Content list available at http://epubs.icar.org.in, www.kiran.nic.in; ISSN: 0970-6429

Indian Journal of Hill Farming

June 2023, Volume 36, Issue 1, Page 185-195

Integrated effect of phosphate solubilizing microorganisms and FYM levels on soil fertility, yield and nutrient uptake of soybean

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ARTICLE INFO

Article history:
Received: 19 January, 2023
Revision: 25 January, 2023
Accepted: 06 February, 2023

Key words: *Phosphorus, PSM, FYM, phosphate solubilization, yield, soil properties*

DOI: 10.56678/iahf-2023.36.01.24

ABSTRACT

Phosphorus (P) limitation in soil, due to P fixation by absorption or precipitation or by both, is a major constraint for agricultural productivity. Application of phosphate solubilizing microorganisms (PSM) along with organic manures can be one of the simple and cost effective approaches to address the above problem in an eco-friendly manner. With this background, an experiment was conducted under control conditions (pots) to study the effect of four PSM strains viz, *Bacillus polymyxa, Pseudomonas striata, Aspergillus awamori and Penicillium digitatum* on some selected soil properties, crop yield and nutrient uptake under three levels of FYM of 0, 5, 10 t ha^{-1}. Seed inoculation of *Bradyrhizobium japonicum* was common for all the treatments. Result of the study revealed that PSM seed inoculation with increasing levels of FYM stimulate the phosphate solubilizing bacterial and fungal population, which lead to slight decreased in rhizosphere soil pH and $CaCO_3$ and slight increase in EC. The efficacy of PSM for improving available Nitrogen, Phosphorus and other soil properties was observed in the order of *Aspergillus awamori* > *Bacillus polymyxa* > *Penicillium digitatum* > *Pseudomonas striata* with higher levels of FYM. The synergistic effect of *Aspergillus awamori* and *Bacillus polymyxa* with *Bradyrhizobium* were most effective among the PSM strains, reflecting highest number of effective root nodules and PSM population. The highest soybean yield and nutrient uptake was observed in *Aspergillus awamori* seed inoculation treatment with 10 t ha^{-1} FYM, followed by *Bacillus polymyxa* seed inoculation treatment. It can therefore be inferred that *Aspergillus awamori* and *Bacillus polymyxa* are the most efficient PSM strain with higher levels of FYM for obtaining higher soybean yield, improving soil N and P fertility and other soil properties.

1. Introduction

Soybean (*Glycine max* L. Merril) is one of the important pulse and oilseed crops of the family *Leguminoseae.* It is an excellent source of crude protein (43.2%) and oil (19.5%). Soybean, being leguminous crop, requires higher quantities of P, next to N, for its growth, development and satisfactory yield performance. It plays vital role in various physiological processes like root system development, root nodulation and N fixation (Li et al., 2021). Availability of P in soil is limited due to its fixation with Al / Fe oxides and hydroxides or absorption on the surface of $CaCO_3$ and clay mineral (Lun et al., 2018). Excess amount of P fertilizer is applied in soil, in order to meet its demand by plant and out of which only 25-30% is available to plants (Penn and Camberato, 2019). However, excessive use of P fertilizer is costly and causes environmental population like eutrophication. Application of PSM inoculants alone or together with organic manures can be suggested as an eco-friendly and cost-effective approach alternative to chemical P fertilizer application (Kennedy et al., 2004; Adnan, 2022).

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PSM helps in increasing the availability of P to plant by solubilizing the organic and inorganic phosphate in soil through production of organic (Chen et al., 2006), inorganic acids (He and Zhu, 1998) and protons (Ryan et al., 2001). The organic acids produced by PSM solubilize insoluble phosphates by chelating the cations like Ca^{2+}, Al^{3+} and Fe^{3+} and competing with phosphate for adsorption sites in the soil (Nahas, 1996). PSM, thus, increase the bioavailability of P to plant in calcareous soil through the above mechanism resulting soil acidification and subsequent P release from $Ca_3(PO_4)_2$. Khan et al. (2022) also reported that application of PSM biofertilizer along with inorganic P fertilizer increase the phosphorus use efficiency in alkaline calcareous soil. Combine application of organic sources like poultry manure and FYM with PSM has also been suggested for improving the wheat growth and properties of calcareous soil.

In soil, out of the total microbial population, P solubilizing bacteria (PSB) constitute 1-50% and P solubilizing fungi (PSF) 0.1-0.5%. However, PSF have higher P-solubilizing activity than PSB as fungi produce more acids than bacteria (Venkateswarlu et al., 1984). P-solubilizing fungi retain the P dissolving activity even when they are repeatedly sub cultured under laboratory conditions, which usually happen with the P-solubilizing bacteria (Sperber, 1958; Kucey, 1983). Among the P-solubilizing microorganisms the bacterial species of *Pseudomonas* and *Bacillus* (Illmer and Schinner, 1992) and fungal species of *Aspergillus* and *Penicillium* are found to be predominant in soil (Wakelin et al., 2004). With the above background, the experiment was conducted with the following objectives: 1. To study the effect of different PSM strains on soil properties of alkaline calcareous soil under varying FYM levels. 2. To assess the impact of P solubilizers and FYM levels on uptake and yield of soybean.

2. Material and Methods

2.1 Soil description

The experimental site is located at Department of Soil Science and Agricultural Chemistry, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri which lies between 73° 15′ 0″ to 76° 22′ 12″ North latitude and 15° 46′ 48″ to 22° 3′ 0″ East longitude. Surface soil (0-15 cm) of Inceptisol order collected from the Soil Test Crop Response Correlation Scheme Research Farm, M.P.K.V., Rahuri was used for the study. The soil was dried under diffused sunlight and processed for experimentation. The soil was alkaline (pH 8.5), non-sodic (0.35 dSm^{-1}), moderately calcareous (5.35% $CaCO_3$) in nature with medium organic carbon content (0.52%), low available nitrogen (194.43 kg ha^{-1}), high available Phosphorus (31.17 kg ha^{-1}) and high available K (560.8 kg ha^{-1}).

2.2 Experimental materials

Well decomposed farm yard manure was procured from the Cattle Project, M.P.K.V., Rahuri and analyzed its chemical properties (Table 1). The farm yard manure was sterilized in the autoclave to ensure complete microbial sterilization. Healthy soybean seed of variety JS 335 was obtained from the Chief Scientist, Central Seed Cell, M.P.K.V., Rahuri for the experiment. Two bacterial P solubilizing strains *viz., Bacillus polymyxa* and *Pseudomonas striata*; and two fungal P solubilizing strains *viz.*, *Aspergillus awamori* and *Penicillium digitatum* and *Bradyrhizobium japonicum* were obtained from Department of plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri.

Table 1. Characterization of FYM

Total N (%)	0.84
Total P (%)	0.52
Total K (%)	0.72
Organic carbon (%)	18.7
C:N ratio	22.26
Fe (mg kg^{-1})	1819.38
Mn (mg kg^{-1})	345.75
Zn (mg kg^{-1})	127.75
Cu (mg kg^{-1})	23.13

2.3 Treatment Details

The experiment was conducted following FCRD with four replications (2 replications upto 45 DAS and 2 replications upto harvesting). The two replications were used for soil sampling and root nodule study at 45 days. The pot experiment consists of two factors- 1) 3 levels of FYM – 0 (F_0), 5 (F_1), and 10 t ha^{-1} (F_2) and 2) 4 PSM strains including one control – uninoculated control (S_0), *Bacillus polymyxa* (S_1), *Pseudomonas striata* (S_2), *Aspergillus awamori* (S_3) and *Penicillium digitatum* (S_4). Before inoculating the seeds with PSM, it was first treated with *Bradyrhizobium japonicum* culture and it was common for all the treatments. The treated seeds were then inoculated with PSM @ 250g $10kg^{-1}$ of seed as per the seed inoculation treatments. The seeds were inoculated separately with each culture by using 40 percent of gum Arabica solution as an adhesive. No recommended dose of fertilizer was added during the experiment. The treated seeds were shown in pots filled with 15 kg sieved soils. Three plants were allowed to grow in each pot. The pots were sited in an experimental net house and periodical randomization was done from time to time. The pots were irrigated with tap water and retained around 60% of field capacity (FC), following the procedure of Wu et al. (2005).

2.4 Effective root nodule count

The root nodule count was recorded at 45 DAS. The plants were uplifted along with the polythene lining from the pot carefully. The soils from intact root system were removed by gentle washing with tap water without disturbing the root system. The big, round, pink coloured active nodules were counted.

2.5 PSM population count

On 45th day after sowing (DAS) the plants were removed from the pots along with the polythene sheet lining and they were uprooted gently and the rhizosphere soil samples for PSM population count were collected by gently shaking. The population of PSM was enumerated by serial dilution and pour plate technique (Salle, 1973) using Pikovskaya's agar media. The medium was supplemented with 30 μg/mL streptomycin for phosphate solubilizing fungal population count. The serial dilution was done up to 10^{-6}-fold, and the respective dilutions were plated separately for bacterial and fungal population count, the plates were inverted and incubated at 28 ± 2 °C for 2–3 days. The visible bacterial colony was counted after 24 h of incubation and fungal colony was counted after 3 days of incubation.

2.6 Soil, FYM and plant analysis

The Soil pH and EC were measured in soil-water suspension (1:2.5) as prescribed by Page et al. (1982); $CaCO_3$ by Piper (1966); soil organic carbon by Walkley and Black (1934); available nitrogen by Subbiah and Asija (1956); available Phosphorus by Watanabe and Olsen (1965); available K by Jackson (1967); DTPA extractable micronutreint (Fe, Mn, Zn and Cu) by Lindsay and Norvell (1978). The FYM Organic matter content was determined using Ignition method (Chopra and Kanwar, 1991). Total N was determined by Macrokjeldahl method (Jackson, 1973), Total P by Vanadomolybdate yellow colour method in nitric acid system (Jackson,1973) and total K by Flame Photometer (Chapman and Pratt, 1961). Micronutreint (Fe, Mn, Zn and Cu) content in FYM was determine by atomic absorption spectophotometer as describe by Zoroski and Burau (1977).

2.7 Statistical Analysis

The data of the present study were subjected to statistical analysis of two factor (PSM strains x FYM levels) completely randomized design (CRD) at 5% level of significance (P = 0.05). Analysis of variance (ANOVA) was used to determine significant treatment effect at 5% significance level. Statistical analysis of the data was done in Microsoft Excel as per the methods described by Snedecor and Cochran (1989).

3. Result and Discussion

3.1 Soil PSM population

It was a significant observation that the bacterial and fungal population in all the treatments increased with increasing levels of FYM at 45 DAS (Table 2). The highest microbial population was observed in 10 t ha^{-1} FYM level followed by 5 t ha^{-1} FYM level. This result may be attributed to the increasing levels of substrate (organic carbon) for microbial proliferation. John et al. (2001) also reported that increase in organic matter content of soil stimulates the growth of productive soil microbes including PSM. The phosphate solubilizing bacterial population in *Bacillus polymyxa* (S_1) and *Pseudomonas striata* (S_2) seed inoculation treatments were significantly higher than the phosphate solubilizing fungal population in treatments S_3 (*Aspergillus awamori*) and S_4 (*Penicillium digitatum*) seed inoculation at 45 DAS. The finding is in same line with Djuuna et al. (2022), who reported that phosphate solubilizing bacterial population is generally higher than the phosphate solubilizing fungal population in soil. The interaction effect between PSM seed inoculation treatment and FYM levels showed that PSM population increased with increasing FYM levels. The highest bacterial population was observed in *Bacillus polymyxa* seed inoculation treatments (S_1) with 10 t ha^{-1} FYM and highest fungal population in *Aspergillus awamori* seed inoculation treatment (S_3) with 10 t ha^{-1} FYM. Patrick and Adeniyi (2016) also reported that organic manures enhance the growth and activity of phosphate solubilizing microorganisms in rhizosphere soil. The result indicates that the organic matter content of the soil significantly influence the population of PSM.

3.2 Effective nodule count

The effect of PSM seed inoculation and levels of FYM on soybean effective root nodulation at 45 DAS are presented in Table 2. Highest number of root nodule was observed in 10 t ha^{-1} FYM level (45.30 $plant^{-1}$) followed by 5 t ha^{-1} FYM level (35.60 $plant^{-1}$). Among the PSM strains, *Aspergillus awamori* significantly increased the number of root nodule (41 $plant^{-1}$) followed by *Bacillus polymyxa* (35.75 $plant^{-1}$). The result showed synergistic effect of *Aspergillus awamori* and *Bacillus polymyxa* with *Bradyrhizobium japonicum* which may increase nodulation. The finding are in same line with Shome et al. (2022) who reported significant increase in nodulation due to combine application of phosphate solubilizing microorganisms with *Rhizobium*. The interaction between all the levels of FYM (0, 5 and 10 t ha^{-1} FYM respectively) with *Aspergillus awamori* recorded significantly higher number of effective root nodulation of soybean crop at 45 DAS (36, 46 and 57 $plant^{-1}$ respectively) followed by *Bacillus polymyxa* (30.50, 41 and 51.50 $plant^{-1}$ respectively). The increase in

Table 2. Effect of conjoint application of Phosphate solubilizing microorganisms (PSM) and FYM on PSM population count and effective root nodule count at 45 DAS stage

Treatments	PSM population (cfu x 10^4 g^{-1} soil)	Effective root nodule $plant^{-1}$
Interaction Effect (PSM × FYM Levels)		
F_0 FYM Level (0 t ha^{-1})		
S_0	4.50	15.50
S_1	24.00	30.50
S_2	21.00	22.00
S_3	11.50	36.00
S_4	9.50	25.00
F_1 FYM Level (5 t ha^{-1})		
S_0	8.50	21.50
S_1	46.50	41.00
S_2	46.00	33.50
S_3	17.00	46.00
S_4	15.00	36.00
F_2 FYM Level (10 t ha^{-1})		
S_0	10.00	29.50
S_1	56.50	51.50
S_2	54.50	42.00
S_3	21.00	57.00
S_4	19.50	46.50
Main Effect (PSM)		
S_0	6.50	18.50
S_1	35.25	35.75
S_2	33.50	27.75
S_3	14.25	41.00
S_4	12.25	30.50
Main Effect (FYM Levels)		
F_0	14.10	25.80
F_1	26.60	35.60
F_2	32.30	45.30
CD (P= 0.05%)		
FYM	1.14	1.27
PSM	1.47	1.64
FYM x PSM	2.54	2.84

* S_0 = uninoculated control, S_1 = *Bacillus polymyxa*, S_2 = *Pseudomonas striata*, S_3 = *Aspergillus awamori*, S_4 = *Penicillium digitatum*

Table 3. Integrated effect of Phosphate solubilizing microorganisms and FYM levels on soil chemical properties and nutrient availability

Treatments	pH (1:2.5)	EC (dS m^{-1})	$CaCO_3$ (%)	Organic Carbon (g CO_2-C h^{-1} kg^{-1} MBC)	Available		
					N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})
Interaction Effect (PSM × FYM Levels)							
F_0 FYM Level (0 t ha^{-1})							
S_0	8.58	0.34	5.30	0.51	61.61	7.79	250.00

S_1	8.51	0.46	5.27	0.49	78.62	10.59	253.00
S_2	8.54	0.41	5.28	0.50	74.11	8.89	251.00
S_3	8.49	0.47	5.26	0.48	80.22	11.47	250.50
S_4	8.52	0.43	5.28	0.50	70.87	9.80	252.00
F_1 FYM Level (5 t ha^{-1})							
S_0	8.54	0.37	5.29	0.56	65.07	9.80	251.50
S_1	8.47	0.46	5.25	0.52	82.92	14.98	256.50
S_2	8.52	0.44	5.26	0.54	80.22	11.46	250.00
S_3	8.45	0.52	5.23	0.53	90.16	16.83	256.00
S_4	8.49	0.43	5.24	0.55	80.87	11.65	252.00
F_2 FYM Level (10 t ha^{-1})							
S_0	8.51	0.39	5.19	0.59	70.19	9.91	256.50
S_1	8.44	0.47	5.15	0.57	85.37	16.61	254.50
S_2	8.48	0.45	5.17	0.58	78.43	12.82	251.00
S_3	8.43	0.51	5.24	0.56	91.61	19.29	257.50
S_4	8.46	0.45	5.26	0.59	79.97	14.49	255.00
Main Effect (PSM)							
S_0	8.56	0.35	5.29	0.54	63.34	8.80	250.75
S_1	8.49	0.46	5.26	0.51	80.77	12.79	254.75
S_2	8.53	0.42	5.27	0.52	77.16	10.17	250.50
S_3	8.47	0.49	5.24	0.51	85.19	14.15	253.25
S_4	8.51	0.43	5.26	0.53	75.87	10.72	252.00
Main Effect (FYM Levels)							
F_0	8.53	0.42	5.27	0.50	73.08	9.71	251.30
F_1	8.49	0.44	5.25	0.54	79.85	12.94	253.20
F_2	8.46	0.45	5.26	0.58	81.11	14.62	254.90
CD (P= 0.05%)							
FYM	0.012	0.013	0.011	0.031	0.97	0.34	2.34
PSM	0.016	0.017	0.014	0.017	1.25	0.44	NS
FYM x PSM	NS	NS	NS	NS	2.16	0.77	NS

* S_0 = uninoculated control, S_1 = *Bacillus polymyxa* , S_2 = *Pseudomonas striata*, S_3 = *Aspergillus awamori*, S_4 = *Penicillium digitatum*

number of root nodule with PSM seed inoculation and increasing level of FYM may be attributed to the increase in soil rhizosphere rhizobial and PSM population due to which the root nodulation was increased. Similar finding was reported by Siddiqui and Debbarma (2022).

3.3 Soil pH

The rhizosphere soil pH was observed to decrease in all the treatments with increasing levels of FYM (Table 3). The reduction in soil pH with increasing levels of FYM may be attributed to the increased population of PSM (Table 2) leading to lowering in rhizosphere soil pH. Moreover, the organic acids release due to the mineralization of FYM may have lowered the soil pH with increasing FYM levels. Similar report was also observed by Kumar et al. (2008). *Aspergillus awamori* seed inoculation treatment significantly decreases the soil pH at harvest (8.47) over all the other treatments followed by *Bacillus polymyxa* seed inoculation treatment (8.49). The high decrease in soil pH with *Aspergillus awamori* and *Bacillus polymyxa* seed inoculation treatments may be attributed to the higher synthesis of organic acids or excretion of H^+ extrusion associated with ammonium assimilation by the two PSM strains (Parks et al., 1990; El-Azouni, 2008). Kpomblekae and Tabatabai (1994) reported that PSM solubilize the insoluble inorganic P through secretion of organic acid and inorganic acid in which the hydroxyl and carboxyl groups of acids chelate cations like Ca^{2+}, Al^{3+} and Fe^{3+} and decrease pH of basic soils. Thus the soil pH was observed to decrease at harvest as compared to initial soil pH due to application of FYM and seed inoculation of PSM. However, the interaction between PSM seed inoculation and FYM levels did not show significant effect on soil pH at harvest.

3.4 Soil EC

The soil EC was slightly increased in all the treatments with increasing levels of FYM (Table 3) and it may be attributed to mineralization of FYM and release of nutrients. Treatment S_3 (*Aspergillus awamori* seed inoculation) was observed to be highly significant in increasing the soil EC followed by treatments S_1 (*Bacillus polymyxa*), S_4 (*Penicillium digitatum*), and S_2 (*Pseudomonas striata*) seed inoculation treatment. The increase in EC with seed inoculation of different PSM may be related to their efficacy of phosphate solubilization. Yahya and Al- Azawi (1989) also reported the increase in soil EC due to dissolution of fixed phosphates and increase in cation and anion concentration in soil. However, soil EC at harvest was not influence by interaction effect of PSM seed inoculation treatments and FYM levels.

3.5 Calcium carbonate ($CaCO_3$) content of soil

Like soil pH, the $CaCO_3$ content of soil was slightly decreased in all the treatment with increased levels of FYM (Table 3). This decreased in $CaCO_3$ content may be attributed to the higher microbial population which secreted organic acids and respired CO_2 leading to the formation of carbonic acid thereby bringing a slight decrease in the $CaCO_3$ content. Similar observation was reported by Mrkovacki et al. (2008). The $CaCO_3$ content of soil in *Aspergillus awamori* and *Bacillus polymyxa* seed inoculation treatments (5.24 and 5.26%) were lower than the other bacterial and fungal strain respectively at harvest of soybean which may be attributed to higher organic acid secretion by these two PSM over the other PSM strains. However, no significant difference was observed in soil $CaCO_3$ content due to the interaction of PSM seed inoculation and FYM levels.

3.6 Organic carbon

A slight increase in soil organic carbon due to application of FYM was observed at harvest over the initial soil status. Saha et al. (2010) also observed significant increase in soil organic carbon in FYM treated soil over the untreated control. Over all the seed inoculation of PSM and its interaction with FYM levels did not influence much on the organic carbon content of the soil at harvest (Table 3).

3.7 Available N

The integrated effect of PSM seed inoculation and levels of FYM on soil available nitrogen in Inceptisol at harvest of soybean are presented in Table 3. The highest available nitrogen content was recorded in 10 t ha $^{-1}$ FYM level (81.11 mg kg^{-1}) followed by 5 t ha $^{-1}$ FYM level (79.85 mg kg^{-1}). The increased in the available soil nitrogen at harvest may be attributed to the mineralization of FYM and released of nitrogen. In general, PSM seed inoculation increased the soil available nitrogen in comparison to uninoculated (control). The highest available nitrogen was recorded in *Aspergillus awamori* seed inoculation treatment followed by *Bacillus polymyxa*, S_1 (*Bacillus polymyxa*), S_4 (*Penicillium digitatum*), and S_2 (*Pseudomonas striata*) seed inoculation treatment. Above all, the highest soil available nitrogen was observed in treatments S_3 followed by treatments S_1 in all the FYM levels (F_0, F_1 and F_2 FYM level respectively). The higher nitrogen accumulation in the soil might also be attributed to higher PSM population (Table 2), which may increase P solubilisation and supply to the root and ultimately lead to significant increase in number of effective root nodule and higher nitrogen fixation. This finding is in the same line with Miao et al. (2007) and Li et al. (2021) who reported that increase in P supply enhance root nodulation and nitrogen fixation capacity in soybean. Higher available nitrogen in *Aspergillus awamori* and *Bacillus polymyxa* in comparison to other *PSM* seed inoculation treatment also highlighted more effective synergistic relationship between these two PSM strains and *Bradyrhizobium japonicum*. Shome et al. (2022) also reported that seed inoculation of PSM and *Rhizobium* increased the root growth and nodules which resulted in higher nitrogen fixation and available N status in soil.

3.8 Available P

The effect of PSM seed inoculation and FYM levels on soil available phosphorus showed similar trend as that of available Nitrogen (Table 3). Higher available phosphorus was observed in 10 t ha^{-1} FYM level (50.56%) followed by 5 t ha^{-1} FYM level (33.26%) than the soil where no FYM is applied. It was observed that available phosphorus in treatment *Aspergillus awamori* seed inoculation treatment (14.15 mg kg^{-1}) was highly significant over all the other treatment followed by *Bacillus polymyxa* (12.79 mg kg^{-1}) *Penicillium digitatum* (10.72 mg kg^{-1}) and *Pseudomonas striata* (10.17 mg kg^{-1}) seed inoculation treatments. The highest available phosphorus status in treatment of *Aspergillus awamori* followed by *Bacillus polymyxa* treatment may be attributed to the secretion of higher amount of organic acids or Proton extrusion by the two PSM strains which resulted better P solubilization (Venkateswarlu et al., 1984; Parks et al. 1990; Luo et al., 1993). Overall, the interaction effect showed that *Aspergillus awamori* seed inoculation treatment showed the highest available phosphorus in all levels of FYM (0, 5, 10 t ha^{-1}) followed by *Bacillus polymyxa* seed inoculation treatment. Phosphorus solubilizing microorganisms enhances the phosphorus availability in soil by mineralization of organic phosphorus in FYM and solubilizing precipitated phosphate (Kang et al., 2002; Pradhan and Shukhla, 2005; Chen et al., 2006). The finding is in same line with Adnan et al. (2022), who reported that application of organic manure in conjunction with PSM improved the P availability in calcareous soil. The result in Table 2, on soil microbial population at 45 DAS growth stage of soybean also supports the observation. The highest microbial population at 45 DAS in fungi was observed in *Aspergillus awamori* and in bacteria was observed in *Bacillus polymyxa.* The higher population of *Aspergillus awamori* and *Bacillus polymyxa* with increasing levels of FYM may also have resulted in efficient phosphate solubilization thereby resulting in higher soil available phosphorus status at harvest.

3.9Available K

The available K status at harvest was not significantly affected due to PSM seed inoculation and its interaction with FYM levels (Table 3). However, a slight increase in soil available potassium was observed with increase in FYM level at harvest of soybean which may be due to the residual effect of applied FYM. Nalatwadmath (2003) also reported build-up of available K in only organic treatments up to 33% as compared to control in Vertisol of Karnataka.

3.10 Grain and straw yield

PSM seed inoculation and FYM application alone or in combination significantly increase the soybean grain and straw yield over control (Table 4). The respected grain and straw yield was significantly higher in 10 t ha^{-1} FYM level (17.66%, 29.41%) followed by 5 t ha^{-1} FYM level (9%, 10.82%) than the untreated control. The highest grain yield and straw was observed in treatment *Aspergillus awamori* seed inoculation followed by *Bacillus polymyxa, Penicillium digitatum* and *Pseudomonas striata* seed inoculation treatment. The lowest grain yield was observed in no PSM inoculation treatment. The higher grain yield in *Aspergillus awamori* and *Bacillus polymyxa* seed inoculation treatment may be associated with solubilization of native inorganic and organic phosphate and their uptake by soybean. The phosphorus uptake by soybean played important role in photosynthesis, nitrogen fixation, root development, flowering, seed formation (Raghothama, 1999) and enhanced the metabolic activities and synthesis of organic constituents like protein, carbohydrate, lipids etc. It was reflected in higher grain yield. The higher atmospheric nitrogen fixation in the soil due to co inoculation of *Rhizobium* and PSM might have also attributed to a significant increase in the number of effective root nodule (Table 2) and increased root growth resulting in higher uptake of nutrients in the grand growth stage. Tagore et al. (2013) and Shome et al. (2022) also reported the increased yield and yield attributing parameters of soybean with co inoculation of *Rhizobium* and PSM

The interaction between PSM seed inoculation and FYM level was observed to be statistically significant for grain and straw yield of soybean. The grain yield was observed to be increased in all the treatment with increasing levels of FYM. The highest grain yield was observed in *Aspergillus awamori* with 10 t ha^{-1} FYM followed by *Bacillus polymyxa.* The result in Table 2 on soil microbial population at 45 DAS growth stage of soybean also supports the observation. The higher population of *Aspergillus awamori* and *Bacillus polymyxa* with increasing levels of FYM may have resulted in efficient phosphate solubilization thereby resulting in higher soil available phosphorus status at 45 DAS and uptake by the crop plant and it was reflected in higher grain yield. Adnan et al. (2022) also observed optimization in grain yield due to the combine effect of PSM seed inoculation along with the application of FYM.

3.11 Nutrient Uptake

The effect of PSM seed inoculation and FYM levels on nutrient (N, P, K) uptake is presented in Table 4. The nitrogen uptake was observed to increase in all the treatment with increase in FYM level and highest nitrogen uptake was observed in 10 t ha^{-1} FYM level followed by 5 t ha^{-1} FYM level treatments, resulting 33.72% and 18.19% respectively increased over control. The main effect of PSM seed inoculation on N uptake showed the same trend as that of the above results *Aspergillus awamori* > *Bacillus polymyxa* > *Penicillium digitatum >Pseudomonas striata.* Kumpawat (2010) also observed that co-inoculation of Rhizobium with PSM increased the dry matter production and N content in grain and straw due to increase uptake of nutrients in legumes. Overall, the interaction effect show that *Aspergillus awamori* seed inoculation treatment significantly increased the nitrogen uptake in all the levels of FYM (0, 5 and 10 t ha^{-1} FYM) among all the other treatment combinations, followed by *Bacillus polymyxa* seed inoculation treatment. The increase in nitrogen uptake with PSM seed inoculation and higher level of FYM may be attributed to increase in rhizobium and PSM population due to which the root nodulation and N fixation increased and thus facilitated the nitrogen uptake in legumes (Linu et al., 2009, Kumpawat, 2010).

Phosphorus and potassium uptake were also increased by addition of FYM and PSM seed inoculation alone or their interactive effect and followed the same trend as that of N.

uptake. The increased in the total uptake of phosphorus increased with PSM seed inoculation and with increasing FYM levels may be attributed to the increase in phosphatase activity of the soil with increasing level of FYM (Manna et al., 2007) making higher availability of P to plant (Adnan et al. 2022). The synergistic effect of PSM and rhizobium also resulted in higher root proliferation leading to higher P uptake by soybean crop (Govindan and Thirumurugan, 2005). In case of potassium uptake, the synergistic effect of N, P and K uptake leading to higher K uptake at grand growth stage

4. Conclusion

Coupling PSM with higher level of FYM enhanced the root nodulation and PSM population, improved the soil available N and P, nutrient uptake and yield in comparison to control. The highest available nitrogen and phosphorus was observed in co-inoculation of *Aspergillus awamori* and *Bradyrhizobium japonicum* followed by *Bacillus polymyxa* seed inoculation treatment indicating a promising synergistic relationship of *Aspergillus awamori* and *Bacillus polymyxa* with *Bradyrhizobium japonicum.* Results confirmed that *Aspergillus awamori* seed inoculation treatment with 10 t ha^{-1}, followed by *Bacillus polymyxa* are the most efficient

Table 4. Effect of conjoint application of Phosphate solubilizing microorganisms and FYM on yield and nutrient uptake

Treatments	Yield		Nutrient Uptake (mg plant^{-1})		
	Grain (g plant^{-1})	Straw (g plant^{-1})	N	P	K
Interaction Effect (PSM × FYM Levels)					
F_0 FYM Level (0 t ha^{-1})					
S_0	5.08	7.23	214.22	21.15	60.85
S_1	7.86	9.31	425.42	57.63	125.69
S_2	7.23	7.84	374.35	49.69	109.21
S_3	8.16	9.87	457.73	62.62	133.96
S_4	7.62	8.23	396.28	54.21	108.75
F_1 FYM Level (5 t ha^{-1})					
S_0	5.41	7.87	264.00	37.77	88.25
S_1	8.60	10.30	504.50	73.91	155.69
S_2	8.07	8.46	440.35	59.95	121.72
S_3	8.84	11.19	528.96	83.71	177.66
S_4	8.27	9.27	470.16	64.61	136.53
F_2 FYM Level (10 t ha^{-1})					
S_0	6.84	9.73	350.87	55.30	121.30
S_1	8.97	11.67	548.93	88.12	192.80
S_2	8.58	9.80	496.65	69.97	152.48
S_3	9.15	13.60	584.31	100.26	227.07
S_4	8.78	10.23	517.13	76.65	165.63
Main Effect (PSM)					
S_0	5.24	7.55	239.11	29.46	74.55
S_1	8.23	9.81	464.96	65.77	140.69
S_2	7.65	8.15	407.35	54.82	115.47
S_3	8.50	10.53	493.35	73.17	155.81
S_4	7.94	8.75	433.22	59.41	122.64
Main Effect (FYM Levels)					
F_0	7.19	8.50	373.60	49.06	107.69
F_1	7.83	9.42	441.59	63.99	135.97
F_2	8.46	11.00	499.58	78.06	171.85
CD (P= 0.05%)					
FYM	0.18	0.21	6.63	2.10	3.90
PSM	0.24	0.27	8.56	2.72	5.04
FYM x PSM	0.41	0.46	14.83	4.71	8.72

* S_0 = uninoculated control, S_1 = *Bacillus polymyxa* , S_2 = *Pseudomonas striata*, S_3 = *Aspergillus awamori*, S_4 = *Penicillium digitatum*

treatment combination for improving the soil available nitrogen, phosphorus and other chemical properties of soil, thereby improving the nutrient uptake and yield of soybean in alkaline soil. Further study on PSM and its combination with different organic sources as an alternative to chemical P fertilizers for legumes and other crops are suggested in various agro climatic conditions to get more in-depth understanding.

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