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Effect of nutrient sources and inoculation of phosphate solubilizing microorganisms on growth enhancement in maize

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Abstract
Phosphorus Solubilizing Microorganisms (PSM) can effectively contribute together in plant nutrient availability. The aim of this investigation was to evaluate the potential of isolated and procured PSM in their phosphate solubilizing capacity and improvement in growth and nutrition in maize upon inoculation with various doses of nutrient sources. Therefore, two isolated PSM strains from phosphorus deficient rhizospheric soils were tested *in vitro* for P solubilizing capacity along with two bacterial stains. *Aspergillus* sp. proved to be the superior nutrient solubilizer in both plate and broth assays. A pot culture experiment was conducted under glass house condition where plant growth and nutrient availability to the plants were observed. Hence, all 15 treatment combinations such as the four isolate inoculated plants, two nutrient sources, controls and a combination of PSM + nutrient sources with three replications were destructively sampled at 60 DAS. Both PSM inoculation and nutrient application and their combinations had synergistic effects on plant growth and nutrient uptake. Plants treated with *Aspergillus* sp. + RP @ 40 kg/ha increased N, P and K uptakes 1.74, 0.29 and 1.07 g /plant, respectively against control. Overall the fungal species upon inoculation with nutrient source proved to be better for plant nutrition than the bacterial ones. PSMs have the potential to be used as a promising biofertilizers for solubilizing mineral nutrients and increasing its availability for improving plant growth and nutrient absorption other than commonly used conventional methods.

Keywords: *Aspergillus*, *Penicillium*, *Bacillus megaterium*, phosphate solubilization, solubilization index

Introduction
Indian soils are inherently low in phosphorus (P) status (80–90% soils are deficient) (Adnan *et al*. 2003) [2], it is important to make P application in soil for its availability in the rhizosphere. P application through chemical fertilizers can be used efficiently to bridge plant nutrition and yield gap but the application cost of P fertilizers gets high due to its inherent nature to make complex with calcium (Ca), aluminum (Al), and iron (Fe) which converts it to unavailable form and hinders its uptake by plants (Herrera *et al*., 2016) [24]. In virgin soil, a notable amount of P (400–1200 mg/ kg) is present (Rodríguez and Fraga 1999) [54]. Besides that, by the addition of chemical fertilizer results in accumulation of a large amount of insoluble P in complex form either with Ca/Mg carbonates in alkaline soil or with Al/Fe mineral complex in acid soil. Organic forms of P constitute about 30–50% of the total P pool in most soils. So to convert the insoluble P pool to soluble one for plant uptake is the current challenge in sustainable agriculture by adopting soil management strategies and minimizing nutrient loss in soil. Phosphorus solubilizing microorganisms (PSM) constitute about 1–50% and 0.1–0.5% of soil biota, respectively which can be effectively utilized for mining of P-minerals (Zaidi *et al*, 2009, Sharma *et al*. 2013) [72, 56]. These PSM are also known for their plant growth promotion and yield enhancement activities (Fasim *et al*. 2002 and Chen *et al*. 2006) [56, 11]. The integration of native PSM in combination with chemical fertilizer (superphosphate and rock phosphate) reduces the dosage requirement by 25–50% (Sundara *et al*. 2002) [60]. Reports have shown that on inoculation of PSM insoluble P may be solubilized from the Ca/Mg/Al/Fe complexes which can then be available to the plant roots for growth enhancement and root proliferation (Liu *et al*. 2016) [35]. The release of enzymes (phytase, and siderophore) and organic acids by PSM dissolves the bond between P and the fixing element to make it available for the plant uptake (Hayes *et al*. 2000) [23]. Several microorganisms such as *Azospirillum*, *Aspergillus*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Penicillium*, *Pseudomonas*, *Trichoderma*, etc., have been identified as phosphate solubilizers in soil.
Traditional farming and extensive use of the chemical fertilizers has developed a one-way movement of the nutrients.

Incorrect P fertilizer application makes them fixed in soil which renders them unavailable for plant uptake whereas the available form of the fertilizer is easily taken up by plant and utilized in biomass production. Its recycling from agricultural residues enhances soil quality and sustainability (Cordell *et al*. 2009 and Metson *et al*. 2014) [40]. Use of such microorganisms in the form of biofertilizer can improve availability of nutrients in the rhizosphere, produce growth stimulants for plants, improve soil stability, provide biological control, biodegrade substances, recycle nutrients, promote mycorrhiza symbiosis, and develop bioremediation processes in soils contaminated with toxic, xenobiotic and recalcitrant substances (Morte *et al.* 2003 and Corpoica 2007) [43, 13].

Materials and Methods

Collection of sample

The soil samples were collected from a depth of 0-15cm from university agricultural land which was deficient in phosphorus status. Collected soil samples were stored in zip lock polythene bags and were immediately stored under freezer and were maintained at 4 ^{0}C for further study.

Isolation of Strains

For isolation of phosphate solubilizing microbes, 1g of surface soil was suspended in 100ml of distilled water from where an aliquot of 1mL was pipette out and were inoculated on Pikovskaya solid medium (0.5g $(NH_4)_2SO_4$, 0.1g $MgSO_4 \cdot 7H_2O$, 0.003g $FeSO_4 \cdot 7H_2O$, 0.003g $MnSO_4 \cdot H_2O$, 5g $Ca_3(PO_4)_2$, 0.02g NaCl, 0.02g KCl, 10.0g glucose, 0.5g yeast extract, 15.0g agar, and 1000 mL distilled water) amended with 1% tricalcium phosphate (TCP) prepared by pour plate technique and incubated at 30 ^{0}C for seven days. Colonies showing clear zone around them were considered as PSM. Single colonies with clear zones which appeared on Pikovskaya agar plates were transferred aseptically to Pikovskaya broth and on agar slants for further experimentation.

Identification of Microbes

For identification of the microbe with phosphate dissolving capacity, a drop of lactophenol cotton blue was placed on a glass slide and observed under microscope. Therefore, based on their morphology two fungi of the strain *Aspergillus* sp. And *Penicillium* sp. were identified and were tested against two bacteria with such phosphate solubilizing property (*Bacillus megaterium* and *Bacillus subtilis*) obtained from Agriculture Research Station in Parbhani.

Analysis of Phosphate Solubilizing Activity

Those isolates with bigger halo producing zone were selected for further study. The qualitative and quantitative analyses of phosphate solubilizing ability of the selected isolates were tested out in plate and broth assays.

Qualitative method

All the selected isolates were screened for their mineral phosphate solubilizing ability on

Pikovskaya agar media amended with one percent tri calcium phosphate were spot inoculated with a sterile toothpick at the centre of the media plate and was incubated at 30 ^{0}C for five days. Diameter of halo zone was measured. The phosphate Solubilization Index (SI) was thus calculated as:

SI = (Halo zone diameter - Colony diameter)/ Colony diameter

All the observations were recorded in triplicate. Isolates that developed see through clear zones around their colonies were identified as PSM.

Quantitative method

Pikovskaya broth mixed with 1% TCP was prepared and sterilized in an autoclave. About 1mL of each isolates was pipetted out and inoculated into the liquid media. The inoculated test tube containing samples were incubated for seven days on rotatory shaker at 37 ^{0}C. After a week of incubation, culture broth was centrifuged at 10,000rpm for 10min. Available phosphorous was determined spectrophotometrically at 410nm with standard KH2PO4. Uninoculated broth served as control.

Pot Trial

A pot culture experiment was conducted in plastic pots (20 cm in diameter). It was of 4kg capacity and was filled with 2.5 kg of sterile soil collected from same field where soils were taken for isolation of microbe purpose. Soils were pre sterilized for two consecutive days in autoclave to remove unwanted microorganisms. Experiment was done in triplicate. Maize seeds were dipped and in cultures and were sown in pots at 5 cm depth. The set up was done in glasshouse condition. Pots were irrigated daily with sterile distilled water for 60 consecutive days. The experimental set up consisted of 15 treatments namely, five treatments of isolates (two each of bacteria and fungi and an uninoculated control) and two nutrient sources of P as Rock Phosphate (RP) @ 12.5 kg/ha and 25 kg/ha along with recommended dose of fertilizer. Five plants were sown in each pot.

Plant Growth Measurement

The crop was harvested after 60 days of sowing (DAS). They were uprooted carefully from each pot and plant growth parameters like plant height, stem girth, chlorophyll content and dry matter yield were noted.

Nutrient Analyses

The plant samples shade dried and ground finely in a mortar and pestle. 0.1g of powdered sample was taken in 150mL conical flask containing a mix of 10mL nitric acid and perchloric acid in the ratio 9:4.The flasks were placed on a hot plate and digested at 300 ^{0}C until the entire material turned into colourless solution and charring was avoided by adding distilled water. The extract was collected in 100 mL volumetric flask and made up to 100mL with distilled water. These samples were then used for estimation of nitrogen and phosphorus by Kjeldahl and Olsen methods respectively and potassium by flame photometer (Tandon, 2001) [62].

Results and Discussion

Isolation an Identification of PSM Isolates

In this study, a total of 9 fungal isolates were obtained from 27 rhizospheric soil samples taken from University farms at CRC, GBPUA&T, Pantnagar. Out of the isolated fungi, a total of two phosphate solubilizing fungal cultures having potential of phosphate solubilization were isolated which belonged to *Aspergillus* sp. and *Penicillium* sp. which were identified based on their morphology studied under microscope.

Qualitative and Quantitative Phosphate Solubilization

The SI of the four isolates ranged from 0.57 to 1.57 after a week of incubation study at 28-30 ^{0}C (Fig. 1). Results

revealed that, among the four isolates, *Aspergillus* sp. and *B. megaterium* in fungal and bacterial cultures, respectively. However, *Aspergillus* sp. proved to be the most efficient P solubilizer among the four isolates (SI= 1.57) on agar plates followed by *Penicillium* sp. (SI= 1.16). The smallest SI of 0.57 was observed for *Bacillus subtilis* Amount of mineral phosphate solubilized by all four isolates were significantly ($p < 0.05$) higher over uninoculated control. The least amount of P solubilized from TCP amended broth was on day 7, afterwards the solubilized P increased up to day 15 of incubation study. Consequently, the phosphate values in the broth ranged between (13.2-73.7 μg/mL) among different isolates during 15 days of incubation time. Maximum quantity of P solubilized was recorded by *Aspergillus* sp. inoculated culture filtrates followed by *Penicillium* sp. (109.3 μg/mL) and *B. megaterium* (32.7 μg/mL). Minimum accumulation of soluble P (16.30 μg/mL) was recorded in the culture of *B subtilis* (Table 1).

Table 1: Solubilization Index (SI) of different inoculants on TCP amended media

Inoculant	Colony Diameter	Halo diameter	SI
Bacillus megaterium	5	9	0.80
Bacillus subtilis	7	11	0.57
Aspergillus sp.	7	18	1.57
Penicillium sp	6	13	1.16

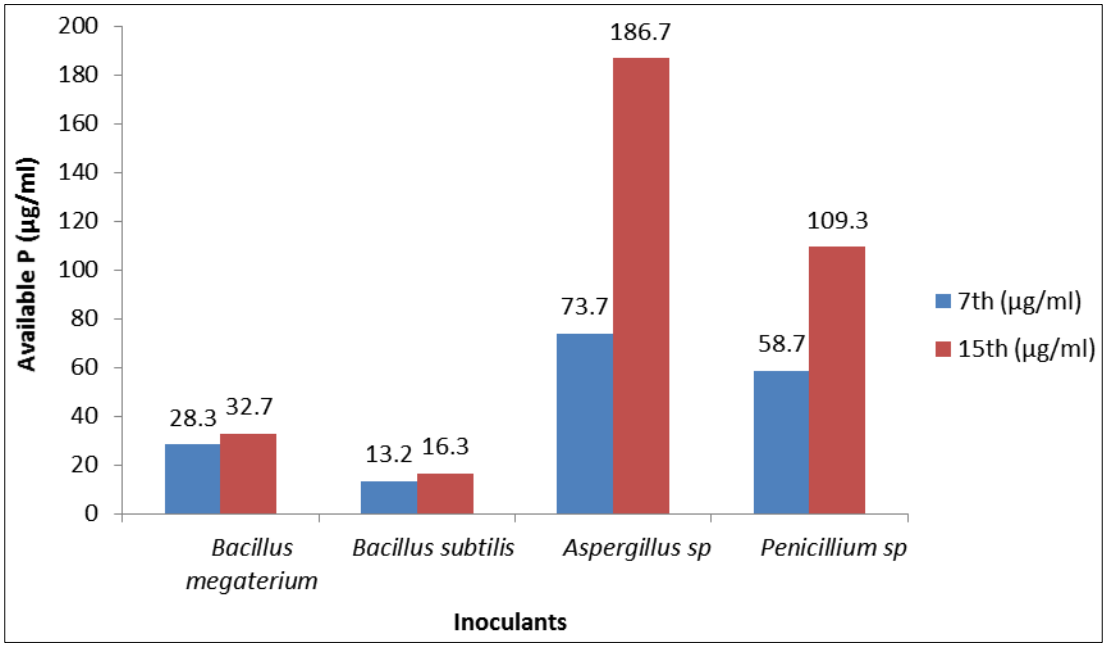

Fig 1: Solubilization of TCP by different isolates in Pikovaskaya broth

All four isolates showed a decline in pH significantly ($p < 0.05$) over control in TCP amended broth assay after 15 days of incubation. The pH values dropped to varying levels during the initial week and later became stagnant at the same level. The maximum drop in pH was recorded in the isolates *Aspergillus* sp. in fungi and *B. megaterium* in bacteria. The decrease in value was from 7.0 to 4.2. Among the four isolates the reduction of pH in culture medium from an initial value of 7.0 to 4.8, 5.2, 6.5 and 6.8 for *Aspergillus sp., Penicillium sp., B. megaterium and B. subtilis*, respectively, after 7 and to 4.2, 4.7, 5.1 and 5.6 respectively, for the same after 15 days of incubation period.

The differences in phosphate solubilization based on SI on agar plate in the present study may be due to of the amount, type and variable diffusion rates of multiple organic acids secreted by all isolates as previously observed by Yadav *et al.* 2011 [71]. Conversely, Mahamuni *et al.* 2012 [36], reported SI for different fungal strains isolated from sugarcane and sugar beet which ranged from

1.13 to 1.59. Similarly, Alam *et al.* 2002 [4] also reported SI that ranged from 1.53 to 1.80 for the fungal cultures isolated from maize rhizosphere. Iman (2008) [25] also reported that the solubilization indices of fungal strains (*Penicillium italicum* and *Aspergillus niger*) were 2.42 and 3.15, respectively. The isolates solubilized the insoluble TCP with gradual increase as the period of incubation period progressed. These observations are in accordance to the reported made by Nenwani *et al*. 2010 [46], who demonstrated a gradual increase in soluble P by fungal isolate F1 in broth cultures. Decrease in P solubilization was at the end of incubation study could be attributed to the availability of soluble phosphate, which had an inhibitory effect on further TCP solubilization, or the exhaustion of carbon source that restricted both the production of organic acids and microbial activity (Kim *et al.* 2005) [31]. Another possibility for reduction in soluble P content could be due to the formation of organo-P compound fostered by released organic metabolites (Chai *et al.* 2011) [9]. Muleta (2010) [44] also stated that solubilized P is employed by fungal cells themselves for their growth and development during this period. Results corroborated with Chakraborty *et al.* (2010) [10] who reported that *Aspergillus niger* (isolate RS/P-14) solubilized maximum amount of TCP (799 μg/mL) and from rock phosphate (385 μg/mL) in PVK broth at 15 days of incubation. Similarly, Pandey *et al.* (2008) [47, 6] recorded mobilized phosphate between 320 μg/mL (*P. oxalicum*) and 500μg/mL (*P. citrinum* and *P. purpurogenum*) from TCP at 15 days of incubation.

Acidification by organic acid production has been reported to be the principle solubilization mechanism of inorganic P by microorganisms (Gong *et al.* 2014) [18]. Reduction in pH values from neutral condition was recorded in all isolates broth assay which might be due to production of various organic acids from the available carbon (glucose) as reported by several researchers (Pandey *et al.* 2008, Yadav *et al.* 2011) [47, 70]. The drop in pH of cultures has been reported a number of times by various research findings (Nautiyal 1999, Pandey

et al. 2008, Malviya *et al*. 2011 and Yadav *et al*. 2011) [45, 47, 37, 70]. The pH values decreased to different levels depending upon the culture and later became stagnant with a decrease in soluble P. This might be due to low glucose concentration in Pikovaskaya broth which is essential for organic acid production. In this aspect, the present observations are in agreement with Nenwani *et al*. 2010 [46] who reported increase in pH value and decrease in solubilized phosphate at the end of incubation time.

Impact of nutrient levels and different inoculants on growth and dry matter yield of maize

Plant height (cm) at 30 and 60 DAS

Varying nutrient levels had a significant influence on plant height of maize at different crop growth periods. At 30 DAS maximum and significant increase was observed due to application of RP @ 40 kg/ha (50.33 cm) followed by RP @ 20 kg/ha (50 cm). RP applications enhanced the plant height by 12.4% over RDF (44.78 cm). At 60 DAS application of RP @ 40kg/ha (137.13 cm) and RP @ 20 kg/ha (135.27 cm) registered significant gain in height over RDF (118.10 cm) by 16.11% and 14.5%, respectively (Table 2).

Inoculation also affected the height of maize plants with maximum significant gain being with *Aspergillus* (54 cm) and *Penicillium* (51.52 cm) over no inoculation (38.5 cm) by 40.2% and 33.1% respectively at 30 DAS. At 60 DAS inoculation with *Aspergillus* significantly increased the plant height by 17.4% followed by *Penicillium* and *B.megaterium* by 16.4% and 11.9% respectively, over no inoculation.

The interaction effect between inoculants and nutrients was significant. The maximum plant height (56.20 cm) was measured due to inoculation with *Aspergillus* sp. + RP @ 40 kg/ha which was greater by 46.2% as compared to uninoculated control at 30 DAS. Also all inoculants performed significantly well with both levels of RP. The best interaction effect was observed with *Aspergillus* sp. + RP @ 40 kg/ha (147 cm) followed by *Penicillium* sp. + RP @ 40 kg/ha (146.33 cm) over RDF (112.83 cm) resulting in an increase by 30.3% and 30% respectively at 60 DAS.

Table 2: Influence of P solubilizers and nutrient levels on Plant height (cm) at 30 and 60 DAS

Nutrient/ Isolate	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average
	30 DAS				60 DAS			
No inoculation	38.43	38.07	39.00	38.50	112.83	119.67	118.67	117.05
B. megaterium	42.00	52.53	51.40	48.64	118.00	136.00	139.00	131
B. subtilis	45.23	53.00	49.27	49.16	118.33	134.00	134.67	129
Aspergillus sp.	50.53	55.27	56.20	54	121.33	144.00	147.00	137.44
Penicillium sp.	47.70	52.83	54.03	51.52	120.00	142.67	146.33	136.33
Average	44.78	50.33	50.00		118.10	135.27	137.13	
	Nutrient		Isolate	Nutrient X Isolate	Nutrient	Isolate		Nutrient X Isolate
S.Em±	0.34		0.34	0.58	0.41	0.41		0.92
CD at 5%	0.96		0.96	2.15	1.17	1.17		2.61

Stem girth (cm) at 30 and 60 DAS

The varying nutrient levels significantly influenced the stem girth. At 30 DAS the maximum and significant increase of 18% over RDF (1.43cm) was recorded with the application of RP @ 40 kg/ha. Effect was also significant with maximum increase of 6.6% (2.44 cm) by application both levels of RP over RDF (2.29cm) at 60 DAS (Table 3).

P solubilizers also significantly affected stem girth at 30 and 60 DAS. At 30 DAS the highest stem girth was resulted due to inoculation with *Aspergillus* (1.72 cm) increasing it by 32.3% over no inoculation (1.30 cm). At 60 DAS, inoculation with *Penicillium* sp., *Aspergillu*s sp. and *B. megaterium* enhanced the girth by 5.3% over no inoculation at 60 DAS.

Interaction effects, at 30 DAS were recorded significant due to all combinations of inoculants and nutrients with highest being with *Aspergillus* sp. + RP @ 40 kg/ha. An increase of 48.7% over RDF was recorded also due to *Penicillium* sp. + RP @ 40 kg/ha. Interaction effects, at 60 DAS, was maximum due to *Penicillium* sp. + RP @ 40 kg/ha and *B. megaterium* + RP @ 20 kg/ha over RDF (2.31 cm) by7.7% and 6.9%, respectivly.

Table 3: Impact of P solubilizing microbes and varying nutrient levels on stem girth (cm) at 30 and 60 DAS

Nutrient\ Isolate	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average
	30 DAS				60 DAS			
No inoculation	1.23	1.33	1.36	1.30	2.31	2.35	2.37	2.28
B. megaterium	1.44	1.56	1.68	1.56	2.31	2.48	2.47	2.42
B. subtilis	1.43	1.51	1.67	1.53	2.26	2.45	2.45	2.38
***Aspergillus* sp.**	1.58	1.70	1.89	1.72	2.38	2.45	2.46	2.43
***Penicillium* sp.**	1.47	1.71	1.83	1.67	2.37	2.42	2.49	2.42
Average	1.43	1.56	1.69		2.29	2.44	2.44	
	Nutrient		**Isolate**	**Nutrient X Isolate**	**Nutrient**	**Isolate**		**Nutrient X Isolate**
S.Em±	0.01		0.01	0.02	0.01	0.01		0.02
CD at 5%	0.03		0.03	0.06	0.02	0.02		0.05

Chlorophyll content (%) at 30 and 60 DAS

Influences of the two varying nutrient levels on chlorophyll content at 30 DAS were significantly higher with an increase of 2.8% over RDF. But at 60 DAS maximum influence was impacted by RP at both levels i.e. @ 20 kg/ha and 40 kg/ha, respectively (0.096% each) (Table 4).

The impact of inoculants on chlorophyll content at 30 DAS were also significant with each other (0.036%). At 60 DAS maximum influence on chlorophyll content was observed by both fungal species i.e. *Aspergillus* and *Penicillium* by 0.096% each. The increase was 6.6% more than uninoculated control by fungi species. Both bacterial inoculants, *B.*

megaterium and *B. subtilis* (0.095% each) performed well, in chlorophyll content.

Though numerical increase due to interactions between nutrient level and inoculants were observed but the increase was not significant at 30 DAS. Significant interactions were observed at 60 DAS between nutrient levels and inoculants with maximum increase in chlorophyll content by *Aspergillus* and *Penicillium* at both levels of RP i.e. 0.098% each, respectively.

Table 4: Effect of nutrient sources and P solubilizers on Chlorophyll content (%) at 30 and 60 DAS of maize

Nutrient\ Isolate	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average
	30 DAS				60 DAS			
No inoculation	0.034	0.035	0.035	0.034	0.090	0.090	0.090	0.090
B. megaterium	0.035	0.036	0.036	0.035	0.093	0.096	0.096	0.095
B. subtilis	0.035	0.036	0.036	0.035	0.093	0.096	0.097	0.095
Aspergillus sp.	0.036	0.037	0.036	0.036	0.094	0.098	0.098	0.096
Penicillium sp.	0.035	0.036	0.036	0.036	0.094	0.098	0.098	0.096
Average	0.035	0.036	0.036		0.093	0.096	0.096	
	Nutrient		Isolate	Nutrient X Isolate	Nutrient	Isolate		Nutrient X Isolate
S.Em±	0.0001		0.0001	0.0003	0.0001	0.0001		0.0002
CD at 5%	0.0004		0.0004	NS	0.0003	0.0003		0.0007

Dry matter yield (g/plant)

The effect of varying nutrient levels on dry matter yield was significant. Maximum and significant increase of yield was obtained by the application of RP @ 40 kg/ha (64.23 g/plant) over RDF (61.21 g/plant) by 4.9% followed by application of RP @ 20 kg/ha (63.39 g/plant) over the same by 3.5% (Table 5).

All inoculants had a significant effect on dry matter yield with maximum input by *Aspergillus* (63.54 g/plant) by 2.9% followed by *Penicillium* (63.40 g/plant) by 2.7% over no inoculation (61.71 g/plant), respectively.

The interaction effect on dry matter yield ranged from 60.50 g/plant to 64.88 g/plant. Significantly maximum yield was obtained on inoculation of *Aspergillus* sp. + RP @ 40 kg/ha followed by significant effects of *B. megaterium* + RP @ 20 kg/ha with increase of 7.2%, over RDF.

Table 5: Effect of nutrient sources and P solubilizers on dry matter yield (g/plant) of maize

Nutrient\Isolate	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average
No inoculation	60.50	62.12	62.51	61.71
B. megaterium	61.24	64.86	63.69	63.26
B. subtilis	61.24	64.68	63.56	63.16
Aspergillus sp.	61.37	64.39	64.88	63.54
Penicillium sp.	61.70	64.23	63.20	63.40
Average	61.21	63.39	64.23	
	Nutrient	Isolate		Nutrient X Isolate
S.Em±	0.069	0.069		0.154
CD at 5%	0.196	0.196		0.439

An increase in overall growth can be attributed to the synthesis and secretion of growth promoting substances by inoculants that carry out stem expansion, increased chlorophyll content and photosynthesis rate (Burd *et al.*, 2000; Panhwar *et al.*, 2011) [7, 48]. P fertilization along with inoculation enhanced chlorophyll content, photosynthetic rate, promoting increased cell division and new tissue development (Taiz and Zeiger, 2013) [63]. Rudresh *et al.* (2005) [53] recorded the highest plant height of 34.6 cm in treatment, which received combined inoculation of *Rhizobium*, PSB and *T. harzianum* with rock phosphate over control in chickpea, Rafi *et al.* (2012) [50] reported dual inoculation with *Azospirillum* strain A2 and PSB isolates resulted in maximum shoot height of foxtail millet (cv. Chitra) over contol. Wu *et al.* (2005) [64] observed co-inoculation with *P. chlororaphis* and *A. pascens* amendment with RP resulted in the highest plant height in walnut seedling, a significant increment in plant height (45%) and shoot length (19%) over control was observed by Viruel *et al.* (2014) [69] in maize treated with *Pseudomonas tolaasii* IEXb with 50 kg P per ha applied as TSP under pot and field trial. Srinivasan *et al.* (2012) [59] reported that *Aspergillus* sp. PSFNRH-2 recorded the highest stem girth (2.63 cm),which was significantly higher than that recorded by all other fungal isolates (0.80–2.20 cm) including the reference strain, *A. awamori* (2.30 cm) but was on par with the SSP control (2.70 cm) in sorghum. Mfilinge *et al*. (2014) [41] reported that *Rhizobium* inoculation with 30 kg/ha P application increased plant girth by 1.3% 6 WAP in field experiment and 5.1% and 11.67% in green house for 3 WAP and 6 WAP respectively in bush bean. Akhtar *et al.* (2014) [3] reported that integrated effect of *Rhizobium* and *Bacillus* spp. on the growth of maize (*Zea mays* L.) with recommended dose of fertilizer (120-60 kg NP/ha) increased stem diameter (15.43mm) over control.

Mehrvarz *et al.* (2008) [38] found significant increase in chlorophyll content of leaves of barley due to positive effect of phosphorous with microbes. Also he found that fungal inoculation was more effective in increasing chlorophyll content over bacterial inoculants due to antagonistic effects on it by chemical fertilizer. Panhwar *et al.* (2011) [48] recorded highest chlorophyll content (29.30) was obtained in treatments with P at 60 kg per ha inoculated with PSB16 *(Bacillus* sp.) compared to uninoculated treatments. Gupta and Gangwar (2012) [20] in chickpea reported highest chlorophyll content (6.20mg/g fresh leaves) was observed with 1.0 kg AM/ha as soil application + *Rhizobium* + PSB +RDF. Abbas *et al.* (2013) [1] also recorded higher chlorophyll content in maize with coinoculation between PGPR and reduced doses of N and P over chemical control. Banik and

Sharma (2014) reported in maize plants grown with 100% recommended dose of fertilizer (RDF) [N: P_2O_5: K2O = 150:60:60 kg/ ha] + AM + *Azospirillum* (T15) produced maximum chlorophyll over uninoculated control. Saxena *et al.* (2015) [55] also recorded high chlorophyll content in maize on coinoculation with TCP over control.

The increase in dry matter yield could be due to PGPR effect of inoculated microbe leading to high uptake of nutrients, increased photosynthesis, and increased growth of root and shoot organs, siderophore and phytohormone production, as well as to their capacity to colonize the root system and interact positively with the plant (Viruel *et al*., 2011). It could be attributed to the increased vegetative growth possibly as a result of effective utilization of nutrients absorbed through extensive root system and prolific shoot development on account of improved nourishment Kumawat *et al*. (2009). Vikram *et al*. (2008) [68] in chickpea reported highest root dry matter by PSBV-5, PSBV-9 and PSBV-13 (all of which recorded 0.59 g) while highest shoot and total dry matter was recorded by PSBV-14 (6.41 and 6.97 g, respectively) with recommended dose of P in the form of MRP in comparison with SSP control and RP control. Kumawat *et al.* (2009) in mung bean reported that application of vermicompost, seed inoculation with PSB and 40 kg P2O5/ha significantly increased dry matter yield over control. Panhwar (2011) [48] reported a significantly higher dry matter (21.48 g) in treatments with 60 kg P2O5 per ha inoculated with PSB16, while the response in the control treatment was very low in aerobic rice. Messele and Pant (2012) [39] recorded that inoculation of *Sinorhizobium ciceri* + *Pseudomonas* sp. with 18/20 kg NP ha-1 as urea and DAP increased dry matter 181.40% respectively over uninoculated control at mid flowering stage in chickpea. Umesha *et al.* (2013) [67] in a field experiment of maize reported that treatment (T13) having recommended dose of NPK + *Azotobacter chroococcum* + *Bacillus megaterium* + *Pseudomonas fluorescence* + enriched compost gave the highest total dry matter production at harvest (375.80 g) over uninoculated control.

Impact of nutrient levels and different inoculants on nutrient content and uptake by maize

N uptake (mg/plant) in maize

Nitrogen uptake was seen to be more at higher level of nutrient application. Maximum and significant uptake of nitrogen by maize was observed when RP @ 40 kg/ha was applied, which was about 78.1% higher followed by application of RP @ 20 kg/ha with an increase of 54% over RDF (Table 6).

Inoculation of all the strains of microbes significantly improved the nitrogen uptake in maze. Inoculation effect was best seen with *Aspergillus* which had a profound effect on N uptake with about 26.2% increase over RDF *i.e*. about 1090.42 mg/plant uptake of N. This was followed by *Penicillium* which contributed to 1345.23 mg/plant N uptake *i.e.* 23.3% higher over RDF.

In general, the interaction between fungi and doses of RP gave better results. Interaction of different nutrient levels and inoculation provided significant impact on uptake of N by maize. Maximum uptake resulted between *Penicillium* sp. + RP @ 20 kg/ha followed by *Aspergillus* sp. + RP @ 40 kg/ha.

P uptake (kg/ha) in maize

Significant influence of nutrient application on P uptake by maize was observed.RP @ 40kg/ha contributing 9.4% increase over RDF. This was followed by RP @ 20 kg/ha which increased by 4% more than RDF (Table 6).

Significant effect of inoculation of microbial strains was observed on P uptake by maize and ranges between 196.34 to 278.09 mg/plant. Maximum Influence on P uptake was observed by *Aspergillus* by 41.6% followed by *Penicillium* by 39.5%, respectively over no inoculation.

Variation on P uptake by interaction of nutrient levels + inoculants over RDF with no inoculation was from 185.74 to 293.56 mg/plant. Most beneficial influence came in case of *Aspergillus* sp. + RP @ 40 kg/ha with an increase of 58.2% with no inoculation closely followed *Penicillium* sp. + RP @ 40 kg/ha.

K uptake (kg/ha) in maize

Impact of varying levels of nutrients on K uptake was most prominent and significant by application of RP @ 40 kg/ha which is 31.4% followed by application of RP @ 20 kg/ha being 27 per cent more over RDF, respectively (Table 6).

Significant efficacy of inoculants was prominently seen by inoculation of *Aspergillus* sp. which is 15% more over no inoculation. *Penicillium* sp. and *B. megaterium* inoculants showed an increase of 11.6% and 11%, respectively over no inoculation.

Combination of nutrient levels with inoculation showed significant increase in K uptake with best results being observed in *Aspergillus* sp.+ RP @ 40kg/ha having 42.4% increase over RDF with no inoculation. Significant results were also seen between *Penicillium* sp. + RP @ 40kg/ha and *Aspergillus* sp. + RP @ 2kg/ha with 39% and 34.4% increase over RDF with no inoculation.

Significantly higher levels of N uptake were observed in maize along with higher levels of RP applied which may be due to IAA production, nitrogenase activity, mechanism of phytohormone production, vitamins, amino acids and beneficial association performed by the microorganisms with plant roots which in turn enhance root growth and increase nutrient uptake Patil *et al.* (2012). Similar observations were reported by Wu *et al.* (2005) [64] and Egambrdiyeva (2007) [16].

Increase in N uptake could be ascertained to the fact that a high N content and chlorophyll content contributes to higher N uptake. Colonization of roots by fungi results in increase in root surface area for nutrient acquisition where the hyphae extend several centimeters in soil to absorb nutrients for the host plant Khan *et al.* (2000) [30], synergistic effect of phosphorus and N and P biofertilizers in the overall improvement N uptake Jat and Ahlawat (2006) [26].

Table 6: Influence of different inoculants and nutrient levels on N, P and K uptake (mg/plant) in maize after harvest

Nutrient Isolate	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average	RDF	RP (20 kg/ha)	RP (40 kg/ha)	Average
	N				P				K			
No inoculation	766.34	1228.68	1276.25	1090.42	185.74	195.13	208.16	196.34	754.27	838.63	906.38	833.09
B. megaterium	898.77	1577.06	1441.03	1305.62	261.74	282.23	285.24	276.4	783.7	974.84	1011.53	925.4
B. subtilis	875.83	1517.82	1393	1262.21	267.18	276.45	279	274.21	755.44	980.99	1000.37	912.26
Aspergillus sp.	898.77	1475.12	1661.82	1345.23	262.65	278.08	293.56	274.05	785.55	1014.4	1074.47	958.14
Penicillium sp.	877.85	1744.89	1508.87	1377.2	259.68	274.6	287.87	278.09	757.39	984.23	1049.42	930.34
Average	873.41	1345.3	1555.57		247.4	257.42	270.77		757.39	966.72	1008.43	
	Nutrient		Isolate	Nutrient X Isolate	Nutrient	Isolate		Nutrient X Isolate	Nutrient		Isolate	Nutrient X Isolate
S.Em±	12.92		12.92	28.91	0.55	0.55		1.23	4.72		4.72	10.57
CD at 5%	36.72		36.72	82.12	1.57	1.57		3.51	13.42		13.42	30.08

Rudresh *et al.* (2005) [53] reported in chick pea that in pot experiment the maximum N uptake in shoots (120 mg/ plant) and in field trial uptake to the shoot (1079 mg/ plant) was observed in plants treated with PSB, *T. harzianum*, rock phosphate and *Rhizobium with rock phosphate fertilization*. Canbolat *et al.* (2005) observed that inoculations with PGPB Bacillus M-13 and fertilizer application increased the N and P contents of barley seedlings compared to the untreated control. Moinuddin *et al.* (2014) [42] in chickpea observed that P @ 30 kg/ha + BNF + BPF resulted in greater N uptake (27.3%) over control. Increase in bio availability of P in soils with rock phosphate minerals has been reported by workers like Liu *et al*. (2002) [34], Han and Lee (2005) [21], Jorquera *et al.* (2008) [29].

Increased P uptake in maize can be attributed to the improvement in root development leading to more nutrient uptake, production of phosphatase enzyme, ACC, diaminase activity thereby increasing phosphorous nutrition in inoculated plants. The increase in P uptake may be due to increased content of P in soil solution with increasing P application Choudhary *et al.* (1997) [12]. Seed inoculation with phosphate solubilizing microorganism also increased P uptake by crop over control due to microbial activity that could have resulted in qualitative and quantitative alteration of root exudates composition and also change in root architecture in plants which changes the soil root ratio hence increases the uptake by plant. Demessie *et al.* (2013) [14] reported in faba bean that the maximum P content and uptake was recorded in plants inoculated with JURB48 (0.07% and 4.79 mg/ plant) with P sources. Devi *et al.* (2012) [15] found phosphorus uptake by stover was maximum (7.52 kg/ ha) with DAP+PSB in soybean. Vikram *et al.* (2008) [68] reported in green gram that the treatment involving inoculation of PSBV-13 with Recorded the highest P content in shoot (0.821%) total P uptake (56.04 mg) in green gram plants at 45 DAS compared to RP control and SSP control.

Potassium uptake was significantly influenced by application of phosphorous solubilizing microorganisms with P_2O_5 at various levels over control was reported by Patil *et al.* (2012). This may be due to synergistic effect between P and K, better root growth. Since uptake is a function of nutrient content and yield of crop, so an increase in anyone positively affects increase in uptake by crop Singh *et al*. (2011) [58], Bhunia *et al*. (2006) [6] and Suri *et al*. (2006) [61]. Similar trend in the results were also reported by Tarafdar (1995) [65]; Han *et al*. (2006) [22]; Yadav *et al.* (2006) [6] and Sharma *et al*. (2012) [58]. Kumawat *et al.* (2009) reported that application of vermicompost at 2t/ha, seed inoculation with PSB and 40 kg P_2O_5/ha significantly increased the N, P and K concentration in seed, straw and their total uptake in mung bean. Kumar *et al.* (2015) reported increased N, P, K content and uptake in mung bean due to PSB inoculation with SSP over uninoculated control. The enhancement in nutrient uptake by inoculation with insoluble sources may be due to the production of low molecular weight, organic acid and subsequent release of Zn from insoluble compounds by reducing sorption of Zn by altering the surface charge of soil colloids Jones (1998) [27]. It may also be due to the fact that initiation of development of lateral roots and increased root weight Rolfe *et al.* (1997) [52], Canbolat *et al.* (2006) [8]. Increased Zn content and uptake by plants due to incorporation of inoculants at various P sources were also reported by Whiting *et al.* (2001), Tariq *et al.* (2007) [66] in wheat, Joshi *et al.* (2013) [28] in wheat, Goteti *et al.* (2013) [19] in maize and Ramesh *et al.* (2013) [51] in soybean and wheat.

Conclusion

On the basis of findings and the performance of maize in pot culture experiment, it is concluded that all parameters *viz.* growth, yield attributing characteristics and nutrient uptake were affected remarkably by the different nutrient levels, inoculants and their combinations. In general, inoculants, so isolated and procured showed a significant and good response with the insoluble source of P mineral in increasing their availability both under laboratory and microcosm experiments. Both the fungal species were superior to the procured bacterial strains in their overall performance with regards to mineral solubilization and nutrient availability. Performance of fungus isolated from soil i.e. *Aspergillus* over all performed best among the used inoculants with varying

nutrient levels in mobilizing P, increasing their availability in plants and maintaining plant health. The success of this experiment could help in bridging the gap of nutrient availability and thereby increased contents in plants especially the availability of P in cereals in the soils where they are severely limited. These inoculants can be further developed as multi nutrient solubilizers biofertilizers for the soils where availability of essential nutrients particularly micronutrients are limiting factor.

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