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IMPLICATION OF MICROBIAL CONSORTIUM ON BIOMASS AND YIELD OF CHICKPEA UNDER SUSTAINABLE AGRICULTURE

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Abstract

Five indigenous soil microbes were isolated from chickpea rhizosphere soils of different location of Jaunpur, Mirzapur, Varanasi and Azamgarh district of eastern Uttar Pradesh. The microbial strains were identified as *Pseudomonas aeruginosa* BHUPSB01, *Pseudomonas putida* BHUPSB04, *Bacillus megaterium* BHUPSB14, *Paenibacillus polymyxa* BHUPSB16 and *Mesorhizobium* sp. BHURC05 by biochemical and molecular characterization. The isolated strains were tested for their plant growth promoting properties. All strains were found positive for indole-3-acetic acid (IAA), ammonia production, and phosphate solubilization. Furthermore, the strain *P. aeruginosa* showed siderophore and hydrogen cyanide (HCN) production, and inhibited the growth of fungal pathogens such as *Fusarium oxysporum* and *Rhizoctonia solani*. The different treatment combinations of soil - bacteria enhanced nodulation, plant growth and yield of chickpea under the glasshouse and field experiments, respectively. The microbial consortium of *P. aeruginosa* and *Mesorhizobium* sp. showed more significant nodulation and biomass dry weight followed by *P. polymyxa* and *Mesorhizobium* as compared to *Mesorhizobium* alone and uninoculated control under glasshouse and field condition, respectively. Similarly, significant increases in grain yield and grain protein were recorded in microbial consortium of *P. aeruginosa* BHUPSB01 and *Mesorhizobium* sp. BHURC05. The present study confirmed that *Mesorhizobium* sp. and *P. aeruginosa* is an effective microbial consortium for biomass and grain production of chickpea in Indo-Gangetic plain of Eastern Uttar Pradesh, India.

Key words: biomass, chickpea, *Mesorhizobium*, microbial consortium, *Pseudomonas*, yield

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1. Introduction

The food security is a challenging problem of ever increasing human population. Furthermore, it has been anticipated that food insecurity will even enhance due to heat and drought stress caused by climate change in tropical and semitropical areas (Battisti and Naylor, 2009). Hence, there is an urgent need to find new agricultural technologies to obtain sufficient yields of good quality crops. It is also very imperative to sustain agricultural productivity under limited resources such as fertilizers (Cordell et al., 2009; Tey et al., 2017). The extensive and indiscriminate use of such agrochemicals in agriculture is currently under debate due to its environmental consequences and human health concerns.

Plant growth promoting rhizobacteria (PGPR) are free living soil bacterial which are beneficial for plant growth and yields (Kloepper and Schroth, 1978). PGPR strains (e.g. *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligens*, *Arthobacter*, *Burkholderia*, *Bacillus* and *Serratia*) have been reported for enhancing plant growth (Joseph et al., 2007; Mia et al., 2010; Mihalache et al., 2016). The PGPR can promote plant growth through

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various mechanism such as biological N_2 fixation, phosphate solubilization, production of phytohormones, vitamins, enzyme, siderophore and HCN, and the production of various types antibiotics for controlling phytopathogens (Ahmad et al., 2008; Akhtar et al., 2012; Krey et al., 2011; Laslo et al., 2012; Lucas et al., 2009; Ramaekers et al., 2010; Vessey, 2003). In addition, PGPR, including free living N-fixing bacteria (NFB), phosphate-solubilizing bacteria (PSB), and K-solubilizing bacteria (KSB), are ubiquitous in soils and can greatly increase nutrient availability and promote plant growth (Wu et al., 2012).

Chickpea (*Cicer arietinum* L.) is an important pulse crop which has the ability to fix 60 – 80% of its nitrogen requirement from the atmosphere under ideal conditions. After the boiling of chickpea seeds are relatively free from various anti-nutritional factors (phytic acid, polyphenols, tannins, saponins, oxalates and trypsin inhibitor activity) (Mittal et al., 2012). It has a high protein digestibility and is richer in phosphorus and calcium as comparison to other pulse crops. Low production in chickpea may be due to loss of microflora (algae, actinomycetes, bacteria, and fungi) and microfauna of soils which help in macro and micronutrient mobilization in soil. The population of soil microflora and microfauna are reducing due to application of higher dose of chemicals and pesticides in agriculture. Other problems of chickpea yield are due to some specific plant disease like root rot, wilting and blight at flowering stage and their casual organism such as *Pythium*, *Fusarium, Rhizoctonia* and *Botrytis,* respectively (Pathak et al., 2007).

PGPR controls soil-borne diseases and also increases the crop yield by the release of enzymes (dehydrogenase, chitinase, cellulase, phosphatase etc.), metabolites (siderophores, antibiotics, HCN), growth promoters (IAA, kinetin). They also induce systemic disease resistance (ISR) (Yadav et al., 2007). Turan et al. (2012) reported that the single inoculation of *Bacillus subtilis* OSU-142, *Bacillus megaterium* M3, or *Azospirillum brasilense* Sp245 have increased grain yield by 24%, 19%, and 19%, respectively, while a mixed inoculation with OSU-142, M3, and Sp245 have also increased grain yield by33% relative to non-inoculated plants. Therefore, the urgent need of efficient and indigenous isolates for enhancing the growth, yield and nutrient contents of chickpea as well as control the chickpea diseases. The indigenous microbes are easily adapted in the agro-climatic region of same place and can be more competitive as compared to the non-native bacteria.

The advantage of using indigenous microbial isolates is the easier adaptation and succession when inoculated into the plant rhizosphere. Hence, the aim of present investigation is evaluation of the effect of environment friendly microbial consortium on biomass and yields of chickpea (*Cicer arietinum* L.).

2. Experimental

2.1. Isolation and collection microbial strains

Soils and fresh root nodules were collected from chickpea rhizosphere (5-20 cm depth) from Indo-gangetic plain of Janupur, Mirzapur, Varanasi and Azamgarh district of Eastern Uttar Pradesh, India during December, 2006. *Rhizobium* was isolated from root nodules of chickpea on Yeast extract mannitol agar media. *Pseudomonas* and *Bacillus* strains were isolated on King's B base and nutrient agar (NA) median, respectively. The bacterial strains BHUPSB01, BHUPSB04, BHUPSB16, BHUPSB14 and BHURC05 were characterized by biochemical using standard methods (Cappuccino and Sherman, 1992).

The studied strains were identified as *P. aeruginosa* BHUPSB01, *P. putida* BHUPSB04, *B. megaterium* BHUPSB14, *P. polymyxa* BHUPSB16 and *Mesorhizobium* sp. BHURC05 by 16S rDNA gene sequencing. The fungal strains *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* were collected from the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi. All the microbiological media and media ingredients were purchased from Hi-Media Lab, Pvt. Ltd., Mumbai, India.

2.2. Molecular Identification of microbial strain

The PGPR strains such as *P. aeruginosa* BHUPSB01, *P. putida* BHUPSB04, *B. megaterium* BHUPSB14, *P. polymyxa* BHUPSB16 were grown in nutrient broth and *Mesorhizobium* sp. BHURC05 grown in YEM broth at 30ºC incubating shaker at 120 rpm for overnight. The extraction of DNA was performed as per manufacturer instructions of Invitogen PureLink^{TM} Genomic DNA Mini Kit USA. For the amplification of 16S rDNA gene in all bacterial isolate were used universal, custom synthesized primer (Bangalore Genei, Bangalore, India). The reaction mixture 50 µl consisted of 50 ng of genomic DNA, 2.5 U of Taq polymerase, 5 µl of 10 X PCR Taq DNA polymerase buffer (100 mM Tris-HCl, 500 mM KCl pH-8.3) (Bangalore Genei, Bangalore, India), 200µM dNTP, 1.5mM $MgCl_2$ and 10 pmoles each primer. The forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1492R (5'-TACGGTTAC CTTGTTACGACTT-3') were used by previously reported (Narde et al., 2004). Amplification was performed under the following PCR (PCR System 2720, Applied Biosystems, Singapore) conditions such as, initial denaturation at 94 °C for 5 min, followed by 34 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1.5 min, extension at 72 °C for 2 min and a final extension at 72 °C for 7 min.

Amplified PCR products (5 μl) were resolved on a 1.5% (w/v) agarose gel at 100 V for 45 min in 1X TAE buffer containing ethidium bromide (EtBr) with 500 bp DNA ladder (Bangalore Genei Pvt., Ltd. Bangalore, India). For the gene sequencing PCR product was purified by using the PCR purification kit (Invitrogen, PureLink^{TM} PCR purification kit USA). Nucleotide sequencing was carried out by Automated DNA Sequencer (Sequence Analyzer Version 2.0, ABI Prism from Chromous Biotech Pvt. Ltd., Bangalore) through methods of Sanger et al. (1977). 16S rDNA sequence was analyzed with the nucleotide database available at the GenBank using the BLAST tool at NCBI (www.ncbi.nlm.nih.gov) for identification of bacteria. 16S rDNA gene sequence was submitted in GenBank with different accession number GU124822, GU124834, GU124821, GU124838 and GU646773.

2.3. *In vitro screening of microbial strains for plant growth promoting activities*

IAA (indole-3-acetic acid) production had been tested with modified methods of Bric et al. (1991). Phosphate solubilization assay had been done on Pikovskaya's agar plates by methods of Gaur (1990) and solubility index estimated by methods of Edi-Premono et al. (1996). NH_3 production evaluated by Cappuccino and Sherman (1992). We have followed the methods of Schwyn and Neilands (1987) for Siderophore assey on the Chrome azurol S agar medium. Also, we had followed the method of Jha et al. (2009) for HCN production and the methods of Rajendran et al. (2008) for Antifungal assay testing.

2.4. *Seed inoculation*

We had obtained seed of chickpea (*Cicer arietinum* L.) var. Radhey from Indian Pulse Research, Kanpur, U.P, India. Seeds were surface sterilized with 0.1% $HgCl_2$ for 2 min and rinsed six times with sterile distilled water. The *Mesorhizobium* sp. was grown in YEM broth and *B. megaterium*, *P. polymyxa*, *P. aeruginosa* and *P. putida* were grown in nutrient broth on shaking incubator (120 rpm) at 28±2°C for 3 days. For field experiment, 100g chickpea seeds for each plot (10m^2) were taken in plastic bag then added 100 ml of sticker solution of gum acacia with proper mixing. The seeds were completely mixed with gum acacia, then 10 ml of broth cultures grown in specific media of respective inoculants (mixed in 1:1 ratio for combined treatments) and left for 5 h. After 5 h, 1g seed were taken and make serial dilution and spread on nutrient agar and yeast extract mannitol agar. The plates were incubated for 28 ± 2°C for 3-4 days. After incubation bacteria population was found 10^7 CFU/seed.

2.4.1. *The physico-chemical and biological properties of soil*

The initial soil samples were collected before the field experiment and final soil sample were collected after crop harvesting. Soil were randomly collected from whole field and mixed it to make a composite sample.

Then three samples were picked for analysis of the physico-chemical and biological properties of the initial and final composite soils. The physico-chemical properties of soil were estimated as texture, water holding capacity, pH and electronic conductivity (EC) (Singh et al., 2005). The content of organic carbon (Walkley and Black, 1934); available N (nitrogen) (Subbiah and Asija, 1956) and available P (phosphorus) (Olsen et al., 1954) in soils were determined. The microbial population of total bacteria, fungi and actinomycetes were also determined in experimental soil (Aneja, 2003). The enzyme activities of soils were determined according to Aneja (2003).

2.4.2. *Glasshouse experiment*

Sandy loam soil collected from Organic Farm, IAS, BHU was passed through a 10-mesh sieve and autoclaved at 121°C, 15 lbs pressure for 2 h. Similarly, earthen pots of 30 cm diameter were also sterilized at 121°C, 15 lbs pressure for 2 h. Sterilized pots were allowed to cool to room temperature before use and each pot were filled with 5 kg of sterilized soil. 20 inoculated seeds were sown in separate 30 cm diameter clay pots containing 1.5 kg steam sterilized soil in the first week of October 2007. Uninoculated seeds treated with nutrient broth were sown in pot as control. Pots were kept in glasshouse at 20 to 25°C. The glasshouse condition was 60-80% humidity, 10 Klux light intensity and 28-37°C to temperature. Pots were irrigated with sterile tap water as needed and the experiment was terminated 70 days after sowing (DAS). Pots were arranged in a randomized block design and each treatment was replicated four times.

2.4.3. *Field experiment*

Since the last two years, fields were used for organic cultivation of mung bean, wheat and red gram. Just before conducting field experiment, green manuring had been done by *Seasbania rostrata*. Healthy seeds weighed 100g for each plot of 10 m^2 (at 100 kg/ha) were surface sterilized with 0.1% $HgCl_2$ for 2 min and rinsed six times with sterile water. The *Mesorhizobium* sp. was grown in YEM broth and *B. megaterium*, *P. polymyxa*, *P. aeruginosa* and *P. putida* were grown in nutrient broth on shaking incubator (120 rpm) at 28±2°C for 3 days.

The field experiments were designed with 6 treatments and 4 replications of indigenous *Mesorhizobium* sp. alone and their appropriate synergistic combination *Mesorhizobium* sp. with *B. megaterium*, *P. polymyxa, P. aeruginosa*, *P. putida* and one uninoculated control. The inoculated seed were sowed in each plot on dated 6 October, 2007. The plot size was 4×2.5m and spacing 25×25cm between row and 10×10 cm between plants. For assessment of root and shoot dry weight, ten plants were randomly uprooted at 70 DAS from inoculated and control plots and the root system were gently rinsed. The plants were cut with a knife above the base of the root

emerging zone. The nodule numbers per root system were counted. For dry weight determination shoot were kept in envelopes at 60°C for 2-3 days in hot air oven. The ends of the growth period, another ten representative plants were randomly harvested from control and inoculated plots to determine grain and straw yield. The protein was determined in grain by Lowery et al. (1951).

2.5. Statistical analysis

Statistical analysis was conducted using one-way analysis of variance (ANOVA). Comparisons of mean were performed by the least significant deferent (LSD) test at $p \leq 0.05$ by using SPSS software version 12.0.

3. Results and discussion

3.1. Isolation and biochemical characterization of microbial strains

Indigenous microbial strains were isolated from soils of chickpea rhizosphere and BHURC05 from root nodules of chickpea. On the basis of morphological and biochemical character the soil isolates were grouped into *Bacillus*, *Pseudomonas* (Holt et al., 1994). Root nodule isolates of chickpea characterized as *Mesorhizobium* sp. Only has shown positive polysaccharide production test. *Mesorhizobium* sp. BHURC05, *Pseudomonas* sp. BHUPSB01 and BHUPSB04 have shown Gram-negative. *Bacillus* sp. BHUPSB14 and BHUPSB16 showed Gram-positive.

All strains have shown positive test for catalase, oxidase and citrate (Table 1). *Bacillus* strains have shown positive for starch hydrolysis as compare to other. *Pseudomonas* has showed negative for lipid hydrolysis than others. All strains have positive for carbohydrate utilization such as glucose, lactose sucrose and mannitol but *Pseudomonas* has negative for mannitol utilization (Table 1).

3.2. Identification of the microbial strains by 16S rDNA gene sequence analysis

16S rDNA gene was amplified by PCR using 16S universal primers. Partial 16S rDNA gene amplicons (1500 bp) sequenced using the facilities provided by Bangalore Genei Pvt. Ltd. Bangalore, India. The 16S rDNA analysis followed by BLAST search and biochemical characterization was in admirable agreement for such characterization, since the isolates exhibited close to 16S rDNA database similarity and were closely associated with known *Rhizobium* spp., *Bacillus* spp. and *Pseudomonas* spp. A comparison with the 16S rDNA sequences available in the GenBank database indicated that the strain BHUPSB01, BHUPSB04, BHUPSB14 and BHUPSB16 showed 99% homology with *Pseudomonas aeruginosa* strain IR-222 (GU586317), *Pseudomonas putida* strain AKMP7 (GU396282) and *Bacillus megaterium* strain LCR50 (FJ976559) and *Paenibacillus polymyxa* strain 1244 (EU982527), respectively. The bacterial strain BHURC05 showed homology 86% with *Mesorhizobium* sp. SOD35 (EF125934). The 16S rDNA gene characterization confirmed the identification of PGPR as *Pseudomonas* group, *P. aeruginosa* strain BHUPSB01 (accession number GU124822), *P. putida* strain BHUPSB04 (accession number GU124834), *Bacillus megaterium* strain BHUPSB14 (accession number GU124821), *P. polymyxa* strain BHUPSB16 (accession number GU124838) and *Mesorhizobium* sp. BHURC05 (accession number GU646773).

3.3. Plant growth promoting properties of isolated microbial strains

Pseudomonas sp. BHUPSB01 showed significant increase in IAA production as compared to other strains, when 150 μg/mL tryptophan was added as the precursor. BHUPSB01 showed two-fold higher IAA secretion when the tryptophan concentration was increasing from 50 to 150μg/mL. IAA production was recorded in broth culture of isolated strains, ranged from 11.48 to 70.80% at 150μg/mL tryptophan concentration. *Pseudomonas* BHUPSB01 showed 70.80, 53.14, 25.25 and 22.09% IAA production as compared to strains BHUPSB16, BHUPSB14, BHURC05 and BHUPSB04, respectively (Tables 2-3). The phosphate solubilization was most frequently encountered by *P. aeruginosa* followed by *P. polymyxa*, *B. megaterium*, *P. putida* and least by *Mesorhizobium* sp. Similarly, high level of IAA production was recorded in *Pseudomonas* sp., *Bacillus* sp., *Rhizobium* and *Mesorhizobium* sp. by other workers (Ahmad et al., 2008; Verma et al., 2010). Solubilization index of bacterial culture were showed maximum significant in BHUPSB01 (6.28) followed by BHUPSB04 (2.95), BHUPSB14 (2.85) and BHUPSB16 (2.27) as compared to BHURC05 (1.95). The phosphate solubilization was most frequently encountered by *P. aeruginosa* followed by *P. polymyxa*, *B. megaterium*, *P. putida* and least by *Mesorhizobium* sp.

The higher solubilization index 6.22 was recorded in *P. aeruginosa* than other strains due to the production of largest halozone around the bacterial colony within 5 days of incubation. Similarly, Ahmad et al. (2008) and Jha et al. (2009) reported that the *P. fluoresecens* NJ101, *P. aeruginosa* and *Bacillus* species is a good phosphate solubilizers. Phosphate solubilization depend on the microbial enzymatic activities phosphates activities. Krey et al. (2011) reported that the application of *P. fluorescens* in the unfertilized soil was enhanced 52% acid phosphatase activities (ACP), 103% alkaline phosphatase activities (ALP), and 133% for phosphodiesterase (PDE). However, the production of ammonia was a common trait in all isolates. *P. aeruginosa* was detected positive for siderophore and HCN production than other strains.

Table 1. Biochemical properties of microbial isolates

Biochemical characters	*Mesorhizobium sp.*	*P. polymyxa*	*B. megaterium*	*P. aeruginosa*	*P. putida*
Colony morphology	Pin head, mucilaginous, Large Gummy, white	Large, irregular flat with an Undulating margin	Serrate margin white	Button shaped fluorescent green	Circular flat margin
Polysaccharide Production	+	−	−	−	−
Gram reaction, Cell shape	− rods	+ rods	+ rods	− rods	− rods
Catalase, oxidase citrate test	+	+	+	+	+
Hydrolysis					
Starch	−	+	+	−	−
Lipid	+	+	+	−	−
Carbohydrates					
Glucose	+	+	+	+	+
Lactose	+	+	+	+	+
Sucrose	+	+	+	+	+
Mannitol	+	+	+	−	−

"+" sign mean positive test; "−" sign means negative test; -rod, negative gram reaction and rod shaped; +rod, positive gram reaction and rod shaped

Table 2. Plant growth promoting properties of bacterial strains

Bacterial strain	*IAA (µg/mL) at different tryptophan (µg/mL)*			*Solubilization Index*	*NH₃*	*Siderophore*	*HCN*	*Antifungal test*
	50	*100*	*150*					
Mesorhizobium sp.	11.3±1.21[cd]	18.33±1.06[b]	25.35±1.11[b]	1.95[a]	+	−	−	−
B. megaterium	8.23±0.70[b]	13.23± 2.04[a]	20.21±1.16[a]	2.85[c]	+	−	−	−
P. polymyxa	5.22±0.32[a]	10.63±2.09[a]	18.12±1.05[a]	2.27[b]	+	−	+	+
P. aeruginosa	13.18±2.25[d]	21.21± 1.12[c]	30.95±2.17[c]	6.22[d]	+	+	+	+
P. putida	10.12±2.05[c]	18.35±1.11[b]	24.71±1.13[b]	2.95[c]	+	−	+	+

"+" sign mean positive test; phosphate solubilization; NH_3, ammonia production; HCN, hydrogen cyanide, Antifungal test; IAA-Indole-3-acetic acid (µg/mL±SD), [a]Values are the mean ± SD, Mean values in each column with the same superscript (s) do not differ significantly by LSD ($P \leq 0.05$)

Table 3. Effect of microbial consortia on the biomass production of chickpea plants under glasshouse condition

Treatment	*Nodule number/plant*	*Nodule dry weight/plant (g)*	*Root dry weight/plant (cm)*	*Shoot dry weight/plant (g)*
Control	0	0	0.219±0.01[a]	0.514±0.03[a]
Mesorhizobium sp.	35±1.2[a]	0.086±0.01[a]	0.298±0.01[b]	0.671±0.01[b]
Mesorhizobium sp. + *B. megaterium*	46±1.1[b]	0.094±0.01[a]	0.342±0.02[c]	0.785±0.02[c]
Mesorhizobium sp .+ *P. polymyxa*	55±1.6[c]	0.121±0.02[b]	0.401±0.06[d]	0.813±0.06[d]
Mesorhizobium sp .+ *P. putida*	41±2.1[b]	0.103±0.03[a]	0.328±0.08[c]	0.789±0.08[c]
Mesorhizobium sp. + *P. aeruginosa*	62±1.8[d]	0.132±0.03[d]	0.428±0.12[d]	1.015±0.08[e]

[a]Value are the mean ± SD, Mean values in each column with the same superscript (s) do not differ significantly by LSD ($P \leq 0.05$)

Experimental results revealed that the strain *P. aeruginosa* BHUPSB01, *P. putida* BHUPSB04 and *P. polymyxa* BHUPSB16 showed HCN production as well as prevent the wilting and root rot disease in chickpea by inhibiting the growth of soil pathogenic fungi *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* while *P. aeruginosa* also has ability of produce siderophore for providing iron to plants (Table 2). The antifungal activity of the test isolates might be due to the production of siderophore and HCN or synergistic interaction of these two or with other metabolites (Blom et al., 2011). Several studies have demonstrated that production of siderophores, other secondary metabolites and lytic enzymes by *Pseudomonas* strains and *Burkholderia cepacia* were most effective for controlling the plant root pathogens including *Fusarium oxysporum* f. sp. *ciceri* and *R. solani* (Vial et al., 2011; Doornbos et al., 2012).

3.4. Effect of microbial consortium on nodulation and biomass production of chickpea under glasshouse

Seed inoculation of chickpea alone with *Mesorhizobium* was recorded significant increase in dry weight of shoot (30.54%) and root (36.07%) of chickpea as compared to uninoculated control at 70 DAS. Co-inoculation of *Mesorhizobium* sp. with *P. aeruginosa*, *P. polymyxa*, *B. megaterium* and *P. putida*, showed 77.14, 57.14, 31.43 and 17.14%, significant increase in nodule number, respectively than *Mesorhizobium* alone. But dry weight of nodules

was significantly increased 51.69% and 40.70%, only in combination of *Mesorhizobium* sp. with *P. aeruginosa* and *P. polymyxa*, respectively over *Mesorhizobium* sp. alone. Similarly, Tagore et al. (2013) reported that the microbial inoculants, the Rhizobium + PSB was found most effective in terms of nodule number (27.66 nodules plant^{-1}), nodule fresh weight (144.90 mg plant^{-1}), nodule dry weight (74.30 mg plant^{-1}), shoot dry weight (11.76 g plant^{-1}), and leghemoglobin content (2.29 mg g^{-1} of fresh nodule) and also showed its positive effect in enhancing all the yield attributing parameters, grain and straw yields under pot and field condition.

The root dry weight was significantly increased by 43.62%, 34.56%, 14.76% and 10.06% while shoot dry weight was also increased by 51.26%, 21.16%, 16.98% and 17.58% in combined inoculation of *Mesorhizobium* sp. and *P. aeruginosa*, *P. polymyxa*, *B. megaterium* and *P. putida*, respectively as compared to *Mesorhizobium* sp. alone at 70 DAS. The co-inoculation of *P. aeruginosa* and *Mesorhizobium* sp. showed more significant nodule numbers, dry weight of nodule, root and shoot as compared to uninoculated control. Other reports on co-inoculation of *P. jessenii* PS06 and *M. ciceri* C-2/2 showed significant increase in nodulation, shoot dry weight, N and P content in shoot of chickpea under greenhouse (Valverde et al., 2006; Verma et al., 2012, Jalilian et al., 2012). Inoculation of capsulated *Pseudomonas putida* MTCC 6842 in soil was enhanced the dry matter of root and shoot of wheat as well as enhanced the availability of phosphorus and auxin in soil under pot condition (Trivedi and Pandey, 2007).

3.5. Effect of microbial consortium on biomass, yield and protein content in grain of chickpea under field experiment

Seed inoculation of chickpea alone with *Mesorhizobium* sp. showed significant increase in nodules number (20%), dry weight of nodules (25.92%), shoot (40.06%) and root (26.57%) as compared to uninoculated control. It also showed increase in yield of grain (6.85%) and straw (23.02%), and protein content (9.37%) in grain than uninoculated control (Table 4). Co-inoculation of *Mesorhizobium* sp. with *P. aeruginosa*, *P. polymyxa*, *B. megaterium* and *P. putida* were found significantly 80.95%, 61.91%, 47.61% and 54.76% nodule number, respectively as compared to *Mesorhizobium* sp. alone. Enhancement in nodulation might be due to synergistic consequence of the microbial consortium for biological nitrogen fixation by *Mesorhizonium* sp. Results of the similar kind have also been reported by Rudresh et al. (2005). The root dry weight had been shown significant increase in co-inoculation of seed of *Mesorhizobium* sp. with *P. aeruginosa* (66.81%), *P. polymyxa* (58.52%), *B. megaterium* (41.48%) and *P. putida* (33.62%), respectively than *Mesorhizobium* sp. inoculation alone at 70 DAS. But significant increase in nodule and shoot dry weight was found in combination of *Mesorhizobium* sp. and *P. aeruginosa* only. The co-inoculation of *Mesorhizobium* sp. with *P. aeruginosa*, *P. polymyxa*, *B. megaterium* and *P. putida* showed significant increase 30.37%, 26.57%, 21.34% and 20.09% grain yield, respectively as compared to control (Table 4). But 54.18%, 46.74%, 42.13% and 31.54% straw yield were observed in combination of *Mesorhizobium* with *P. aeruginosa*, *P. polymyxa*, *B. megaterium* and *P. putida,* respectively than control. Significant increase in protein content was found in treatments combination of *Mesorhizobium* with *P. aeruginosa* (30.19%), *P. polymyxa* (25.61%), *B. megaterium* (19.78%) and *P. putida* (17.18), respectively as compared to control (Table 4).

The combined treatment of *Mesorhizobium* sp. with *P. aeruginosa* showed higher grain, straw and protein content followed by *Mesorhizobium* sp. with *P. polymyxa*, *B. megaterium* and *P. putida* as compared to low in control. Rudresh et al. (2005) was also recorded that the combined inoculation of *Rhizobium* and PSB showed significant nodulation, plant growth, yield and nutrient uptake in chickpea. Co-inoculation studies with PGPR and *Rhizobium*/*Bradyrhizobium* spp. have been shown to increased root and shoot biomass, nodule dry matter, nitrogenase activity, N_2-fixation, and grain yield in chickpea (Gull et al., 2004).

Table 4. Effect of PGPR and *Mesorhizobium* sp. inoculation on biomass production, yield and protein content in grain of chickpea at field experiment

Treatment	***Nodule/ plant***		***Dry weight/plant (g)***		***Yield (q/ha)***		***Protein % in grain***
	Number	***Dry weight (g)***	***Root dry***	***Shoot dry***	***Grain***	***Straw***	
Control	31±1.5^{a}	0.081±0.013^{a}	0.407±0.03^{a}	0.978±0.09^{a}	21.60±1.13^{a}	13.03±0.06^{a}	19.21±1.24^{a}
Mesorhizobium sp.	42 ±1.8^{b}	0.102±0.03^{b}	0.458±0.02^{b}	1.21±0.08^{b}	23.08±1.81^{b}	16.03±0.42^{b}	21.01±0.56^{b}
Mesorhizobium sp. + *B. megaterium*	62±1.5^{c}	0.123±0.019^{b}	0.648±0.016^{c}	1.63±0.09^{c}	26.21±1.87^{c}	18.52±1.91^{b}	23.01±1.34^{c}
Mesorhizobium sp .+ *P. polymyxa*	68±2.8^{c}	0.131±0.015^{b}	0.726±0.019^{d}	1.75±0.05^{c}	27.34±1.23^{c}	19.12±1.84^{c}	24.13±1.64^{c}
Mesorhizobium sp .+ *P. putida*	65±3.6^{c}	0.118±0.011^{b}	0.612±0.011^{c}	1.31±0.02^{b}	25.94±1.52^{c}	17.14±1.71b^{b}	22.51±0.45^{b}
Mesorhizobium sp. + *P. aeruginosa*	76±2.6^{d}	0.144±0.016^{c}	0.764±0.028^{d}	1.95±0.07^{d}	28.16±1.25^{d}	20.09±1.51^{c}	25.01±1.31^{d}

[a]Values are the mean ± SD, Mean values in each column with the same superscript (s) do not differ significantly by LSD (P≤0.05)

Combination of *Mesorhizobium* sp. with *P. aeruginosa* were found significant plant growth, yield and protein content in grain followed by combinations of *Mesorhizobium* with *P. polymyxa*, *B. megaterium* and *P. putida* as compared to alone and uninoculated control in glasshouse and field experiment, respectively. Similarly, Wani et al. (2007) have been also reported that the synergistic effect of nitrogen fixing and phosphate-solubilizing rhizobacteria on plant growth, yield, grain protein, and nutrient uptake of chickpea plants. It is also due to the fact that phosphate solubilizing bacteria by virtue of their property of producing organic acids solubilize insoluble or fixed form of phosphorus in the rhizosphere and make it available to the growing plants, which promotes root development in plants (Subba Rao, 1986; Lavakush et al., 2014).

In the present study, a significant response of dual inoculation with Rhizobium and PSB was observed with respect to shoot dry weight per plant. Observations of the similar kind have been recorded by Gupta and Namdeo (2000); Barea et al. (2005); Rokhzadi and Toashih (2011).

3.6. The physico-chemical and biological properties of composite soils of initial and final

The physico-chemical properties of the initial soil before experiment and experimental soil after crop harvesting were recorded as sandy clay loam in texture, soil pH (7.05 and 7.14) and electronic conductivity (0.135 and 0.145 dS/m), respectively. The content of organic carbon (0.60 and 0.625%), available N (212.48 and 228.54 kg/ha) and available P (20.81 and 26.38 kg/ha), respectively in initial soil and experimental soil after crop harvesting were determined (Table 5).

The total bacterial population (8.3×10^5 and 21.3×10^5 CFU/g soil), fungi (4.1×10^4 and 10.6×10^4 CFU/g soil), actinomycetes (6.6×10^4 and 15.8×10^4 CFU/g soil) and alkaline phosphatase (28.49 and 33.27 µgPNP/g soil/h), and dehydrogenase (115.67 and 138.25µgTPF/g soil/day), respectively in initial soil and experimental soil after crop harvesting were estimated (Table 5). The physico-chemical properties like pH, EC and organic carbon in soil showed slight increment in crop harvested field trial application with microbial consortium as compared to initial soil samples of field. But the available nitrogen, phosphorus, microbial population (bacteria, fungi and actinomycetes) and enzyme activities (alkaline phosphatase and dehydrogenate) showed enhancement in inoculated field after harvesting as compared to initial soil sample. The increased yield due to application of chemical fertilizers in conjunction with PGPR may be due to improved vegetative growth, better availability of nutrients, enhanced photosynthetic activity and improvement in soil physical properties which led to better soil physical health (Sharma, 1986).

Increase in organic carbon, electrical conductivity and slight decrease in pH due to more root excretions and deposition of organic residues were seen in treatments of microbial consortium than control treatments.

Increasing trend in soil organic carbon due to seed inoculation of soil microbes in peas was reported by Ram and Sanoria (1979). Microbial mineralization of organic residues might be the reason for positive differences of available N and P in the soil as also reported in mungbean (Wein et al., 1979; Ahmad et al., 2008) under inoculation condition. Available nitrogen and phosphorus were found significantly increased in PGPR combinations than control and some other treatments due to symbiotic and asymbiotic N_2-fixers which provided nitrogen and phosphate solubilizers provided available phosphorus in rhizosphere soil. The combined inoculation of *P. aeruginosa* BHUPSB01 and *Mesorhizobium* sp. BHURC05 help in provided nitrogen, phosphorus, iron and other plant nutrient for the growth and yield of chickpea which save approximate 25% chemical fertilizer input. A further study also indicated that PGPR such as *Pseudomonas fluorescens* and *Pseudomonas putida*, could improve wheat yield and reduce the dependence on inorganic N by 25%, whilst giving an increase in wheat grain yield of 96% (Naveed et al. 2008). The application of PGPR strains MK5 isolate at 75% recommended dose of NP fertilizers not only increased the yields of cauliflower by 24% but also saved 31kg N /ha and 8 kg P/ha fertilizers over control (Kaushal and Kaushal, 2013).

Table 5. The physico-chemical and biological properties of initial soil sample before chickpea field experiment

Soil sample	***pH***	***EC (dSm^{-1})***	***Org C (%)***	***Avail N kg ha^{-1}***	***Avail P kg ha^{-1}***	***Alk Phosphatase (µg PNP g^{-1}soil h^{-1}***	***Dehydrogenase (µg TPF g^{-1}soil day^{-1}***	***Total microbial count (CFU/g soil)***		
								Bacteria	***Fungi***	***Actinomycetes***
Initial soils	7.05^a	0.134^b	0.60^a	212.48^a	20.81^a	28.49^a	115.67^a	8.3×10^{5a}	4.1×10^{4a}	6.6×10^{4a}
Soil after crop harvesting	7.14^a	0.145^b	0.63^a	238.54^b	28.38^b	43.27^b	148.25^b	21.3×10^{5b}	10.6×10^{4b}	15.8×10^{4b}

EC: Electrical conductivity, Org. C: organic carbon, Avail N: available nitrogen, Avail P: available phosphorus, Alk Phosphatase: alkaline phosphatise, PNP: para-Nitrophenol, TPF: *1,3,5-triphenylformazan, CFU: colony forming unit, Data are presented as mean (n=3), Mean values in each column with the same superscript (s) do not differ significantly by Duncan post hoc multiple comparison tests (P≤0.05)*

Erikson (2005) and Chen et al. (2003) reported that application of green manuring with PGPR to soil are considered as an effective management practice in any agricultural system due to stimulation of soil microbial growth and activity with subsequent mineralization of plant nutrient and increased biological nitrogen fixation in soil.

4. Conclusions

The indigenous microbial consortium of *Mesorhizobium* sp. BHURC05 and *P. aeruginosa* BHUPSB01 could be effective biofertilizer for enhancing biomass and yield of chickpea as well as protein content in grain.

Chickpea seed is an important pulse as nutrient rich and protein source in diet of human being. These type microbial consortia can substantially enhance yield and nutrient content of chickpea. It is environment friendly microbial consortium for chickpea production.

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