P_2O_5 ($kg\ ha^{-1}$)	Available K_2O ($kg\ ha^{-1}$)
T_1	Absolute control	164.53	13.96	545.57
T_2	Only RDF	165.17	13.98	549.33
T_3	RDF + *Rhizobium phaseoli*	180.84	14.12	563.65
T_4	T_3 + *Bacillus megaterium*	192.34	15.08	602.13
T_5	T_3 + *Bacillus subtilis*	179.80	15.23	594.52
T_6	T_3 + *Bacillus polymyxa*	165.16	14.08	570.58
T_7	T_3 + *Pseudomonas striata*	197.29	15.68	622.71
T_8	T_3 + *Pseudomonas flurescens*	182.93	16.87	612.58
T_9	T_3 + *Azotobacter chroococcum*	182.93	14.13	597.08
T_{10}	T_3 + *Azospirillum lipoferum*	150.18	14.14	584.63
	S.E.±	4.06	0.33	8.33
	C.D. at 5 %	10.05	1.19	23.73

Table.3 Effect of co-inoculation of different bacterial cultures with *Rhizobium phaseoli* on DTPA extractable micronutrients in soil after harvest of black gram

Sr. No.	Treatments	DTPA Zn (mg kg^{-1})	DTPA Fe (mg kg^{1})	DTPA Cu (mg kg^{1})	DTPA Mn (mg kg^{1})
T_1	Absolute control	0.41	4.41	3.22	5.92
T_2	Only RDF	0.43	4.55	3.39	6.32
T_3	RDF + *Rhizobium phaseoli*	0.46	4.73	3.43	6.48
T_4	T_3 + *Bacillus megaterium*	0.58	5.03	3.99	6.94
T_5	T_3 + *Bacillus subtilis*	0.54	4.82	3.70	6.31
T_6	T_3 + *Bacillus polymyxa*	0.50	4.77	3.58	6.32
T_7	T_3 + *Pseudomonas striata*	0.59	5.12	4.10	7.04
T_8	T_3 + *Pseudomonas flurescens*	0.57	4.96	3.83	6.93
T_9	T_3 + *Azotobacter chroococcum*	0.54	4.66	3.45	6.42
T_{10}	T_3 + *Azospirillum lipoferum*	0.49	4.68	3.44	6.30
	S.E.±	0.01	0.07	0.11	0.13
	C.D. at 5 %	0.04	0.21	0.31	0.39

The treatment T_7 (5.12 mg ka^{-1}) receiving co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* found significant over rest of treatments and found at par with treatment T_8 (4.96 mg kg^{-1}) receiving co-inoculation of *Pseudomonas flurescens* with *Rhizobium phaseoli* and T_4 (5.03 mg kg^{-1}) receiving co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli*.

DTPA extractable copper

The maximum available Cu (4.10 mg kg^{-1}) was observed under treatment T_7 (RDF + *Rhizobium phaseoli* + *Pseudomonas striata*) which significantly differed from all other treatments. Treatments T_4 (3.99 mg kg^{-1}) receiving co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli* and T_8 (3.83 mg kg^{-1}) receiving co-inoculation of *Pseudomonas flurescens* with *Rhizobium phaseoli* found at par with T_7.

DTPA extractable manganese

Regarding to Mn the availability range varied from 5.92 to 7.04 mg kg^{-1} and treatment T_7 (7.04 mg kg^{-1}) receiving co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* found significant over rest of treatments and found at par with treatments T_4 (6.94 mg kg^{-1}) having co-inoculation of *Bacillus megaterium* with *Rhizobium phaseoli* and T_8 (6.93 mg kg^{-1}) having co-inoculation of *Pseudomonas flurescens* with *Rhizobium phaseoli*. The availability of micronutrient (Zn, Fe, Cu and Mn) is might be due co-inoculation of bacterial cultures helps in mineralization which leads to organic forms into inorganic forms of nutrient hence its availability increases (Singh *et al.*, 2016).

Our results are similar to the results reported by Kranthikumar *et al.*, (2017b) that the available micronutrients in soil after harvest of soybean crop were influenced significantly with the inoculation of microbial cultures and recorded maximum in treatment receiving RDF + *Rhizobium* + Other microbial cultures. and it is due to production of growth-promoting substances and high colonization ability of rhizobacteria such as *Pseudomonas* because they enhance the nitrogen fixation when co-inoculated with *Rhizobium*. The pH, EC and $CaCO_3$ after harvest of black gram indicates non-significant results. But in case of soil organic carbon co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli* and RDF found superior over rest of the treatments. Significant increase in nutrient availability in soil after harvest of black gram crop was also recorded with co-inoculation of bio-inoculants. Significantly highest value of available P_2O_5 were noted in treatment receiving *Pseudomonas fluorescens* along with *Rhizobium phaseoli* and RDF and availability of N, P_2O_5 and K_2O enhanced due to application of *Pseudomonas striata* along with *Rhizobium phaseoli*. The DTPA extractable micronutrients (Fe, Mn and Cu) found significantly increased by co-inoculation *Pseudomonas striata* with *Rhizobium phaseoli*. In case of soil DTPA Zn treatment having co-inoculation of *Pseudomonas flurescens* with *Rhizobium phaseoli* shows significant result over others and found at par with treatment having co-inoculation of *Pseudomonas striata* with *Rhizobium phaseoli*.

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