Int.J.Curr.Microbiol.App.Sci (2023) *12*(11): 42-54

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 12 Number 11 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1211.005

Development of Microbial Consortia of Nitrogen Fixing, Phosphate Solubilizing and Potash Mobilizing Bacteria for Optimizing Nutrient Supplementation to chickpea

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Keywords

Consortium, Rhizobium, PSB, KSB, Inoculation, Chickpea

Article Info

Received:
22 September 2023
Accepted:
25 October 2023
Available Online:
10 November 2023

A B S T R A C T

The present study was aimed with the formulation of suitable culture media for growth of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria in a consortium. Among different culture media, MS III media having Glucose (10 g l^{-1}), Mannitol (10 g l^{-1}), Ammonium sulphate (0.5 g l^{-1}) and Yeast extract (6 g l^{-1}) recorded maximum growth and microbial count of Rhizobium, PSB and KMB. These three beneficial microorganisms found compatible with each other when grown on MS III culture media. Furthermore, a field experiment was conducted to study the effect of seed inoculation of consortium of Rhizobium, PSB and KMB on growth parameters and yield of chickpea. Among different inoculation treatments, seed inoculation with consortium of Rhizobium, PSB and KMB + 75% RDF was found to be the most effective as it recorded significantly highest germination (97.46%), shoot length (16.53 cm), root length (7.45 cm) and plant vigour index (2336.68) at 15 days after sowing, plant height (33.58 cm and 44.95 cm), root length (13.50 cm and 19.53 cm), dry weight of shoot (7.75 g $plant^{-1}$ and 9.02 g $plant^{-1}$) and dry weight of root (910.33 mg $plant^{-1}$ and 968 mg $plant^{-1}$) at flowering and harvest stage of the crop, number of branches (22.67 $plant^{-1}$), number of nodules (24.93 $plant^{-1}$), number of pods (55.47 $plant^{-1}$), 1000 seed weight (127.86 g), and seed yield (20.48 q ha^{-1}) of chickpea and found statistically indistinguishable with the treatment of seed inoculation with consortium + 100% RDF for growth parameters and seed yield of chickpea. The results indicated 25% saving of nitrogen, phosphorus and potassium dose of chemical fertilizers to chickpea. Moreover, MS III culture medium proved effective with respect to population stability of individual strain and effectiveness of consortium of Rhizobium, PSB and KMB on growth.

Introduction

Microorganisms usually need different types of culture media for their growth under *in- vitro* condition. Yeast extract mannitol agar media, Pikovskaya's media and Alexandrov media are suitable for individual growth of *Rhizobium*, phosphate solubilizing bacteria and potash mobilizing bacteria, respectively. But it needs to be essential to formulate such a culture medium which

found suitable for growth of these three beneficial microorganisms in a consortium. It is well known that phosphate solubilizing bacteria and *Rhizobium* have synergistic effect on legume crops. Development of consortia containing one strain of *Rhizobium*, PSB and PGPR has been attempted (Bansal, 2015). The present study was undertaken for formulation of suitable culture media for growth of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria in a consortium and its application as seed inoculation under glasshouse experiment with chickpea crop.

Materials and Methods

Isolation of *Rhizobium* from root nodules of chickpea

The healthy, unbroken, firm and pink nodules from chickpea roots were selected for isolation of *Rhizobium* by using yeast extract mannitol agar (YEMA) media as described by Rajendran *et al.*, (2008).

Nitrogen fixing ability of the rhizobial isolate

The 48 hour old culture of freshly isolated *Rhizobium* strain was inoculated to 5 ml of yeast extract mannitol medium. It was incubated for 48 hours. One ml of this broth was inoculated to 50 ml yeast extract mannitol medium. Then it was incubated for 15 days. Ten ml of this culture was used for N estimation by following the standard procedure of Microkjeldhal technique (Reis *et al.*, 1994). The formula for N_2 estimation is:

$$N_2\ (mg/g) = \frac{\text{ml of } H_2SO_4 \text{ in the sample x Normality of } H_2SO_4 \text{ x } 14.01}{\text{Weight of the sample (carbon used in g)}}$$

Biochemical and physiological characterization of rhizobial isolate

Pure culture of the isolate was made and then subjected to Gram reaction. The Gram negative isolates were further subjected to biochemical tests including catalase, oxidase, gelatin hydrolysis, indole tests and growth on different carbon sources for confirmation. The biochemical characterization of the isolates was carried out as per the procedures outlined by Cappuccino and Sherman (1987) in their 10th edition of Microbiology: A Laboratory Manual.

Isolation of phosphate solublizing bacteria (PSB) from rhizosphere soil of chickpea

The isolation of phosphate solublizing bacteria on Pikovskaya's medium was carried out by serial dilution of soil and agar plating method (Aneja, 2003). The formation of clear zone of P-solublization around the colonies grown on Pikovskaya's medium were selected, purified, subcultured and maintained on the slants of Pikovskaya's agar for further use.

Phosphate solublizing ability of the bacterial isolates

The ability of the bacterial isolates to solubilize insoluble inorganic phosphate was tested by spotting overnight cultures on Pikovskaya's agar plates and incubating at 28-30°C for 2-3 days. The isolates which showed clear zone of solublization of tricalcium phosphate (TCP) around the colony were noted as phosphate solubilizers. The diameter of the zone of TCP solublization was measured and expressed in millimeters. The bacterial isolates positive for P solublization on Pikovskaya's agar medium were subjected to quantification of Pi released from TCP in broth medium.

Biochemical characterization of the PSB isolate

The biochemical characterization of the isolate was carried out as per the procedures outlined by Cappuccino and Sherman in their 10th edition of Microbiology: A Laboratory Manual. Catalase test, Oxidase test, Indole production test, Methyl red test, Voges-Proskaeur (VP) test, Urea hydrolysis, Nitrate reduction test, Gelatin hydrolysis test, Starch hydrolysis, Casein hydrolysis and H_2S production test were performed.

Isolation of potash mobilizing bacteria from rhizospheric soil of chickpea

One gram of rhizosphere soil was mixed thoroughly in 100 ml sterile water and was processed following serial dilution agar plate technique (Aneja, 2003). A suitable dilutions (10^{-5} and 10^{-6}) of both rhizosphere and rhizoplane solutions were plated on Alexandrov medium (Hu *et al.,* 2006). The plates were incubated at room temperature (30±1°C) for 3 days and the colonies exhibiting clear zones of solubilization of muscovite mica were selected purified, subcultured and maintained on the slants of Alexandrov medium for further use.

Quantitative estimation of 'K' solublization

The isolates showing zone of solublization on Alexandrov agar medium were further examined for their ability to release K from broth media. The amount of K released from muscovite mica in the broth by the isolates was studied at 7, 15 and 20 days after incubation (DAI) under laboratory condition (Parmar *et al.,* 2016).

Biochemical characterization of KMB isolate

The biochemical characterizations of the KMB isolate was carried out as per the procedures outlined by Bergey's Manual of Systematic Bacteriology 9th Edition (1993). Sugar utilization, Methyl red test, Voges-Proskauer (VP) test, Urea hydrolysis, Nitrate reduction test, Gelatin hydrolysis test, catalase test, starch hydrolysis, Casein hydrolysis and H_2S production test were performed.

Selection of culture medium

The culture media (MS I, MS II, MS III, MS IV and MS V) of various compositions were formulated as described by Shete *et al.,* (2019) and screened for growth of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria in broth by using various carbon sources like glucose, sucrose and nitrogen sources like ammonium sulphate and yeast extract in different concentrations along with different micronutrients (Table 1). The pH of all culture media was maintained in the range of 6.9 to 7.1.

In vitro studies

Broth of each culture media *viz*., MS I, MS II, MS III, MS IV and MS V were inoculated with efficient strains of *Rhizobium*, PSB and Potash mobilizing bacteria separately as well as in consortia and kept for incubation at 28±2°C for 5 days.

The cfu count of *Rhizobium*, PSB and potash mobilizing bacteria was recorded after incubation period of 5 days by using direct plate count technique. Before development of consortium, all strains were examined *in vitro* for their compatibility on selective medium by cross streak method (Ganesan and Gnanamanickam, 1987).

Observations on growth and cfu count of *Rhizobium*, PSB and Potash mobilizing bacteria in each culture media were recorded.

Preparation of consortium of *Rhizobium*, PSB and KMB on a selective medium

Inoculum of *Bradyrhizobium japonicum*, *Bacillus subtilis* and *Frateuria aurantia* was prepared in selective medium MS III (Shete *et al.,* 2019). The media was inoculated in 500 ml conical flask containing 150 ml medium and incubated at 28 ± 2°C under shaking at 100-150 rpm for three days to give an optical density of 0.5 recorded at 535 nm.

Lignite powder used as carrier was sterilized at 121°C and 1.04 kg/cm^2 pressure for one hour and inoculated with broth cultures of *Rhizobium ciceri*, *Bacillus subtilis* and *Frateuria aurantia* (100 ml per 500 g of lignite powder). Lignite powder based inoculum was incubated at 28 ± 2°C for three days by adding 10% sugar solution to increase the population of respective microbe. Inoculum of *Rhizobium ciceri*, *Bacillus subtilis* and *Frateuria aurantia* having cfu of 2 x 10^7 per gram of lignite powder were applied to soybean as seed coating.

Field experiment

A field experiment was conducted during *Rabi*, 2021 in the field at College of Agriculture, Pune to study the effect of seed inoculation with consortium of *Rhizobium*, PSB and KMB on growth parameters, nutrient uptake and yield of chickpea. The chickpea variety *Phule Digvijay* was used as a test crop. The experiment was laid out in randomized block design with three replications and eight treatments.

Treatment details

The chickpea seeds were treated before sowing as follows:

T_1: Consortium of *Rhizobium*, PSB and KMB

$T_{2:}$ Consortium of *Rhizobium*, PSB and KMB + 100% RDF

T_3: Consortium of *Rhizobium*, PSB and KMB + 75% RDF

T_4: *Rhizobium* + 75% recommended N + 100% recommended P_2O_5 and K_2O

T_5: PSB + 75% recommended P_2O_5 + 100% recommended N and K_2O

T_6: KMB + 75% recommended K_2O +100% recommended N and P_2O_5

T_7: 100% RDF

T_8: Absolute control

Observations

The observations on germination (%), shoot length (cm), root length (cm) and plant vigour index at 15 days after sowing, plant height (cm), root length (cm), dry weight of shoot (g plant^{-1}) and dry weight of root (mg plant^{-1}) at flowering and harvest stage of the crop, number of branches, number of nodules and number of pods per plant, 1000 seed weight, NPK uptake (kg ha^{-1}) and seed yield (q ha^{-1}) of chickpea were recorded. Plant vigour index was computed at 15 days after sowing using the formula: Plant vigour index= Germination% x [shoot length (cm) + root length (cm)]. Nitrogen content of plant was estimated by following Modified Kjeldahl's process and accordingly N uptake (kg ha^{-1}) was estimated as N% x total dry matter yield (kg ha^{-1})/100.

Microbial count of Rhizobium, PSB and KMB at flowering stage of chickpea

Fresh root nodules of soybean 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 chickpea 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$\pm$2 ^{0}C temperature for 72 hours and colonies were counted. The population was expressed as cfu g^{-1} soil.

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

Isolation of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria

The isolation of *Rhizobium* from root nodule of chickpea (var. *Phule Digvijay*) was done using yeast extract mannitol agar medium. The isolation

procedure was carried out for all the three samples and three isolates were obtained as RH-I, RH-II and RH-III. Moreover, isolation of phosphate solublizing bacteria on Pikovskaya's medium was carried out by serial dilution of soil and agar plating method (Aneja, 2003). The isolation procedure was carried out for all the three rhizosphere soil samples and the plates were observed for the appearance of bacterial colony showing clear zone of solublization of tricalcium phosphate purified (TCP) on Pikovskaya's medium. Three isolates were obtained as P-I, P-II and P-III. Furthermore, isolation of potash mobilizing bacteria was carried out on Alexandrov medium. The isolation procedure was carried out for all the three rhizosphere soil samples on Alexandrov medium (Hu *et al.,* 2006). The plates were observed for the appearance of bacterial colony showing clear zone of solublization of insoluble potassium bearing mineral (mica). Three isolates were obtained as K-I, K-II and K-III.

Nitrogen fixing ability of *Rhizobium* isolate

All the three *Rhizobium* isolates of chickpea along with MPKV strain (*Rhizobium ciceri*) were subjected to know the nitrogen fixation by Microkjeldhal method (Table 2). The isolate RH-1 fixed highest amount of nitrogen (149.88 µg of nitrogen/mg of carbon used). This was followed by MPKV strain, RH-II and RH-III isolate (147.01, 130.99 and 123.07 µg of nitrogen/mg of carbon used, respectively). The results of the present investigation are in agreement with results of Hema and Savalgi (2017) who reported that isolate from maize GdM5 fixed about 142 µg of nitrogen/mg of carbon used.

Phosphate solubilizing ability of the PSB isolates

All the three PSB isolates along with MPKV strain (*Bacillus subtilis*) were tested for their ability to solubilize inorganic phosphate both qualitatively and quantitatively and their results are presented in Table 3. Quick analysis of P-solubilization was carried out on Pikovskaya's agar medium. All the three isolates were able to form zone of P-solubilization on the medium. The diameter of the zone of P-solubilization ranged from 3-6 mm in different isolates.

Quantitative estimation of Pi released from TCP for bacterial isolates

The amount of Pi released from tri-calcium phosphate by the PSB isolates along with MPKV strain (*Bacillus subtilis*) in Pikovskaya's broth was estimated at 10 days after inoculation. The amount of Pi released from TCP by the isolates at 10 DAI ranged from 12.57 to 30.52 per cent (Table 3). The isolate P-I recorded highest P-solubilization (30.52%) than the other isolates tested.

Decrease in pH of medium during phosphate solubilization

The decrease in pH of TCP broth from initially adjusted pH of 7.0 was also noted at 10 days after inoculation. The maximum reduction in pH of the medium i.e. pH 3.46 was recorded by P-I isolate followed by MPKV strain (*Bacillus subtilis*), P-II and P-III isolates (3.48, 4.07 and 4.09, respectively) (Table 3). The decrease in pH of the medium with the amount of Pi released had positive correlation.

Quantitative estimation of 'K' solubilisation of the KMB isolates

The isolates showing zone of solubilization on Alexandrov agar medium were further examined for their ability to release 'K' from broth media. The amount of 'K' released from muscovite mica in the broth by the isolates along with MPKV strain (*Frateuria aurantia*) were studied at 7, 15 and 20 days after incubation (DAI) in lab condition and found in the range of 7.62 to 41.94 µg ml^{-1} (Table 4). The results indicated that the amount of released 'K' increased as the days of incubation increases and the highest amount of 'K' present at 20 DAI. The maximum solubilization of muscovite mica was observed in K-I isolate (41.94 µg ml^{-1}) followed by MPKV strain (*Frateuria aurantia*) (40.38 µg ml^{-1}) at 20 DAI. The results of the present investigation are

in agreement with the results of Parmar *et al.*, (2016) who isolated 25 potassium solubilizing bacterial isolates from the rhizosphere of maize from various areas of Navsari district and tested quantitative estimation of 'K' solubilisation of the highly efficient KMB isolates. He further reported the amount of 'K' released from muscovite mica in the broth by the isolates in the range of 1.89 to 46.52 µg ml^{-1}.

On the basis of nitrogen fixing, phosphate solubilising and potash mobilizing ability, highly efficient nitrogen fixing *Rhizobium* isolate (RH-I), phosphate solubilising isolate (P-I) and potash mobilizing isolate (K-I) were further tested for different biochemical characterization.

Biochemical characterization of Rhizobium, PSB and KMB isolate

The highly efficient nitrogen fixing rhizobial isolate (RH-I) was tested for different biochemical characters *viz*., gram staining, motility test, gelatin hydrolysis, catalase test, oxidase test, indole production test, starch hydrolysis, H_2S production, Voges-Proskaeur test and growth on different carbon sources (Table 5). The cells of nitrogen fixing rhizobial isolate were rod shape, motile and gram negative in reaction. The nitrogen fixing rhizobial isolate was positive for catalase test, oxidase test, indole production test, starch hydrolysis, H_2S production and Voges-Proskaeur test but was negative for gelatin hydrolysis.

Table.1 Composition of culture media for consortia of *Rhizobium*, PSB and KMB

S.N.	Chemicals	Composition of culture media (g)				
		MS I	MS II	MS III	MS IV	**MS V**
1	Glucose	5	15.	10	0	**20**
2	Mannitol	15	5	10	20	**0**
3	Tri calcium phosphate	5	5	5	5	**5**
4	Ammonium sulphate	0.1	0.3	0.5	0.7	**0.9**
5	Yeast extract	2	4	6	8	**10**
6	Magnesium sulphate	0.1	0.1	0.1	0.1	**0.1**
7	Potassium chloride	0.2	0.2	0.2	0.2	**0.2**
8	Manganese sulphate	0.001	0.001	0.001	0.001	**0.001**
9	Ferrous sulphate	0.1	0.1	0.1	0.1	**0.1**
10	Calcium carbonate	2	2	2	2	**2**
11	Potassium alumino silicates	2	2	2	2	**2**
12	Dipotassium hydrogen orthophosphate	0.1	0.1	0.1	0.1	**0.1**
13	Sodium chloride	0.1	0.1	0.1	0.1	**0.1**
	pH	6.9	7.1	7.2	6.9	**7.1**
	Distilled water	**1lit**	**1lit**	**1lit**	**1lit**	**1lit**

Table.2 Nitrogen fixing ability of *Rhizobium* isolate of chickpea by Microkjeldhal method

Sr. No.	Rhizobium isolate	Nitrogen fixing ability (µg of Nitrogen/mg of Carbon)
1.	RH-I	**149.88**
2.	RH-II	**130.99**
3.	RH-III	**123.07**
4.	**MPKV strain (*Rhizobium ciceri*)**	**147.01**

Table.3 Zone of P solubilization on Pikovskaya's agar and per cent Pi released from TCP brothby the PSB isolates

Sr. No.	PSB Isolate	Zone of P solubilization on TCP (mm)	% Pi released from TCP after10 days	Decrease in pH of medium (from initial pH7.0) after 10 days
1	P-I	6.0	30.52	**3.46**
2	P-II	5.0	14.37	**4.07**
3	P-III	3.0	12.57	**4.09**
4.	**MPKV strain (*Bacillus subtilis*)**	**6.0**	**29.49**	**3.48**

Table.4 Solubilization of muscovite mica by the KMB isolates

Sr. No.	KMB isolate	7 DAI (µg ml^{-1})	15 DAI (µg ml^{-1})	20 DAI ((µg ml^{-1})
1.	K-I	25.69	38.39	**41.94**
2.	K-II	13.63	22.71	**34.88**
3.	K-III	7.62	20.73	**31.55**
4.	**MPKV strain (*Frateuria aurantia*)**	**24.38**	**36.23**	**40.38**

Table.5 Selective biochemical tests of nitrogen fixing, phosphate solubilizing and potashmobilizing bacterial isolate

Sr. No.	Biochemical tests	Rhizobium isolate (RH-I)	PSB isolate (P-I)	KMB isolate (K-I)
1.	Cell shape	Rod shape	Rod shape	**Rod shape**
2.	Gram reaction	Gram negative	Gram positive	**Gram negative**
3.	Motility	+	+	+
4.	Gelatin hydrolysis	-	+	+
5.	Catalase test	+	+	+
6.	Oxidase test	+	-	
7.	Indole production test	+		
8.	Starch hydrolysis	+	+	+
9.	H2S production	+	-	-
10.	Voges-Proskaeur test	+	+	-
11.	Urea hydrolysis			+
12.	Caesin hydrolysis test			+
13.	Nitrate reduction test			+
14.	Methyl red test			+
15.	Growth on carbon sources			
	a) Glucose	+		
	b) Sucrose	+		+
	c) Mannitol	+		+
	d) Maltose			+

Glucose, sucrose and mannitol were used as a sole carbon source for growth by the nitrogen fixing rhizobial isolate. Based on biochemical and physiological characterization, the nitrogen fixing rhizobial isolate was identified as *Rhizobium ciceri*.

The results of the present investigation are in conformity with results of Jadhav (2013) who isolated rhizobia from root nodule of soybean cultivated in Latur area and further characterized these isolates biochemically for specific characters of *Bradyrhizobium japonicum* according to Burgey's
Manual of Systematic Bacteriology. All the isolates were positive for most of characters specific for *Bradyrhizobium japonicum*. Further all isolates tested negative for gelatin hydrolysis.

The highly efficient phosphate solubilizing bacterial isolate (P-I) was tested for different biochemical characters *viz*., gram staining, motility test, gelatin hydrolysis, catalase test, oxidase test, starch hydrolysis, H_2S production and Voges-Proskaeur test (Table 5). The cells of phosphate solubilizing bacterial isolate were rod shape, motile and gram positive in reaction.

The phosphate solubilizing bacterial isolate was positive for gelatin hydrolysis, catalase test, starch hydrolysis and Voges-Proskaeur test but was negative for oxidase test and H_2S production. Based on biochemical and physiological characterization (Claus and Berkeley, 1986), the phosphate solubilizing bacterial isolate was identified as *Bacillus subtilis*.

Table.6 Growth of *Rhizobium*, PSB and KMB on different culture media

Sr. No.	Culture media	*Rhizobium*	PSB	KMB
1.	MS I	+	+	+
2.	MS II	++	+	-
3.	MS III	+++	+++	+++
4.	MS IV	+	-	-
5.	**MS V**	-	+	+

Table.7 Microbial count of *Rhizobium*, PSB and KMB in a consortium on different culturemedia

Sr. No.	Culture media	*Rhizobium* (*cfu* g^{-1})	PSB (*cfu* g^{-1})	KMB (*cfu* g^{-1})
1.	MS I	1×10^3	1×10^3	**1×10^3**
2.	MS II	1×10^5	1×10^3	-
3.	MS III	11×10^7	6×10^7	**8×10^7**
4.	MS IV	1×10^3	-	**1×10^7**
5.	**MS V**	-	**1×10^3**	**1×10^7**

Table.8 Inoculation effect of consortium of *Rhizobium*, PSB and KMB on growth parameters of chickpea

Tr. No	Treatment details	Germination (%)	Plant vigour index	Plant height (cm)		Root length (cm)		Dry weight of shoot(g plant^{-1})	
				Flowering	Harvest	Flowering	Harvest	Flowering	**Harvest**
T_1	Consortium of Rhizobium, PSB and KMB	93.33	1850.25	31.58	42.72	12.07	16.68	6.59	**7.94**
T_2	Consortium of Rhizobium, PSB and KMB + 100% RDF	96.72	2112.36	32.61	43.84	12.67	18.33	7.39	**8.53**
T_3	Consortium of Rhizobium, PSB and KMB + 75% RDF	97.46	2336.68	33.58	44.95	13.50	19.53	7.75	**9.02**
T_4	Rhizobium + 75% recommended N + 100% recommended P_2O_5 & K_2O	93.01	1881.02	31.48	41.99	11.95	16.48	6.05	**7.34**
T_5	PSB + 75% recommended P_2O_5 + 100% recommended N and K_2O	91.47	1806.23	30.98	41.70	11.80	16.34	5.72	**7.09**
T_6	KMB + 75% recommended K_2O +100% recommended N and P_2O_5	90.55	1704.43	30.89	41.34	11.65	15.93	5.60	**6.60**
T_7	100% RDF	91.88	1794.21	32.31	42.99	12.30	17.99	6.95	**8.26**
T_8	Uninoculated control	85.30	1352.38	27.98	39.45	9.25	13.44	4.05	**5.61**
	S.E.	2.19	74.10	0.34	0.43	0.30	0.45	0.20	**0.21**
	C.D.at 5%	6.63	225.32	1.02	1.32	0.90	1.36	0.62	**0.64**
	C.V.	**4.10**	**6.01**	**1.86**	**1.78**	**4.31**	**4.62**	**5.64**	**4.84**

PSB = Phosphate solubilizing bacteria, KMB = Potash mobilizing bacteria

Table.9 Inoculation effect of consortium of *Rhizobium*, PSB and KMB on growth and yield attributing characters of chickpea

Tr. No	Treatment details	Dry weight of root (g plant^{-1})		Number of branches plant $^{-1}$	Number of nodules plant $^{-1}$	Number of pods plant $^{-1}$	1000 seed weight (g)	Seed yield(q ha^{-1})
		Flowering	Harvest					
T_1	Consortium of Rhizobium, PSB and KMB	803.33	869.67	22.00	22.20	50.21	122.50	**18.04**
T_2	Consortium of Rhizobium, PSB and KMB + 100% RDF	855.00	908.33	22.13	23.85	53.44	125.54	**19.44**
T_3	Consortium of Rhizobium, PSB and KMB + 75% RDF	910.33	968.00	22.67	24.93	55.47	127.86	**20.48**
T_4	Rhizobium + 75% recommended N + 100% recommended P_2O_5 & K_2O	793.67	843.00	21.97	22.13	49.11	122.26	**17.62**
T_5	PSB + 75% recommended P_2O_5 + 100% recommended N and K_2O	772.00	825.00	21.70	21.13	48.44	121.39	**17.33**
T_6	KMB + 75% recommended K_2O +100% recommended N and P_2O_5	747.33	819.67	21.63	20.83	48.24	119.77	**17.21**
T_7	100% RDF	817.33	885.33	22.07	18.37	51.54	124.36	**18.35**
T_8	Uninoculated control	382.00	402.00	17.60	11.77	30.57	109.94	**15.62**
	S.E.	27.17	26.15	0.20	0.39	0.81	0.89	**0.69**
	C.D.at 5%	82.42	79.32	0.62	1.17	2.47	2.69	**2.09**
	C.V.	**6.19**	**5.56**	**1.65**	**3.23**	**2.92**	**1.26**	**16.63**

The highly efficient potash mobilizing bacterial isolate (K-I) was tested for different biochemical characters *viz*., gram staining, motility test, methyl red test, Voges-Proskaeur (VP) test, urea hydrolysis, nitrate reduction test, gelatine hydrolysis test, catalase test, starch hydrolysis, casein hydrolysis, H_2S production test and growth on different carbon sources (Table 5).

The potash mobilizing bacterial isolate was rod shape, motile and gram negative in reaction. The potash mobilizing bacterial isolate was positive for gelatin hydrolysis, catalase test, starch hydrolysis, urea hydrolysis, casein hydrolysis test, nitrate reduction test and methyl red test but was negative for H_2S production and Voges-Proskaeur test.

Sucrose, mannitol and maltose were used as a sole carbon source for growth by the potash mobilizing bacterial isolates. Based on biochemical and physiological characterization (Parmar *et al.,* 2016), the potash mobilizing bacterial isolate was identified as *Frateuria aurantia*.

Growth of Rhizobium, PSB and KMB on different culture media

Broth of each culture media *viz*., M I, M II, M III, M IV and M V were inoculated with efficient strains of *Rhizobium*, PSB and Potash mobilizing bacteria separately as well as in consortia and kept for incubation at 28±2^oC for 5 days. The data presented in Table 6 revealed that the maximum growth of *Rhizobium*, PSB and potash mobilizing bacteria was found on MS III culture media. Moreover, *Rhizobium*, PSB and KMB were found to be compatible with each other on MS III culture media (Fig. 2). Singh *et al.,* (2014) reported maximum growth of rhizobia in media containing 12.5 g l^{-1} sucrose at 29.4ºC for 7 days. Further, Kucuk *et al.*, (2006) reported that *Rhizobium* strains were able to utilize glucose and sucrose more efficiently than normal YEM medium. Moreover, Sagervanshi *et al.,* (2014) studied the effect of different nitrogen sources *viz*., ammonium sulphate, casein, sodium nitrate and urea and found best optimized source was ammonium sulphate for the maximum 'P' solubilisation. Furthermore, Sugumaran and Janarthanam (2007) reported that *B. mucilaginosus* isolated from soil, rock and mineral samples recorded 4.29 mg l^{-1} release of potassium in media supplemented with muscovite mica. Results of the present investigation are in agreement with results of these researchers.

Microbial count of *Rhizobium*, PSB and KMB in a consortium on different culture media

The data on microbial count of *Rhizobium*, PSB and potash mobilizing bacteria is presented in Table 7. Among all the culture media, MS III culture medium recorded maximum count of *Rhizobium*, PSB and KMB ($11x10^7$, 6×10^7 and 8×10^7 cfu g^{-1}, respectively).

Preparation of consortium of Rhizobium, PSB and KMB

Inoculum of *Rhizobium* (*Rhizobium ciceri*), PSB (*Bacillus subtilis*) and KMB (*Frateuria aurantia*) was prepared in a selective medium MS III. The media was inoculated in 500 ml conical flask containing 150 ml medium and incubated at 28 ± 2^oC under shaking at 100-150 rpm for three days to give an optical density of 0.5 recorded at 535 nm. Lignite powder used as carrier was sterilized at 121^oC and 1.04 kg/cm^2 pressure for one hour and inoculated with broth cultures of *Rhizobium ciceri*, *Bacillus subtilis* and *Frateuria aurantia* (100 ml in 500 g lignite powder). Lignite powder based inoculum was incubated at 28 ± 2^oC for three days by adding 10% sugar solution to increase the population of the respective microbes. The inoculum of *Rhizobium ciceri, Bacillus subtilis* and *Frateuria aurantia* having 2×10^7 cfu g^{-1} of lignite powder was applied to chickpea as seed coating.

Inoculation effect of consortium of Rhizobium, PSB and KMB on growth and yield of chickpea

The results in respect of growth and yield attributing characters of chickpea are presented in Table 8 and 9. The results of the present investigation revealed that among the different inoculation treatments, T_3

i.e. seed inoculation with consortium of *Rhizobium*, PSB and KMB + 75% RDF was found to be the most effective as it recorded significantly highest germination (97.46%), plant vigour index (2336.68) at 15 days after sowing, plant height (33.58 cm and 44.95 cm), root length (13.50 cm and 19.53 cm), dry weight of shoot (7.75 g plant^{-1} and 9.02 g plant^{-1}) and dry weight of root (910.33 mg plant^{-1} and 968 mg plant^{-1}) at flowering and harvest stage of the crop, number of branches (22.67 plant^{-1}), number of nodules (24.93 plant^{-1}), number of pods (55.47 plant^{-1}), 1000 seed weight (127.86 g) and seed yield (20.48 q ha^{-1}) of chickpea, however it was statistically at par with the treatment T_2 i.e. seed inoculation with consortium + 100% RDF for growth parameters and seed yield of soybean. Bansal (2009) reported that presowing inoculation of mungbean seeds with different inoculants (*Rhizobium*, PGPR and PSB) alone or in combination, significantly increased the plant height, root length, dry matter production, number of nodules/plant, 1000 seed weight, nutrient uptake and seed yield over uninoculated control. Moreover, Qureshi *et al.*, (2011); Argaw (2012) and Tarafder *et al.*, (2016) reported increased growth parameters, nutrient uptake and seed yield in different legume crops due to seed inoculation of *Rhizobium*, PGPR and PSB alone or in combination. Results of the present investigation are in agreement with results of these researchers.

In conclusion MS III culture medium proved effective with respect to population stability of individual strain and effectiveness of consortium of *Rhizobium*, PSB and KMB on growth and yield of chickpea. Moreover, from the present investigation it can be concluded that seed inoculation with consortium of *Rhizobium*, PSB and KMB + 75% RDF was found to be the most beneficial for getting higher seed yield of soybean with 25% saving of nitrogen, phosphorus and potassium dose of chemical fertilizers to chickpea.

Acknowledgement

The authors express their sincere thanks and gratitude to the Head, Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune for extending research facilities and financial support for conducting the present study.

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How to cite this article:

Thite, A. S., D. R. Murumkar and Chetan Shinde. 2023. Development of Microbial Consortia of Nitrogen Fixing, Phosphate Solubilizing and Potash Mobilizing Bacteria for Optimizing Nutrient Supplementation to chickpea. *Int.J.Curr.Microbiol.App.Sci.* 12(11): 42-54. **doi:** https://doi.org/10.20546/ijcmas.2023.1211.005