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Effect of phosphorus and phosphate solubilizing microorganisms on soil fertility and yield of Mungbean [*Vigna radiata* (L.) Wilczek]

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Abstract
A field experiment entitled "Effect of Phosphorus and Phosphate Solubilizing Microorganisms on Soil Fertility and Yield of Mungbean [*Vigna radiata* (L.)Wilczek]" was conducted during *Kharif* season 2018 at Agronomy farm, S.K.N. College of Agriculture, Jobner (Rajasthan). The experiment consisted 16 treatments combination of four levels of phosphorus *viz*., Control, 20, 40 and 60 kg P_2O_5 ha^{-1} and four levels of phosphate solubilizing microorganisms (PSM) *viz*., Control, *Pseudomonas fluorescens*, *Aspergillus awamori* and *Pseudomonas fluorescens* + *Aspergillus awamori* and replicated thrice in randomized block design. Mungbean var. IPM-03-2 was taken a test crop. Results indicated that the number of total and effective root nodules, fresh and dry weight of root nodules, leghaemoglobin content in root nodules, protein content, seed and straw yield in soil at harvest of crop were found significantly maximum upto the level of 40 kg P_2O_5 ha^{-1} over control and 20 kg P_2O_5 ha^{-1}. Among the treatments of different PSM, *Pseudomonas fluorescens* + *Aspergillus awamori* resulted in significantly maximum increase in the number of total and effective root nodules, fresh and dry weight of root nodules, leghaemoglobin content in root nodule, protein content, seed and straw yield in soil at harvest of crop as compared to control, as well as individual inoculation of *Pseudomonas fluorescens* and *Aspergillus awamori.* However seed inoculation with *Pseudomonas fluorescens* and *Aspergillus awamori* individuallyat par.

Keywords: phosphorus, phosphate solubilizing microorganisms, *Pseudomonas fluorescens*, *Aspergillus awamori*

Introduction
Pulses are important source of dietary protein and have unique ability of maintaining and restoring soil fertility through biological nitrogen fixation as well as addition of ample amount of residues to the soil. Pulse crops leave behind reasonable quantity of nitrogen in soil to the extent of 30 kg ha^{-1}. They contain more protein than any other plant and also serve as a low-cost protein to meet the needs of the large section of the people. They have, therefore, been justifiably described as 'the poor man's meat'. Mungbean [*Vigna radiate* (L.) Wilczek] is a self-pollinated leguminous crop, which is grown during kharif as well as summer seasons in arid and semi-arid regions of India. It is tolerant to drought and can be grown successfully on drained loamy to sandy loam soil in areas of erratic rainfall.

Phosphorus is an important nutrient next to nitrogen for plants. Indian soils are poor to medium in available phosphorus. At present 5% of the Indian soils have adequate available P, 49.3% are under low category, 48.8% under medium and 1.9% under high category (Pattanyak *et al.*, 2009). The P input in Indian agriculture comes from fertilizers, organic manures and to a very small extent from crop residues. The overall uptake of phosphorus in cereals, pulses, oil seeds and tubers varies between 2 and 24 mg kg^{-1}. Only 25% to 30% of the applied P is available to crops and remaining P is converted into insoluble P (Sharma and Khurana, 1997) [20]. Phosphorus application to legume not only benefits the current crop but also has favourable effect on succeeding non-legume crop. It's also improved the crop quality and resistance to diseases. It is a part of ADP, ATP, nucleic acid, thiamine phosphate, flavin nucleotides, phospholipids and phosphorylated sugar etc. It acts as energy storage and transformation. It is also essential for cell division, protein synthesis, root development flowering, fruiting and seed formation.

Phosphorus solubilizing microorganisms (bacteria and fungi) enable P to become available for plant uptake after solubilization. Several soil bacteria, particularly those belonging to the genera *Bacillus and Pseudomonas* and fungi belonging to the genera *Aspergillus and Penicillium* possess the ability to bring insoluble phosphates in soil into soluble forms by secreting organic acids such as formic, acetic, propionic, lactic, glycolic, fumaric, and succinic acids.

Bacteria of the genus *Pseudomonas* are widely distributed in soil and can colonize plant rhizospheres to produce different metabolites. These bacteria are usually present around the roots and assist in nutrient uptake by the plants. The use of growth-promoting bacteria can increase the growth and yield of crops through direct and indirect mechanisms. It has been proven that these bacteria increase the qualitative/quantitative yields of most crops through biological fixation of nitrogen, biological control of plant pathogens (Saravanakumar *et al.*, 2007) [18], vitamin production, increased iron absorption by siderophore secretion (Shaharoona *et al*., 2008; Braud *et al.*, 2009) [19, 5] and production of the plant hormones cytokinin, auxin and gibberellin (Dey *et al.*, 2004) [8], and thus increasing the phosphorus solubility. Seed inoculation with the *Aspergillus awamori* and phosphate solubilizing bacteria (PSB) significantly increased growth, yield, nutrient content and their uptake, after harvest over the rest of the treatments with increasing level of phosphate application (Venkata Rao *et al.*, 2018) [25].

Materials and Methods

The experiment was laid out at Agronomy farm of S.K.N. College of Agriculture, Jobner which is situated at 45 km west of Jaipur at 26^0 05" North latitude and 75^0 28" East longitude at an altitude of 427 meters above mean sea level. In Rajasthan, this area falls under Agro-climatic zone-IIIA (Semi-Arid Eastern Plains) of Rajasthan. The experiment comprising four levels of phosphorus *viz*., Control, 20, 40 and 60 kg P_2O_5 ha^{-1} and four treatments of phosphate solubilizing microorganisms (PSM) *viz.*, Control, *Pseudomonas fluorescens*, *Aspergillus awamori* and *Pseudomonas fluorescens* + *Aspergillus awamori* and replicated thrice in randomized block design. Mungbean var. IPM-03-2 was taken a test crop with seed rate 16 kg ha^{-1}. As per treatments, the seed were inoculated with *Psuedomonas inflorescence, Aspergillus awamori and Psuedomonas inflorescence* + *Aspergillus awamori* before sowing using standard method and dried in shade (Paul et al., 1971) [14]. The whole quantity of phosphorus was applied through SSP as per treatment details prior to sowing and incorporated manually in top 15cm of soil. Total no. of root nodules and effective root nodule were recorded at flowering stage. Effective root nodules obtained from the five plants of each plot were weighed with the help of an electronic balance and average was worked out and recorded as fresh weight of effective root nodule per plant. Effective root nodule so obtained from the five plants in each plot were subjected to oven dry at 70 ^{0}C till a constant weight was obtained and then average was worked out. Leghaemoglobin content in nodules was estimated as hemochrome as described by Appleby *et al.* (1986) [1] and Bergersen (1982) [3]. Protein content in seed was calculated by multiplying per cent nitrogen content in the grain with a constant factor 6.25 (A.O.A.C., 1960) [2]. The yield attributed and yield were recorded as per standard statistical procedures.

Results and Discussion

Effect of phosphorus on mungbean growth stages

Application of phosphorus upto 40 kg P_2O_5 ha^{-1} significantly enhanced the total nodules, effective nodules, dry weight and fresh weight of root nodules per plant (Table. 1) over control and 20 kg P_2O_5 ha^{-1}. The leghaemoglobin content was also increased significantly upto 40 kg P_2O_5 ha^{-1} (Table. 2). However, the maximum content was obtained with 60 kg P_2O_5 ha^{-1}. Phosphorus is an indispensable constituent of nucleic acid, ADP and ATP. It has beneficial effects on nodulation, root development, leghaemoglobin content, growth and also hastens maturity as well as improves quality of crop produce (Choudhary *et al.* 2015) [6]. Such increase in nodulation, increase the nitrogen content by nitrogen fixation and finally enhance productivity of green gram (Prasad *et al.*, 2014) [16]. Besides, improvement in nodulation, the larger canopy development under the influence of increased P levels seems to have increased absorption and utilization of radiant energy resulting in higher effective and total nodules (Escalante *et al.*, 2014) [9].

Phosphorus encourage formation of new cells, promote plant vigour and hastens leaf development, which help in harvesting more solar energy and better utilization of nitrogen, which help towards higher nodulation. These findings are found relevant to Malik *et al.* (2013) [10], Escalante *et al.* (2014) [9], Rathore *et al.* (2015) [17].

Increase in protein content in seed of mungbean might be due to enhanced uptake and translocation of nitrates which provide nitrogen for amino acid synthesis. Moreover, phosphorus is involved in the synthesis of ATP that is required in nitrogen uptake and protein synthesis. Higher seed protein content in mungbean in response to N and P applications have also been reported by Dewangan (1992) [7] and Nazir (1993) [12].

Phosphorus is an important element in all biological system, participating in most metabolic pathways and as a structural component of nucleic acids, coenzymes, phosphoproteins and phospholipids. Thus, phosphorus application realized spectacular improvement in seed and straw yield of mungbean (Table 2). Significantly highest seed yield of mungbean were recorded with treatment 40 kg P_2O_5 ha^{-1} over control and 20 kg P_2O_5 ha^{-1}. This might be due to the role of phosphorus on promotion of root growth and there by enhancement in renewable of nitrogen by the crop. The improvement in yield and protein content is attributed to increase in root nodulation due to phosphorus application (Bhatt *et al.* 2013) [4]. These results are in agreement with the findings of Bhatt *et al.* (2013) [4], Tiwari *et al.* (2015) [23], Rathour *et al.* (2015) and Choudhary *et al.* (2015) [6].

Effect of PSM on grain and straw yield of mungbean

The increment in nodulation of mungbean crop (Table 1) might be due to the higher phosphatases activity in the rhizosphere and production of organic acids by *Aspergillus niger* might have solubilized the insoluble and native phosphate and brought into soluble form. This resulted in increased P uptake by the crop. Apart from increased enzymatic activities, the beneficial effects of *Aspergillus niger* on plant growth might also include the production of plant growth promoter. Greater phosphatases activity has been directly implicated in the acquisition of more P by plants (Yadav and Tarafdar, 2010) [26].

A positive influence of *Pseudomonas fluorescens +Aspergillus awamori* on nodulation and leghaemoglobin content was observed (Table 2) due to higher enzyme activities in the rhizosphere and better nutrient availability besides the production of the plant growth regulators. The production of organic acids such as lactic, glycolic, and succinic acids in the soil medium of *Aspergillus awamori* can solubilize unavailable inorganic phosphates. The results obtained in the investigation are in line with the findings of Pramanik *et al.* (2014) [15], Vanita *et al.* (2014).

The increase in seed yield of mungbean due to increase in P availability through solubilization of phosphate rich compound. The *Pseudomonas fluorescens* secrete a number of organic acids which may form chelates resulting in effective solubilization of phosphate, favoured higher nitrogen fixation, dry matter accumulation, rapid growth, higher absorption and utilization of P and other plant nutrients and ultimately positive effect on growth and finely on yield attributes (Rathour *et al.* 2015).

Higher seed and straw yield with higher dose of phosphorus contributed more removal of N, P and K by the crop (Bhatt *et al.*, 2013) [4]. The combined inoculation of *Pseudomonas fluorescens* with *Aspergillus awamori* also significantly increased the uptake of phosphorus which might be due to enhanced availability of phosphorus, which is known to be positively related with phosphorus uptake. Significant role of these treatments in root enlargement, better microbial activities resulted in more availability and uptake of nitrogen and thereby increased protein content in seed (Rathour *et al.* 2015). The observations recorded were found similar with Meena *et al.* (2015) [11], Singh *et al.* (2013) [21], Yadav (2011) [27].

Table 1: Effect of phosphorus and PSM on total number and effective root nodules, fresh weight and dry weight of root nodule

Treatments	Total root nodules per plant	Effective root nodules per plant	Fresh weight (mg plant^{-1})	Dry weight (mg plant^{-1})
Phosphorus level				
P_0 (Control)	22.80	19.02	66.02	15.32
P_1 (20 kg P_2O_5 ha^{-1})	26.02	21.50	75.16	18.44
P_2 (40 kg P_2O_5 ha^{-1})	28.40	23.02	79.45	20.67
P_3 (60 kg P_2O_5 ha^{-1})	29.50	24.00	82.13	21.63
S.Em +	0.73	0.60	2.07	0.52
CD (P=0.05)	2.10	1.74	5.99	1.51
PSM				
M_0 (Control)	22.37	18.29	65.82	15.75
M_1 (*P. fluorescens*)	26.85	21.79	74.36	18.52
M_2 (*A. awamori*)	27.36	23.11	78.93	19.92
M_3 (*P. fluorescens+ A. awamori*)	30.14	24.34	83.65	21.87
S.Em+	0.73	0.60	2.07	0.52
CD (P=0.05)	2.10	1.74	5.99	1.51

Table 2: Effect of phosphorus and PSM on leghaemoglobin content in root nodule, protein content in seed, seed and straw yield of mungbean

Treatments	Leghaemoglobin content (mg/plant)	Protein content (%)	Seed yield (q/ha)	Straw yield (q/ha)
Phosphorus level				
P_0 (Control)	1.29	17.63	7.72	17.28
P_1 (20 kg P_2O_5 ha^{-1})	1.47	20.25	11.78	21.41
P_2 (40 kg P_2O_5 ha^{-1})	1.56	21.56	13.88	25.26
P_3 (60 kg P_2O_5 ha^{-1})	1.62	21.94	14.43	26.14
S.Em+	0.04	0.55	0.25	0.62
CD (P=0.05)	0.12	1.59	0.71	1.78
PSM				
M_0 (Control)	1.23	17.69	7.50	17.98
M_1 (*P.fluorescens*)	1.45	20.38	12.37	22.85
M_2 (*A.awamori*)	1.59	20.69	12.94	23.74
M_3 (*P. fluorescens+ A. awamori*)	1.67	22.63	14.98	25.51
S.Em+	0.04	0.55	0.25	0.62
CD (P=0.05)	0.12	1.59	0.71	1.78

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