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Response of Chickpea (*Cicer arietinum* **L***.***) to Phosphate Solubilizing Microorganisms and Phosphorus Levels on Nutrient Availability, Uptake and Seed Yield in Inceptisol**

Waghmode B. G. ^a , Waghmare M. S. ^b , Ugile S. K. c* and Puri A. N. ^a

^a Department of Soil Science and Agricultural Chemistry, Collage of Agriculture, Latur, V.N.M.K., Parbhani, India.

^bDepartment of Soil Science and Agricultural Chemistry, Collage of Agriculture, Dharashiv, V.N.M.K., Parbhani, India.

^cDepartment of Soil Science and Agricultural Chemistry, Collage of Agriculture, Badnapur, V.N.M.K., Parbhani, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: skugile@gmail.com;*

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ABSTRACT

The present investigation was carried out for the "Response of chickpea (*Cicer arietinum* L.) to phosphate solubilizing microorganisms and phosphorus levels on nutrient availability, uptake and seed yield in inceptisol." The experiment was conducted in factorial randomized block design (FRBD) with three replications*.* during Rabi 2019-20 at department research farm of SSAC, College of Agriculture, Latur. The treatments comprises four main (absolute control, *Bacillus megaterium, Aspergillus niger* and *Aspergillus awamori* at 10 ml kg-1 seed treatment) and four sub treatments (0,45,60 and 75 P_2O_5 kg ha⁻¹). The results indicates that, the incorporation of phosphate solubilizing microorganisms *viz. Aspergillius awamori* at 10 ml kg-1 seed in combination with soil application of 75 P₂O₅ kg ha⁻¹ found to be significantly higher availability of nutrient, increased total N, P and K uptake of chickpea as compared to *Aspergillus niger* and *Bacillus megaterium* along with 60 P2O⁵ kg ha⁻¹ and over control .Further results revealed that seed yield was significantly improved with the seed treatment of *Aspergillius awamori* at 10 ml kg⁻¹ seed in combination with application of 75 P₂O₅ kg ha-1 .

Keywords: Chickpea; microorganisms; nutrient; phosphorus; uptake; yield.

1. INTRODUCTION

Pulses are an important group of food crops that can play an important role to address national food and nutritional security and also tackle environmental challenges. Among the pulses India is one of the major chickpea producing country in the world and ranked first in area and production with 34 per cent and 26 per cent respectively, as compare to total world production. Chickpea is the *rabi* pulse crop grown as a supplementing protein (17-25 per cent), amino acid, Vit. A, Vit. C, Vit, B, Vit. K, source of folic acid demand of vegetarian diet. Chick pea is not only important human diet but also it plays a significant role in improving soil fertility by fixing the atmospheric nitrogen. Chickpea meet about 80 per cent of nitrogen requirement from symbiotic nitrogen fixation from air. It leaves substantial amount of residual nitrogen for subsequent crops and add plenty of organic matter to maintain and improve soil health and fertility [1].

Phosphorus can be termed as 'life mineral' because of its crucial role in metabolic and energy transfer reactions in plant. Phosphorus is an essential element in DNA and RNA that contain the genetic code of the plant to produce protein and other compounds essential for plant structure, seed yield and genetic transfer. It is also associated with increased root growth, chlorophyll content, straw strength and crop maturity in cereals and N_2 -fixation in legumes. Thus, phosphorus is essential for vigorous growth and development of reproductive parts in crops. The phosphorus deficiency leads to stunted root and shoot growth, bluish green

coloration of leaf, delayed maturity and poor grain development in cereals. Thus, phosphorus has become a major constrain in agricultural production mainly because of its fixation in soils involving both adsorption and precipitation reactions. The rate and magnitude of phosphate adsorption depends upon the properties of soils and phosphorus resources Barros et al., [2], Boparai [3]. Use of phosphorous in soils phosphorous solubilizing microorganisms (PSMs) are capable to convert insoluble phosphorous to soluble forms and increase the native phosphorous in soil [4] . Low fertility, particularly phosphorus deficiency is one of the major constrains to increase the chickpea productivity [5]. Use of biofertilizers is low cost renewable source of plant nutrients, which supplement chemical fertilizer. Biofertilizers solubilize plant nutrients like N and P through their activities in the soil or rhizosphere and make available to plant in gradual manner. PSB solubilize insoluble phosphorus compounds by exerting organic acids, which is the primary mechanism of solubility of insoluble inorganic phosphates. Besides organic acids, production of chelating substances, mineral acids and proton extrusion also involved [6]. Phosphate solubilizing microorganisms, such as *Aspergillus* and *Bacillus,* play a crucial role in solubilizing soil phosphorus through mechanisms like pH reduction and organic acid secretion Bharadkar et al., [7] Mittal et al*.,* [8] found that seed inoculation of chickpea with *Aspergillus awamori* increased shoot height by 7-12 per cent, a nearly 3 fold increase in seed weight as compared to uninoculated control. Seed inoculation with *Aspergillus awamori* increased total P content and biomass of mungbean [9].

2. MATERIALS AND METHODS

2.1 Study Site

The field experiment was conducted during *Rabi* 2019-20 at research farm departmental farm of SSAC, College of Agriculture, Latur using chickpea crop (Var.BDNG-797). In order to evaluate the response of chickpea (*Cicer arietinum* L*.*) to phosphate solubilizing microorganisms and phosphorus levels on nutrient availability, uptake and seed yield in inceptisol.

2.2 Experimental Soil

The experimental soil pH was found to be 7.8, Electrical conductivity (0.28 dSm-1), organic carbon 4.6 g per kg, and $CaCO₃$ 5.6 per cent. Available N, P_2O_5 and K₂O were 143.25, 6.28 and 317.19 kg ha-1 , respectively. The experimental soil was clayey in texture, moderately alkaline in reaction, low in available nitrogen and phosphorus and high in available potassium.

2.3 Preparation and Analysis methods of Experimental Soil

Soil pH was determined by 1:2.5 soil water suspension ratio using a digital pH meter [10]. Electrical conductivity of soil was determined by 1:2.5 soil: water suspension ratio using the Conductivity Bridge [10]. Soil organic carbon was determined by modified method of Walkley-Black methods [11]. The calcium carbonate content in soil was determined by Rapid titration method [10]. Available nitrogen was determined by alkaline potassium permanganate method [12]. Available phosphorus was extracted from the soil with 0.5 M Sodium bicarbonate by Olsen's method [10]. Available potassium was determined with neutral normal ammonium acetate and potassium in the extract was determined on Flame Photometer [3].

2.4 Experimental Details

After completion of preparatory tillage operations, the experiment was laid out in factorial randomized block design (FRBD) with sixteen treatments plots replicated thrice. Organic manures i.e. FYM was applied at the rate of 5 t ha⁻¹ prior to 15 days of sowing of chickpea crop and all the plots were fertilized with

recommended dose of fertilizer NPK (25:50:00 kg ha-1) was applied as a basal dose through urea, SSP treatment wise at the time of sowing.
The treatments comprises as a seed The treatments comprises as a seed treatment T0: Control, T1: *Bacillus megaterium* @ 10 ml kg-1 seed, T2: *Aspergillus niger* @ 10 ml kg-¹ seed, T3: *Aspergillus awamori* @ 10 ml kg-1 seed as a main treatments and application P_0 : 0 P kg ha⁻¹, P₁:45P kg ha⁻¹, P₂:60P kg ha⁻¹, P₃:75P kg ha⁻¹ as a sub main treatments. Seed was inoculated with *Bacillus megaterium Aspergillus niger* and *Aspergillus awamori,*@ 10 ml / kg seed.

Chickpea seed was sown on 09 October 2019 by dibbling method as per randomly replicated plot having size $3x + 2$ m² maintained row to row spacing 30 cm and plant to plant 10 cm and using a seed rate of 80 kg ha⁻¹. The crop was harvested at maturity stage on 22 January 2020. The observation recorded the seed yield, fodder yield were recorded at harvest stage. The data collected from the above observation were analysed statistically by the procedure prescribed by Panse and Sukhatme [13].

2.5 Methods of Plant and Grain Analysis

For the determination of nutrient contents in plant samples fresh plant samples and grain sample were collected at harvest stage from each net plot and processed with following standard procedure of washing, sun drying and then oven drying and grinding. The grind plant materials were stored in the paper bags and used for further chemical analysis. One gram of fine powered plant and grain sample, 15 ml of tri-acid mixture (HNO $_3$:H $_2$ SO $_4$ HCLO $_4$ in a ratio of 10:1:4) was added and digested plant and grain sample as described by Piper [14]. It was kept in digestion chamber till complete digestion of the sample. The residue was dissolved in doubledistilled water and after filtration; final volume was made to 50 ml. Nutrient content in plant and grain sample were analyzed for the total nitrogen content determined by Microkjeldahl's method A.O.A.C., [15], total phosphorus vanadomolybdo phosphoric acid yellow colour method spectrophoto-metrically as described by (Jackson, 1973), total potash estimated by di-acid extract [14] by using flame photometer. Nutrient uptake (kg ha⁻¹) by using the following formula - {Nutrient uptake (kg ha⁻¹) =Nutrient content (%) X yield (kg ha⁻¹) /100}.

3. RESULTS AND DISCUSSION

3.1 Effect of Phosphate Solubilizing Microorganisms and Phosphorus Levels on Available Nutrients

3.1.1 Available nitrogen

The available nitrogen was influenced significantly due to seed inoculation of phosphate solubilizing microorganism (Table 1). The data indicated that the maximum available of N content in soil was found with seed inoculation of Aspergillus awamori (166.68 kg ha⁻¹) followed by *Aspergillus niger* (165.81 kg ha-1) and *Bacillus* megaterium (158.44 kg ha⁻¹). The lower value of (154.89 N kg ha-1) available N was recorded in control. The application of different phosphorus levels significantly increased the available N kg ha⁻¹. The highest available N was recorded with the application of phosphorus $@$ 75 kg P_2O_5 ha⁻¹ $(163.52 \text{ kg} \text{ ha}^{-1})$. As the levels of phosphorus decreases the available N content was also decreases. While minimum available N (158.01 kg ha-1) was recorded in control. The data showed that the interaction effect of phosphate solubilizing microorganism and phosphorus levels on available nitrogen showed nonsignificant. Inoculation of phosphate solubilizing microorganism and application of phosphorus levels resulted in reduction in denitrification and leaching losses of N and that result in improve the soil health.

Increased available nitrogen observed due to phosphorus play an important role in nodule initiation and root proliferation. Nodule formation increases the nitrogen fixation and ultimately increases soil available nitrogen [16]. Similar results were reported by Kumar et al., [17] studied that the application of 60 kg P2O5 and

inoculation of biofertilizers significantly increased available N (230.5 kg ha⁻¹) and (224.7 kg ha⁻¹) respectively in the soil. N status increased with increase in the levels of P and biofertilizers (PSB and AM). This might be attributed to the application of P and biofertilizers which enhanced and established better root system. Nutrients possibly stimulate the modulating bacteria for more fixation of atmospheric N₂ resulting in increase of its contents in the soil over control.

3.1.2 Available phosphorus

The data on effect of phosphate solubilizing microorganisms on available phosphorus in chickpea after harvest was reported in (Table 2). The data indicated that seed inoculation with phosphate solubilizing microorganisms influenced significantly. Among the phosphate solubilizing microorganisms seed inoculation with *Aspergillus awamori* recorded higher value of available phosphorus (9.68 kg ha-1) followed by *Aspergillus niger* (9.25 kg ha-1) and *Bacillus* megaterium (8.67 kg ha⁻¹). While minimum available phosphorus (7.89 kg ha-1) was recorded in control after harvest of chickpea.

The application of different phosphorus levels significantly increased the available phosphorus in soil. The maximum available phosphorus was recorded with application of phosphorus @ 75 kg $P₂O₅$ ha⁻¹ (9.42 kg ha⁻¹) followed by application of phosphorus $@60$ kg P₂O₅ ha⁻¹ (9.12 kg ha⁻¹) and 45 kg P_2O_5 ha⁻¹ (8.73 kg ha⁻¹). The lowest value of available phosphorus was recorded in control after harvest of crop. Thus, it was observed that the available phosphorus in soil was decreased significantly as decrement in the levels of phosphorus.

Key: PSM = phosphate solubilizing microorganism

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Further data shows that the interaction effect of phosphate solubilizing microorganisms and phosphorus levels on available phosphorus found significant. The interaction *Aspergillus* awamori and application of 75 kg P₂O₅ ha⁻¹ found maximum availability than rest of the interactions. The increase in the available phosphorus might be due to the fact that increase in the solubilization of inorganic P by secreting more organic acids in the soil which helps to increase the P availability Yadav et al*.* [18] reported that, the application of *Aspergillus awamori* + *Psedomonas striata* to munbean increased P content in soil as compared to control. *Pseudomonas strita* stimulate the microbial activity in soil and after decaying of their bodies in soil increases the P content in soil. Similar findings was also reported by Laharia et al., [19].

3.1.3 Available potassium

The results obtained in respect to phosphate solubilizing microorganism and phosphorus levels on available potassium after harvest of crop reported in (Table 3). The inoculation of microorganism *Aspergillus awamori* recorded maximum available potassium (784.25 kg ha⁻¹), followed by *Aspergillus niger* (283.71 kg ha-1) and *Bacillus megaterium* (280.69 kg ha-1) while minimum available potassium recorded in control (278.96 kg ha-1) after harvest of chickpea. However, available potassium could not reach to level of significance due to use of different phosphate solubilizing microorganisms but increase in potassium status in soil. The effect of application of different phosphorus levels were shows non- significant differences in available K. The maximum available K was recorded with application of phosphorus $@$ 75 kg P_2O_5 ha⁻¹ $(283.35 \text{ kg} \text{ ha}^{-1})$, followed by 60 kg P₂O₅ ha⁻¹ $(295.82 \text{ kg} \text{ ha}^{-1})$ and 45 kg P₂O₅ ha⁻¹ (280.89 kg ha- ¹).

The minimum available K content was recorded in control after harvest of crop. However increase in potassium status noticed as the levels of phosphorus increased. The interaction effect of phosphate solubilizing microorganism and phosphorus levels on available potassium was found non-significant. The availability of

Key : PSM = phosphate solubilizing microorganism

potassium was decreased as compared to initial available K $(317.19 \text{ kg} \text{ ha}^{-1})$ due to the unavailability of K content in fertilizer that resulted in decrease the available K content in soil. Laharia et al., [19] reported that application of 125% RDP + PSB recorded available potassium in soil after harvest of chickpea (381.07 kg ha-1). Available potassium increased in soil with increasing phosphorus level with PSB. Similar findings were also reported by Kumar et al*.,* [17] and Jahangir et al., [16].

3.2 Effect of Phosphates Solubilizing Microorganism and Phosphorus Levels on Nutrient Uptake by Chickpea

3.2.1 N uptake

The data pertaining to effect of phosphates solubilizing microorganism on N uptake by chickpea presented in (Table 4). The significantly maximum N uptake (58.65 kg ha-1) was recorded with seed inoculation with *Aspergillus awamori* as compare to *Aspergillus niger* (55.81 kg ha-1) and Bacillus megaterium (55.21 kg ha⁻¹). While minimum total N uptake was noticed in control. The data on effect of different phosphorus levels on chickpea shows that application of 75 kg P_2O_5 ha⁻¹ recorded significantly higher N uptake (60.90 kg ha⁻¹) against the application of 60 kg P₂O₅ ha⁻¹ $(56.41 \text{ kg} \text{ ha}^{-1})$ and 45 kg P₂O₅ ha⁻¹ (54.97 kg ha-1). Whereas, minimum N uptake was recorded in control (51.99 kg ha-1).

The combined effect of different phosphate solubilizing microorganism and phosphorus levels shows significant effect on N uptake. The interactive effect of seed inoculation with
Aspergillus awamori and application of Aspergillus awamori and phosphorus $@$ 75 kg P_2O_5 ha⁻¹ found significant

on uptake of N as compare to rest of interactions. This may be attributed to synergistic effect of microorganisms and phosphorus levels on increase in the availability of N in soil which helps to enhance vegetative growth of plants thereby increase in N uptake by chickpea. Similarly increase in N uptake owing to significant increase in seed and straw yield. Laharia et al., [19] stated that N uptake by chickpea was significantly influenced with increasing levels of P along with PSB over control.

3.2.2 P uptake

The data pertaining to effect of phosphate solubilizing microorganism on P uptake by chickpea presented in (Table 5). The significantly maximum P uptake (26.23 kg ha-1) was recorded with seed inoculation with *Aspergillus awamori* as compare to *Aspergillus niger* (23.17 kg ha-1) and *Bacillus megaterium* (18.86 kg ha⁻¹) while low total P uptake was found in control. Application of different phosphorus levels influenced significantly on P uptake by chickpea. Among the different P levels application of phosphorus $@75$ kg P_2O_5 ha⁻¹ recorded significantly higher P uptake $(22.21 \text{ kg} \text{ ha}^{-1})$ as compare to application of phosphorus @ 60 kg P₂O₅ ha⁻¹ (21.06 kg ha⁻¹) and 45 kg P₂O₅ ha⁻¹ (54.97 kg ha-1).Whereas minimum value of P uptake was recorded in control (19.23 kg ha⁻¹).

The combined effect of different phosphate solubilizing microorganism and phosphorus levels shows significant effect on P uptake. The effect of seed inoculation of *Aspergillus awamori* and application of phosphorus $@$ 75 kg P₂O₅ ha⁻¹ found significant on P uptake. This relates to synergistic effect between P and phosphate solubilizing microorganisms thereby higher biomass production as well due to more plant

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accessible by phosphate solubilizing microorganism from native and applied phosphorus. Vidhyashree et al*.,* [20] reported that *Aspergillus awamori* and *Aspergillus niger* increase the growth parameters of plant due to higher phosphatase activity in the rhizosphere and production of organic acid might have solubilized the insoluble and native phosphate and brought into soluble form. This type of results was also reported by Paratey and Wani [21].

3.2.3 K uptake

The data presented in Table 6 indicated that the total K uptake of chickpea increased due to application of different phosphate solubilizing microorganisms. The maximum K uptake (60.24 kg ha-1) was recorded with seed inoculation with *Aspergillus awamori* as compare to *Aspergillus niger* (59.20 kg ha-1) and *Bacillus megaterium* (55.94 kg ha-1) while low total K uptake was found in control. It is found that the application of different phosphate solubilizing microorganisms not reached upto the mark of significance but recorded maximum value of K uptake as compared to control. The data revealed that among the different P levels application of phosphorus $@$ 75 kg P₂O₅ ha⁻¹ recorded higher K uptake (59.63 kg ha-1) as compare to application of phosphorus $@$ 60 kg P_2O_5 ha⁻¹ $(58.63 \text{ kg} \text{ ha}^{-1})$ and 45 kg P₂O₅ ha⁻¹ (57.96 kg ha-1). While, low uptake of P was recorded in control (57.11 kg ha-1).

The combined effect of different phosphate solubilizing microorganism and phosphorus levels shows non-significant effect on K uptake. The higher value of K uptake due to application of phosphate solubilizing microorganism and phosphorus levels might be due to fact that more microbial activity in the rhizosphere and addition of FYM at the time of sowing of chickpea increase the available K in soil ultimately increase the uptake of nutrients [19]. P favors to better root proliferation in soil which helps to absorb more nutrient from soil and transported to plant thereby increase in uptake of K [22]. Similar findings also reported by Biswas et al., [23].

Key : PSM = phosphate solubilizing microorganism

3.3 Effect of Phosphate Solubilizing Microorganisms and Phosphorus Levels on Yield and Yield Attributes of Chickpea

3.3.1 Seed yield

The seed yield of chickpea as influenced by phosphate solubilizing microorganism narrated in Table 7. The seed inoculation with *Aspergillus awamori* produced higher seed yield (1502.68 kg ha-1) as compared to application of *Aspergillus niger* (1406.6 kg ha-1) and *Bacillus megaterium* (1344.53 kg ha-1). Significant improvement in yield was noticed with the application different phosphorus levels. Among the different phosphorus levels application of phosphorus @ 75 kg P_2O_5 ha⁻¹ recorded higher seed yield (1451.0 kg ha-1) as compare to application of phosphorus @ 60 kg P₂O₅ ha⁻¹ (1403.25 kg ha⁻¹) and 45 kg P_2O_5 ha⁻¹ (1346.18 kg ha⁻¹). While, minimum seed yield was recorded in control (1322.77 kg ha-1). Interaction between phosphate solubilizing microorganisms and phosphorus levels was found to be significant. The seed

inoculation with *Aspergillus awamori* and application of phosphorus $@$ 75 kg P₂O₅ ha⁻¹ recorded highest value of seed yield than rest of the interactions. This may attributed *Aspergillus awamori* increase more values of growth parameters at almost all growth stages and helped in reducing P fixation by its chelating effect and also solubilized the unavailable form of P leading to more uptake of nutrients resulted in better growth of the plant [24]. The increase in seed yield due to increase in P level may be attributed to increase in the availability of P in soil. Similar findings are noted by Nawange et al*.* [25].

3.3.2 Straw Yield

It is evidenced from the data presented in Table 8. The straw yield of chickpea as significantly influenced by phosphate solubilizing microorganism, The seed inoculation with *Aspergillus awamori* produced maximum straw yield of chickpea (1803.2 kg ha-1) as compared to application of *Aspergillus niger* (1688.0 kg ha-¹) and *Bacillus megaterium* (1613.4 kg ha⁻¹). Among the different phosphorus levels

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application of phosphorus ω 75 kg P₂O₅ ha⁻¹ recorded higher straw yield (1741.19 kg ha-1) as compare to application of phosphorus @ 60 kg P2O5 ha⁻¹ (1683.90 kg ha⁻¹) and 45 kg P2O5 ha⁻ 1 (1615.41 kg ha -1). However, the minimum straw yield was recorded in control (1587.32 kg ha⁻¹).

Interaction between phosphate solubilizing microorganisms and different phosphorus levels on straw yield was found to be significant. The seed inoculation with *Aspergillus awamori* and application of phosphorus $@$ 75 kg P_2O_5 ha⁻¹ recorded higher value of straw yield than rest of the interactions. This was mainly due to the fact that *Aspergillus awamori* and application of phosphorus $@$ 75 kg P_2O_5 ha⁻¹ increase in the availability of N and P caused better root development, better growth and development of plants and better diversion of photosynthates towards sink Tagore et al*.,* [26]. Kumar et al*.,* [17] reported that the straw yield of chickpea increased due to increase in phosphorus levels might be because of increase in the microbial activity in the root environment which accelerates cell division and formation of meristem.

4. CONCLUSION

It can be inferred and concluded that, incorporation of phosphate solubilizing microorganisms *viz. Aspergillius awamori* in combination with application of phosphorus @ 75 kg P_2O_5 ha⁻¹ improved N,P and K availability, uptake and seed yield of chickpea.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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