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

AS Thite and DR Murumkar

Abstract
A field trial was conducted to investigate the impact of seed inoculation with a combination of *Rhizobium*, PSB, and KMB on the growth parameters, nutrient absorption, and yield of chickpeas. Among various inoculation methods, the most effective was found to be seed inoculation with the *Rhizobium*, PSB, and KMB consortium along with 75% recommended dose of fertilizer (RDF). This treatment demonstrated significantly superior results, including the highest germination rate (97.46%), shoot length (16.53 cm), root length (7.45 cm), and plant vigor index (2336.68) at 15 days after sowing. Furthermore, it exhibited increased plant height (33.58 cm and 44.95 cm), root length (13.50 cm and 19.53 cm), shoot dry weight (7.75 g plant^{-1} and 9.02 g plant^{-1}) and root dry weight (910.33 mg plant^{-1} and 968 mg plant^{-1}) during the flowering and harvest stages of the crop. The treatment also resulted in higher numbers of branches (22.67 plant^{-1}), nodules (24.93 plant^{-1}), pods (55.47 plant^{-1}), 1000-seed weight (127.86 g), as well as increased NPK uptake (50.19, 13.73, and 24.30 kg ha^{-1}, respectively) and seed yield (20.48 q ha^{-1}) for chickpeas. Importantly, these outcomes were statistically comparable to the treatment involving seed inoculation with the consortium plus 100% RDF for the various growth parameters, nutrient uptake, and seed yield of chickpeas. The findings suggest a noteworthy 25% reduction in the application of nitrogen, phosphorus, and potassium doses from chemical fertilizers for chickpea cultivation.

Keywords: Consortium, *Rhizobium*, PSB, KMB, chickpea, yield

Introduction
In order to enhance chickpea growth, a combination of 25 kg N, 50 kg P2O5 and 30 kg K2O is deemed necessary. The positive impact of combining phosphate solubilizing bacteria (PSB) and *Rhizobium* on legume crops has been well-established, as evidenced by Cao *et al.*'s findings in 2016 [5]. Previous studies have endeavored to formulate a consortium comprising a *Rhizobium* strain, PSB, and PGPR. Additionally, research by Sheng and Huang (2002) [19] has indicated that potash mobilizing bacteria can enhance soil 'K' availability and mineral uptake by plants. Biofertilizers, encompassing nitrogen fixation, phosphate solubilization, potash mobilization, and plant growth-regulating substances, contribute to maintaining a soil environment rich in various micro and macro nutrients, as highlighted by Javaid in 2009 [11]. Shete *et al.* (2019) [20] developed the MS III culture medium specifically for cultivating a consortium of nitrogen-fixing, phosphate-solubilizing, and potash-mobilizing bacteria. Building on these insights, the current research aims to explore the impacts of a consortium comprising nitrogen-fixing, phosphate-solubilizing and potash-mobilizing bacteria on the growth, nutrient absorption, and yield of chickpeas.

Materials and Methods
Isolation of *Rhizobium* from root nodules of chickpea
Chickpea roots with robust, intact, and pink nodules were chosen for the isolation of *Rhizobium*, employing yeast extract mannitol agar (YEMA) media, as outlined in the methodology by Rajendran *et al.* (2008) [16].

Biochemical and physiological characterization of rhizobial isolates: A pure culture of the isolated microorganism was established and subjected to Gram staining. Subsequently, the Gram-negative isolates underwent additional biochemical tests, including catalase, oxidase, gelatin hydrolysis, indole tests, and growth on various carbon sources for confirmation. The characterization of these isolates followed the procedures outlined by Cappuccino and Sherman (1987) [6] in the 10th edition of "Microbiology: A Laboratory Manual."

To assess the nitrogen-fixing ability of the rhizobial isolate, a 48-hour-old culture of the freshly isolated *Rhizobium* strain was inoculated into 5 ml of yeast extract mannitol medium. After a 48-hour incubation, 1 ml of this broth was transferred to 50 ml of yeast extract mannitol medium and the mixture was further incubated for 15 days. Ten ml of this culture underwent N estimation using the standard Microkjeldhal technique (Reis *et al.*, 1994) [17]. The formula for N2 estimation is.

$$N2\ (mg/g) = \frac{\text{ml of } H_2SO_4 \text{ in the sample x Normality of} H_2SO_4 \text{ x } 14.01}{\text{Weight of the sample (carbon used in g)}}$$

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

Phosphate-solubilizing bacteria were isolated using Pikovskaya's medium through a process involving the serial dilution of soil and agar plating, as outlined by Aneja (2003) [1]. Colonies exhibiting the formation of a clear zone of P-solubilization on Pikovskaya's medium were chosen, purified, subcultured, and subsequently preserved on Pikovskaya's agar slants for future applications.

Phosphate solubilizing ability of the bacterial isolates

The capacity of the bacterial isolates to solubilize insoluble inorganic phosphate was assessed by applying 10 µl of overnight cultures onto Pikovskaya's agar plates and then incubating them at 28-30 °C for 2-3 days. Those isolates demonstrating a distinct zone of tricalcium phosphate (TCP) solubilization around the colony were identified as phosphate solubilizers. The diameter of the TCP solubilization zone was measured and expressed in millimeters. Bacterial isolates that exhibited positive results for phosphate solubilization on Pikovskaya's agar medium underwent quantification of the released phosphate ions (Pi) from TCP in a broth medium.

Biochemical characterization of the PSB isolates

The isolates underwent biochemical characterization following the procedures detailed in the 10th edition of "Microbiology: A Laboratory Manual" by Cappuccino and Sherman. Various tests, including the Catalase test, Oxidase test, Indole production test, Methyl red test, Voges-Proskauer (VP) test, Urea hydrolysis, Nitrate reduction test, Gelatin hydrolysis test, Starch hydrolysis, Casein hydrolysis and H2S production test, were conducted as part of the characterization process.

Isolation of potash mobilizing bacteria from rhizospheric soil of chickpea

One gram of soil from the rhizosphere was thoroughly mixed in 100 ml of sterile water and processed using the serial dilution agar plate technique as outlined by Aneja (2003) [1]. Appropriate dilutions (105 and 106) of both rhizosphere and rhizoplane solutions were plated on Alexandrov medium following the method described by Hu *et al.* (2006) [10]. The plates underwent an incubation period at room temperature (30±1 °C) for 3 days, during which colonies displaying clear zones of muscovite mica solubilization were carefully selected. These chosen colonies were then purified, subcultured, and preserved on slants containing Alexandrov medium for subsequent applications.

Biochemical characterization of KMB isolates: The isolates underwent biochemical characterizations following the protocols specified in Bergey's Manual of Systematic Bacteriology 9th Edition (1993). Various tests, including 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 conducted.

Quantitative estimation of 'K' solublization

The isolates that exhibited a zone of solubilization on Alexandrov agar medium underwent additional scrutiny regarding their capacity to liberate potassium (K) from broth media. The assessment of potassium release from muscovite mica in the broth by these isolates was conducted at 7, 15, and 20 days after incubation (DAI) in a laboratory setting, as described by Parmar *et al.* (2016) [14].

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

An inoculum consisting of *Rhizobium ciceri*, *Bacillus subtilis*, and *Frateuria aurantia* was developed using the selective medium MS III (Shete *et al.*, 2019) [20]. The medium was introduced into a 500 ml conical flask with 150 ml of medium and then subjected to incubation at 28 ± 2ºC with shaking at 100-150 rpm for three days until it reached an optical density of 0.5, measured at 535 nm. Lignite powder, employed as a carrier, underwent sterilization at 121oC and 1.04 kg/cm2 pressure for one hour. Subsequently, it was inoculated with broth cultures of *Rhizobium ciceri*, *Bacillus subtilis*, and *Frateuria aurantia* (100 ml per 500 g of lignite powder). The lignite powder-based inoculum was further incubated at 28±2 ºC for three days, during which 10% sugar solution was added to enhance the population of the respective microorganisms. The resulting inoculum of *Rhizobium ciceri*, *Bacillus subtilis*, and *Frateuria aurantia*, with a colony-forming unit (cfu) count of 2 x 107 per gram of lignite powder, was then applied as seed coating for soybeans.

Field experiment

In the Rabi season of 2021, a field trial took place at the College of Agriculture in Pune to investigate the impact of seed inoculation using a combination of *Rhizobium*, PSB, and KMB on the growth parameters, nutrient absorption, and yield of chickpeas. The test crop employed for this study was the Phule Digvijay chickpea variety. The experimental setup followed a randomized block design, featuring three replications and eight distinct 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

The data collection encompassed observations on various

parameters, including germination percentage, shoot length (in centimeters), root length (in centimeters), and plant vigor index at 15 days after sowing. Additionally, measurements were taken for plant height (in centimeters), root length (in centimeters), dry weight of shoot (in grams per plant), and dry weight of root (in milligrams per plant) at both the flowering and harvest stages of the crop. Other recorded metrics involved the number of branches, number of nodules, and number of pods per plant, as well as 1000-seed weight, NPK uptake (in kilograms per hectare), and seed yield (in quintals per hectare) for chickpeas. The plant vigor index at 15 days after sowing was calculated using the formula: Plant vigor index = Germination percentage × [shoot length (in centimeters) + root length (in centimeters)]. The nitrogen content of the plant was determined through the Modified Kjeldahl's process, and subsequently, the nitrogen uptake (in kilograms per hectare) was calculated as N percentage × total dry matter yield (in kilograms per hectare) / 100.

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

Analysis of fresh chickpea root nodules during the flowering stage involved assessing the rhizobial population on yeast extract mannitol agar media, following the method outlined by Rajendran *et al.* (2008) [16]. Additionally, microbial populations of phosphate-solubilizing bacteria (PSB) and potash-mobilizing bacteria (KMB) in rhizospheric soil samples at the chickpea flowering stage were examined using the soil serial dilution and agar plating method described by Aneja (2003) [1]. Enumeration of PSB and KMB populations took place on Pikovskaya's media and Alexandrov's agar media, respectively, at 10^6 dilutions. Incubation of the plates occurred at a temperature of 28±2 °C for 72 hours, after which colonies were counted. The population was expressed as colony-forming units (cfu) per gram of soil.

Statistical analysis

The data collected on different parameters underwent statistical analysis using the standard method of analysis of variance. The significance level employed in the 'F' and 't' tests was set at P = 0.05. Critical difference (CD) values were computed in cases where the 'F' test yielded significance, as per the approach outlined by Panse and Sukhatme (1985) [13].

Results and Discussion

Isolation of nitrogen fixing, phosphate solubilizing and potash mobilizing bacteria

Rhizobium was isolated from the root nodules of chickpeas (variety: Phule Digvijay) using a yeast extract mannitol agar medium. This isolation process was conducted for all three samples, resulting in three isolates designated as RH-I, RH-II, and RH-III. Additionally, the isolation of phosphate-solubilizing bacteria on Pikovskaya's medium involved serial dilution of soil and agar plating following the method described by Aneja (2003) [1]. The procedure was applied to all three rhizosphere soil samples, and the plates were monitored for bacterial colonies displaying a clear zone of tricalcium phosphate solubilization (TCP) on Pikovskaya's medium. Three isolates, identified as P-I, P-II, and P-III, were obtained. Furthermore, the isolation of potash-mobilizing bacteria was executed on Alexandrov medium, following the procedure outlined by Hu *et al.* (2006) [10]. This isolation process was carried out for all three rhizosphere soil samples on Alexandrov medium, with observation focused on the appearance of bacterial colonies exhibiting a clear zone of solubilization of insoluble potassium-bearing minerals (mica). Three isolates, designated as K-I, K-II, and K-III, were obtained through this method.

Nitrogen fixing ability of *Rhizobium* isolate

The nitrogen fixation of all three *Rhizobium* isolates from chickpeas, in addition to the MPKV strain (*Rhizobium ciceri*), was assessed using the Microkjeldhal method (see Table 1). RH-1 exhibited the highest nitrogen fixation, recording 149.88 µg of nitrogen per milligram of carbon utilized. Following this, the MPKV strain, RH-II, and RH-III isolates demonstrated nitrogen fixation amounts of 147.01, 130.99, and 123.07 µg of nitrogen per milligram of carbon used, respectively. These findings align with the results reported by Hema and Savalgi (2017) [9], who observed a nitrogen fixation of approximately 142 µg per milligram of carbon used in the isolate GdM5 from maize.

Phosphate solubilizing ability of the PSB isolates

The solubilization capability of all three PSB isolates, together with the MPKV strain (*Bacillus subtilis*), was evaluated for both qualitative and quantitative inorganic phosphate solubilization. The outcomes are detailed in Table 2. Rapid assessment of P-solubilization was conducted using Pikovskaya's agar medium. Each of the three isolates demonstrated the ability to create a zone of P-solubilization on the medium, with the diameter of the solubilization zone varying between 3-6 mm across different isolates.

Quantitative estimation of Pi released from TCP for bacterial isolates

The quantification of phosphorus released from tri-calcium phosphate (TCP) by the PSB isolates, in conjunction with the MPKV strain (*Bacillus subtilis*), was assessed in Pikovskaya's broth after 10 days of inoculation. The percentage of phosphorus (Pi) released from TCP by the isolates at 10 days after inoculation (DAI) varied between 12.57% and 30.52% (refer to Table 2). Notably, the isolate designated as P-I exhibited the highest level of phosphorus solubilization, recording a rate of 30.52%, surpassing the performance of the other tested isolates.

Decrease in pH of medium during phosphate solubilization

A decline in the pH of the TCP broth was observed ten days after inoculation, starting from the initially adjusted pH of 7.0. The P-I isolate exhibited the most significant reduction in the medium's pH, reaching pH 3.46, followed by the MPKV strain (*Bacillus subtilis*), P-II and P-III isolates, which recorded pH values of 3.48, 4.07, and 4.09, respectively (refer to Table 2). The decrease in the medium's pH showed a positive correlation with the amount of released Pi.

Quantitative estimation of 'K' solubilisation of the KMB isolates

The isolates displaying a zone of solubilization on Alexandrov agar medium underwent further assessment for their capacity to release potassium ('K') from broth media. The investigation involved studying the amount of 'K' released from muscovite mica in the broth by these isolates, along with the MPKV strain (*Frateuria aurantia*), at 7, 15,

and 20 days after incubation (DAI) under laboratory conditions. The results, presented in Table 3, revealed a range of 7.62 to 41.94 µg ml^{-1} for the 'K' released. The findings suggested that the quantity of released 'K' increased with the duration of incubation, reaching its peak at 20 DAI. Notably, the K-I isolate demonstrated the highest solubilization of muscovite mica, releasing 41.94 µg ml^{-1}, followed closely by the MPKV strain (*Frateuria aurantia*) at 40.38 µg ml^{-1} at 20 DAI. These results align with a prior study by Parmar *et al.* (2016) [14], where 25 potassium-solubilizing bacterial isolates were obtained from the maize rhizosphere in various areas of Navsari district. Parmar *et al.* (2016) [14] conducted quantitative estimation of 'K' solubilization by highly efficient KMB isolates, reporting 'K' release from muscovite mica in the broth within 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 nitrogen-fixing rhizobial isolate, RH-I, known for its high efficiency in nitrogen fixation, underwent a series of biochemical tests to assess various characteristics, including gram staining, motility, gelatin hydrolysis, catalase activity, oxidase activity, indole production, starch hydrolysis, hydrogen sulfide (H_2S) production, Voges-Proskauer test, and growth on different carbon sources (see Table 4). The cells of the nitrogen-fixing rhizobial isolate exhibited a rod shape, motility and a gram-negative reaction. Positive results were obtained for the catalase test, oxidase test, indole production test, starch hydrolysis, H_2S production and Voges-Proskauer test, while the gelatin hydrolysis test yielded negative results. For growth, the nitrogen-fixing rhizobial isolate utilized glucose, sucrose, and mannitol as sole carbon sources. Through biochemical and physiological characterization, the nitrogen-fixing rhizobial isolate was identified as *Rhizobium ciceri*. These findings align with the research conducted by Jadhav (2013) [24], who isolated rhizobia from soybean root nodules in the Latur area and characterized them biochemically based on specific traits of *Brady Rhizobium japonicum* as outlined in Bergey's Manual of Systematic Bacteriology. All isolates exhibited positive results for most characteristics specific to *Rhizobium ciceri*, and none of the isolates tested positive for gelatin hydrolysis.

The extensively proficient phosphate-solubilizing bacterial isolate (P-I) underwent various biochemical assessments, including gram staining, motility testing, gelatin hydrolysis, catalase testing, oxidase testing, starch hydrolysis, H_2S production and the Voges-Proskauer test (refer to Table 4). The cells of the phosphate-solubilizing bacterial isolate exhibited a rod shape, motility, and a positive gram reaction. This isolate demonstrated positive results for gelatin hydrolysis, catalase testing, starch hydrolysis, and the Voges-Proskauer test, while showing negative results for oxidase testing and H_2S production. In accordance with biochemical and physiological characterization (Claus and Berkeley, 1986) [7], the phosphate-solubilizing bacterial isolate was identified as *Bacillus subtilis*. Similarly, the highly efficient potash-mobilizing bacterial isolate (K-I) underwent diverse biochemical assessments, including gram staining, motility testing, methyl red test, Voges-Proskauer (VP) testing, urea hydrolysis, nitrate reduction testing, gelatine hydrolysis testing, catalase testing, starch hydrolysis, casein hydrolysis, H_2S production testing, and growth on different carbon sources (refer to Table 4). The potash-mobilizing bacterial isolate exhibited a rod shape, motility, and a gram-negative reaction. It tested positive for gelatin hydrolysis, catalase testing, starch hydrolysis, urea hydrolysis, casein hydrolysis testing, nitrate reduction testing, and the methyl red test, while testing negative for H_2S production and Voges-Proskauer test. Notably, the potash-mobilizing bacterial isolates demonstrated growth on sucrose, mannitol and maltose as sole carbon sources. In line with biochemical and physiological characterization (Parmar *et al.*, 2016) [14], the potash-mobilizing bacterial isolate was identified as *Frateuria aurantia*.

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

The broth from each of the culture media, namely M I, M II, M III, M IV, and M V, underwent inoculation with efficient strains of *Rhizobium*, PSB, and Potash mobilizing bacteria, both individually and in consortia. The inoculated broths were then incubated at a temperature of 28±2 ºC for a duration of 5 days. The data, as presented in Table 5, disclosed that the optimal growth of *Rhizobium*, PSB, and potash mobilizing bacteria occurred in the MS III culture media. Additionally, it was observed that *Rhizobium*, PSB, and KMB exhibited compatibility with each other when cultivated on MS III culture media. Singh *et al.* (2014) [21] previously reported the highest growth of rhizobia in media containing 12.5 g l^{-1} sucrose at 29.4 ºC for a period of 7 days. Furthermore, Kucuk *et al.* (2006) [12] found that *Rhizobium* strains displayed more efficient utilization of glucose and sucrose compared to the normal YEM medium. Moreover, Sagervanshi *et al.* (2014) [18] conducted a study on different nitrogen sources, such as ammonium sulfate, casein, sodium nitrate, and urea, determining that ammonium sulfate was the most optimized source for achieving maximum 'P' solubilization. In line with these findings, Sugumaran and Janarthanam (2007) [22] reported that *B. mucilaginosus*, isolated from soil, rock, and mineral samples, demonstrated a release of 4.29 mg l^{-1} potassium in media supplemented with muscovite mica. The results obtained from the current investigation align with and support the outcomes reported by these researchers.

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

Table 6 presents the microbial count data for *Rhizobium*, PSB and potash mobilizing bacteria across various culture media. Notably, the MS III culture medium exhibited the highest counts for *Rhizobium*, PSB, and KMB, reaching 11 x 10^7, 6 x 10^7, and 8 x 10^7 cfu g^{-1}, respectively, among all the culture media.

Preparation of consortium of *Rhizobium*, PSB and KMB

A consortium inoculum comprising *Rhizobium* (*Rhizobium ciceri*), PSB (*Bacillus subtilis*), and KMB (*Frateuria aurantia*) was cultivated in a specialized medium MS III. The medium was introduced into a 500 ml conical flask with 150 ml of medium and then incubated at 28 ± 2oC, with continuous shaking at 100-150 rpm for three days, achieving an optical density of 0.5 recorded at 535 nm. Lignite powder,

chosen as a carrier, underwent sterilization at 121 °C and 1.04 kg/cm2 pressure for one hour. Subsequently, it was inoculated with broth cultures of *Rhizobium ciceri*, *Bacillus subtilis*, and *Frateuria aurantia* (100 ml in 500 g lignite powder). The lignite powder-based inoculum underwent incubation at 28±2 °C for three days, with the addition of a 10% sugar solution to boost the population of the respective microbes. The resulting inoculum, containing 2 x 10^7 cfu g-1 of lignite powder for *Rhizobium ciceri*, *Bacillus subtilis*, and *Frateuria aurantia*, was then applied as a seed coating for chickpeas.

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

The findings concerning the growth and yield-related characteristics of chickpeas are outlined in Tables 7 and 8. The study revealed that, among the various inoculation treatments, T3 entailing seed inoculation with a consortium of *Rhizobium*, PSB, and KMB along with 75% RDF emerged as the most effective. This treatment demonstrated significantly superior outcomes, including the highest germination rate (97.46%), plant vigor 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), shoot dry weight (7.75 g plant^{-1} and 9.02 g plant^{-1}), and root dry weight (910.33 mg plant^{-1} and 968 mg plant^{-1}) during the flowering and harvest stages of the crop. Additionally, T_3 exhibited increased numbers of branches (22.67 plant^{-1}), nodules (24.93 plant^{-1}), pods (55.47 plant^{-1}), 1000-seed weight (127.86 g), and seed yield (20.48 q ha^{-1}) for chickpeas. However, these results were statistically comparable to those of treatment T_2, involving seed inoculation with a consortium plus 100% RDF, in terms of growth parameters and seed yield for chickpeas. According to Bansal (2009) [3], pre-sowing inoculation of mungbean seeds with various inoculants (*Rhizobium*, PGPR, and PSB), either alone or in combination, significantly increased plant height, root length, dry matter production, the number of nodules per plant, 1000-seed weight, nutrient uptake, and seed yield compared to the uninoculated control. Moreover, Qureshi *et al.* (2011) [15], Argaw (2012) [2], and Tarafder *et al.* (2016) [23] reported enhanced growth parameters, nutrient uptake, and seed yield in different legume crops resulting from seed inoculation with *Rhizobium*, PGPR, and PSB, either individually or in combination. The results of this study align with the findings of these researchers.

Table 1: 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 2: Zone of P solubilization on Pikovskaya's agar and percent Pi released from TCP broth by the PSB isolates

Sr. No.	PSB Isolate	Zone of P solubilization on TCP (mm)	% Pi released from TCP after 10 days	Decrease in pH of medium (from initial pH 7.0) 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 3: 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 4: Selective biochemical tests of nitrogen fixing, phosphate solubilizing and potash mobilizing 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			+

Table 5: 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 6: Microbial count of *Rhizobium*, PSB and KMB in a consortium on different culture media

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 7: 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 P2O5 & K2O	93.01	1881.02	31.48	41.99	11.95	16.48	6.05	7.34
T_5	PSB + 75% recommended P2O5 + 100% recommended N and K2O	91.47	1806.23	30.98	41.70	11.80	16.34	5.72	7.09
T_6	KMB + 75% recommended K2O +100% recommended N and P2O5	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 8: 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	808.33	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 P2O5 & K2O	793.67	843.00	21.97	22.13	49.11	122.26	17.62
T_5	PSB + 75% recommended P2O5 + 100% recommended N and K2O	772.00	825.00	21.70	21.13	48.44	121.39	17.33
T_6	KMB + 75% recommended K2O +100% recommended N and P2O5	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

Table 9: Inoculation effect of consortium of *Rhizobium*, PSB and KMB on available nutrients and nutrient uptake at harvest and microbial population at flowering stage of soybean

Tr. No.	Treatment details	Available nutrients (kg ha^{-1})			Nutrient uptake (kg ha^{-1})			Microbial population at Flowering (x 10^6)		
		N	P_2O_5	K_2O	N	P_2O_5	K_2O	*Rhizobium*	PSB	KMB
T_1	Consortium of *Rhizobium*, PSB and KMB	170.63	18.90	72.94	27.51	6.28	15.18	28.07	20.65	17.44
T_2	Consortium of *Rhizobium*, PSB and KMB + 100% RDF	199.74	24.71	102.85	45.52	12.15	22.04	34.20	30.65	27.65
T_3	Consortium of *Rhizobium*, PSB and KMB + 75% RDF	205.30	28.66	107.00	50.19	13.73	24.30	37.88	33.91	30.31
T_4	*Rhizobium* + 75% recommended N + 100% recommended P2O5 & K2O	184.30	23.76	87.41	40.47	11.52	20.39	28.01	20.63	17.30
T_5	PSB + 75% recommended P2O5 + 100% recommended N and K2O	177.96	18.81	81.18	41.75	10.08	18.92	27.18	13.86	9.78
T_6	KMB + 75% recommended K2O +100% recommended N and P2O5	174.13	21.21	77.33	41.24	11.84	20.96	19.01	20.66	14.55
T_7	100% RDF	167.44	18.74	69.81	40.14	10.98	21.66	17.09	19.81	16.68
T_8	Uninoculated control	159.30	15.45	61.89	20.94	4.74	11.90	8.56	10.20	8.09
	S.E.	2.39	2.58	1.46	1.71	0.96	0.85	3.22	4.07	4.03
	C.D.at 5%	7.18	7.79	4.42	5.19	2.89	2.66	9.74	12.30	12.18

Inoculation effect of consortium of *Rhizobium*, PSB and KMB on available NPK and nutrient uptake after harvest of chickpea

Table 9 presents the findings regarding available NPK levels and nutrient uptake following the harvest of chickpeas. The outcomes of this study indicate that among the various inoculation treatments, T_3 (consortium + 75% RDF) exhibited the significantly highest available NPK values (205.30, 28.66, and 107.00 kg ha^{-1}, respectively) and nutrient uptake (50.19, 13.73, and 24.30 kg ha^{-1}, respectively) compared to the other treatments. However, it is noteworthy that T3 was statistically equivalent to T_2 (consortium + 100% RDF) concerning available NPK levels (199.74, 24.71, and 102.85 kg ha^{-1}, respectively) and nutrient uptake (45.52, 12.15, and 22.04 kg ha^{-1}, respectively) by chickpeas at the time of harvest. The inoculation of *Rhizobium*, PGPR, and PSB, either individually or in combination, has been reported by various researchers to result in increased available soil nutrients and nutrient uptake after the harvest in different legume crops (Bansal, 2009; Qureshi *et al.*, 2011; Argaw, 2012; Tarafder *et al.*, 2016; Shete *et al.*, 2019) [3, 15, 2, 23, 20]. The present study's results align with the findings of these researchers.

Inoculation effect of consortium on microbial population of *Rhizobium*, PSB and KMB at flowering stage of chickpea

During the flowering stage, an analysis of fresh root nodules in chickpeas was conducted to assess the rhizobial population, while soil samples were scrutinized for the microbial population of phosphate solubilizing bacteria (PSB) and potash mobilizing bacteria (KMB). The collected data is presented in Table 9. Among the various inoculation treatments, T3 (consortium + 75% RDF) exhibited the significantly highest populations of *Rhizobium*, PSB, and KMB (37.88, 33.91, and 30.31 x 106 cfu g^{-1} soil, respectively) during the chickpea flowering stage. Interestingly, T3 was statistically comparable to T2 (Consortium + 100% RDF) for microbial populations of *Rhizobium*, PSB, and KMB (34.20, 30.65, and 27.65 x 106 cfu g^{-1} soil, respectively). These findings align with the research of Cao *et al.* (2016) [5], who observed increased populations of *Rhizobium* and PSB during the flowering stage of soybeans with the application of rhizobial and/or PSB inoculants. Similarly, Shete *et al.* (2019) [20] reported elevated populations of *Rhizobium*, PSB, and KMB during the flowering stages of crops, specifically mungbeans, when inoculated with the mentioned consortium, surpassing counts observed in uninoculated control groups. In conclusion, the present investigation suggests that seed inoculation with the consortium of *Rhizobium*, PSB, and KMB + 75% RDF is most beneficial for achieving higher chickpea seed yields, accompanied by a significant 25% reduction in nitrogen, phosphorus, and potassium doses from chemical fertilizers compared to untreated controls.

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