Research Article

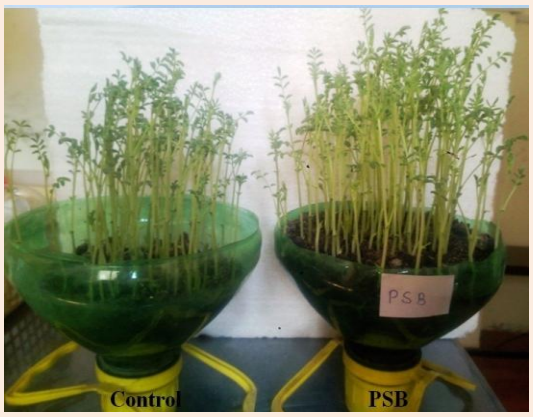
Effect of Phosphate Solubilizing Microbes Isolated from Soil on the Growth of *Cicer Arietinum*

Parul Jain and Dharmendra Singh Khichi

Department of Chemistry, S. V. Polytechnic College, Bhopal (M.P.), India

Abstract

In the present study the effect of PSM on the growth of *Cicer arietinum* was observed. PSM was isolated from soil. Standard methods were used for physicochemical analysis and isolation of PSM respectively and cultured separately in their corresponding selective media. The efficiency of PSM was checked by treating them with seeds of *Cicer arietinum*. In terms of its growth and yield parameters, it was observed that microbial biofertilizer showed maximum growth as compared to control plant. This study concludes that the biofertilizers clearly enhanced shoot biomass and their vigour index.



***Correspondence**
Parul Jain
Email: drparuljainsv@gmail.com

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Introduction

For optimum plant growth, nutrients must be available in sufficient and balanced quantities. Soils contain natural reserves of plant nutrients, but these reserves are largely in forms unavailable to plants, and only a minor portion is released each year through biological activity or chemical processes [1]. Phosphorus (P) is second only to nitrogen as most essential macro-nutrient required by plants [2]. It is a key nutrient for higher and sustained agriculture productivity which limiting plant growth in many soils. However, Phosphorus deficiency is the most common nutritional stress in many regions of the world, affecting 42% of the cultivated land over the world. Phosphorus, the master key element is known to be involved in a plethora of functions in the plant growth and metabolism. It's deficiency results in the leaves turning brown accompanied by small leaves, weak stem and slow development [3].

Application of biofertilizers provides effective implementation of biological mechanisms of plant nutrition, growth promotion and protection. These are important arguments for the use of biofertilizers as prospective elements for nutrient management in organic agriculture. Low cost and safety for the environment make fertilizers advantageous as an alternative to mineral fertilizers [4]. They are extremely beneficial in enriching the soil with those micro-organisms, which produce organic nutrients for the soil and help to combat diseases. Biofertilizers are products containing living cells of different types of microorganisms which when, applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen, phosphorus) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock phosphate [5]. Biofertilizers are important components of integrated nutrients management [6]. Both bacterial and fungal strains exhibiting P solubilizing activity are detected by the formation of clear halo (a sign of solubilization) around their colonies. Production of a halo on a solid agar medium should not be considered the sole test for P solubilization [7].

Chickpea (*Cicer arietinum*) is the most important staple food in several developing countries and chemical fertilizers are the most important input required for chickpea cultivation. In order to make its cultivation sustainable and less dependent on chemical fertilizers, it is important to know now to use PGPR that can biologically fix nitrogen, solubilize phosphorus and induce some substances like indole acetic acid (IAA) that could contribute to the improvement of chickpea growth. Thus the aim of this study was to determine the effect of phosphate solubilizing microbes for improvement of germination and plant Growth of chickpea (*Cicer arietinum*) [8].

Materials and Method

Collection of samples and Isolation of phosphate solubilizing microorganism:

For the isolation of PSM soil samples were collected from different forest area around Bhopal district. Collected soil samples were analyzed for determination of their physicochemical properties and quantity of available phosphate. Phosphate solubilizing microorganism isolated from soil that contain high quantity of phosphorus. The soil samples were serially diluted upto 10-7 dilution. 0.1 ml suspension was spread on Pikovskaya's medium and incubated at 28◦ C for 7 days.

Phosphate solubilizing efficiency of the microorganisms was calculated based on the holozones produced around the colonies on pikovskaya medium. The pH of the growth medium was determined at regular intervals by using digital pH meter [9].

Experiment design and inoculums treatment of seed:

The field experiment was conducted during November - December in pots at laboratory. The soil sample of pots was sandy loam and alkaline (pH - 8.2). The healthy and best quality of *Cicer arietinum* seeds was taken. Isolated phosphate solubilizing inoculant was mixed with small quantity of water and treated with seed. In case of seed dipping inoculums suspension was prepared in the ratio of 1:10. The seeds were dipped in suspension and kept immersed for about 12 hours. The seeds were taken out from the suspension and dried on air. The remaining suspension of slurry was directly sprayed on the pot. Control pot of experiment used without treatment of inoculants.

The effect of microorganisms on growth *Cicer arietinum:*

The plant height, stem diameter and dry weight (at successive developmental stage), was measured from the base of the plant at ground level to the point of the attachment of the capitulam after 8 days of cultivation. The root was absorbing nutrients, minerals from the soil. Based on the nutrients uptake, the plant growth and yield parameters were varied. The root length was measuring from tip of the root and then mean and standard deviation were calculated.

Statistical analyses

Growth and yield parameters were measured (plant weight, shoot length, root length, plant dry weight.) and analyzed by statistics (Mean, and standard deviation of least significant differences were calculated.)

Results and Discussion:

The diversified investigations about different types of soil collected from various locations are reported in **Table 1**. The Physico-Chemical values of the soil samples were calculated. The result indicates that the quality of soil considerably varies from location to location and sample to sample. S2 sample was show best results as compared to others. Due to the best physicochemical properties and high phosphate content S2 soil sample further used for the isolation of PSM.

Table 1 Physico-chemical analysis of collected soil samples

Parameters	Soil Samples			
	S1	**S2**	**S3**	**S4**
pH	7.6	8.4	5.8	6.6
EC (ds/m)	.39	.30	.41	.48
Moisture %	25	35	28	19
Soil colour	Brown	Black	Shadow black	yellow
Nitrogen (Kg/hac.)	172	215	119	194
Phosphorus (Kg/hac.)	32	48	27	21
Organic Carbon %	49	65	52	44

Out of four Samples, only one soil sample was used for the isolation of PSM. In this sample isolate exhibiting halozone was found showing the capability of P solubilization was obtained from S2 sample of 10^{-7} dilution. It has been reported that organisms capable of doing phosphate solubilization give clear zone around the colony. PSM colony gave clear zone by which it was confer that they can solubilize phosphate **Figure 1**.

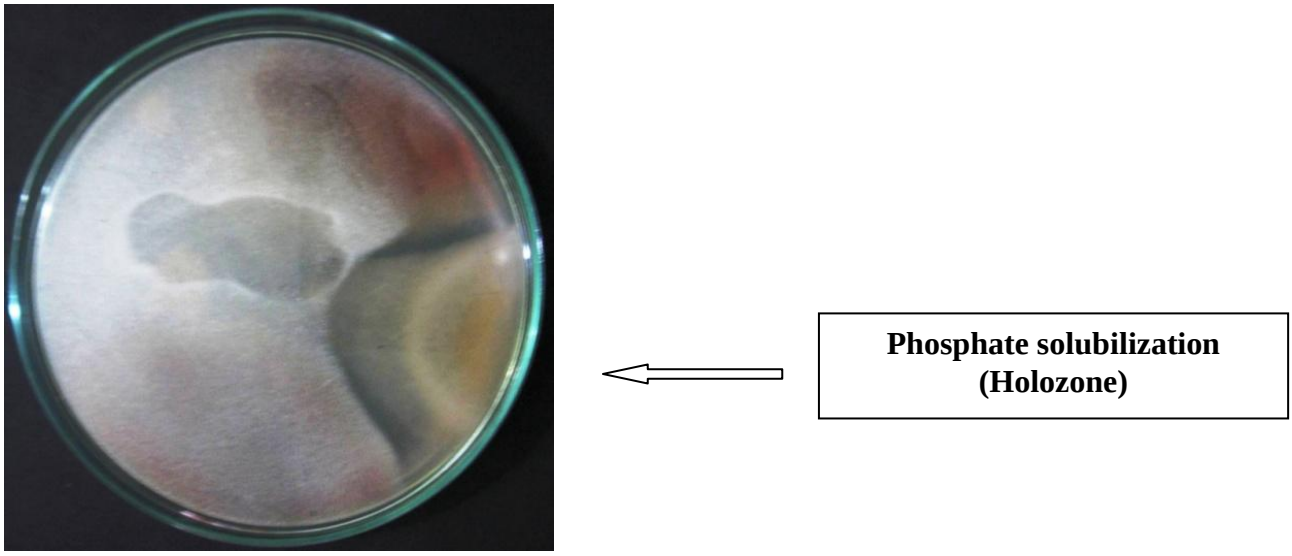


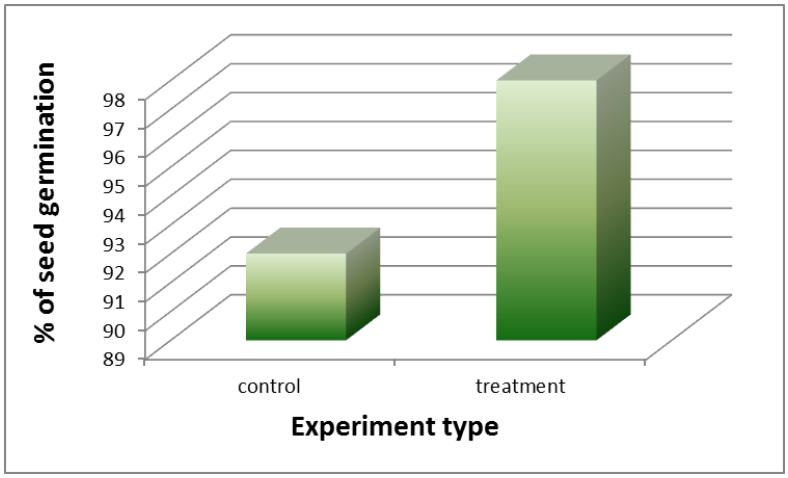
Figure 1 PSM exhibiting holozone of P solubilization

The results of the present study found that application of isolated PSM influences the growth of *Cicer arietinum* plants. Considering the impact of PSM fertilizers on *Cicer arietinum* plant, one of the major growth factor is soil pH, which was decreased to 8 from 8.4 after the one month treatment of inoculum [10]. The soil pH was changed from 8.0 to 7.6 due to the biofertilizer treatment. In the present study, the initial pH of the *Cicer arietinum* soil was 8.4 but after the application of control and biofertilizer (test), the control retained the pH as 7.4 but the biofertilizer treated soil pH turned in to 5.3 **(Table 2)** this may be due to the biofertilizer interaction with cheakpea plant and due to acid production by PSM. Results show that the effect of soil amendments was significant for all roots and shoots growth parameters at the three growth stages (**Graph 3 and 4**). In general, plant growth was better in PSB treated Pot. The PSB treated *Cicer arietinum* plants were showed better germination than the control (**Graph 1**). The root length was gradually increased in treated plants and the minimum level was observed in control (**Figure 3**). The shoot length was recorded and maximum levels were observed in PSB treated plants (**Graph 3 and Figure 2**). In general, PSB inoculated plants showed increased seed germination, Number of shoots, Shoot length (cm), Plant height (cm), Root length (cm), Number of nodules when compared to uninoculated control plants. All the inoculated plants showed

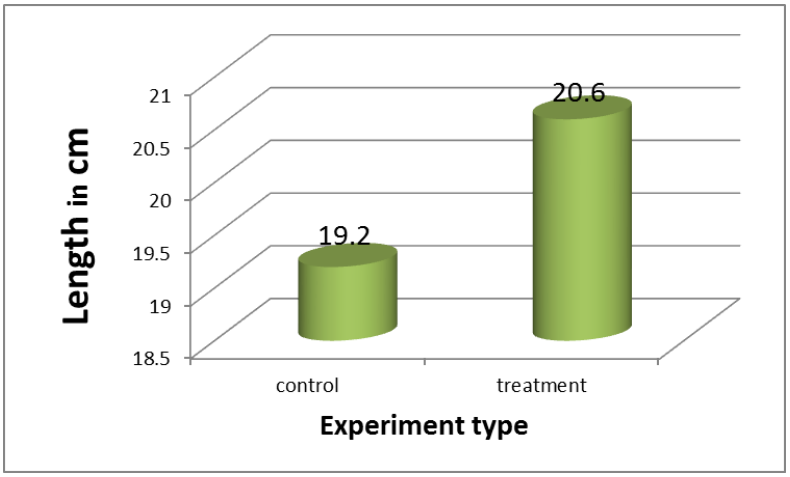
better growth and higher Vigour index than the uninoculated plants (**Table 1**). Similar observations have been made in plant growth promotion and suppression of pests and diseases in other agricultural crops [11, 12, 13]. Soil microorganisms play an important role in soil biogeochemical processes which determine plant productivity and successful functioning of introduced microbial bioinoculants and their influence on soil health. Exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behavior in soil habitats [14, 15].

Table 2 Plant ecology and growth Pattern of *Cicer arietinum* after 8 days of inoculation

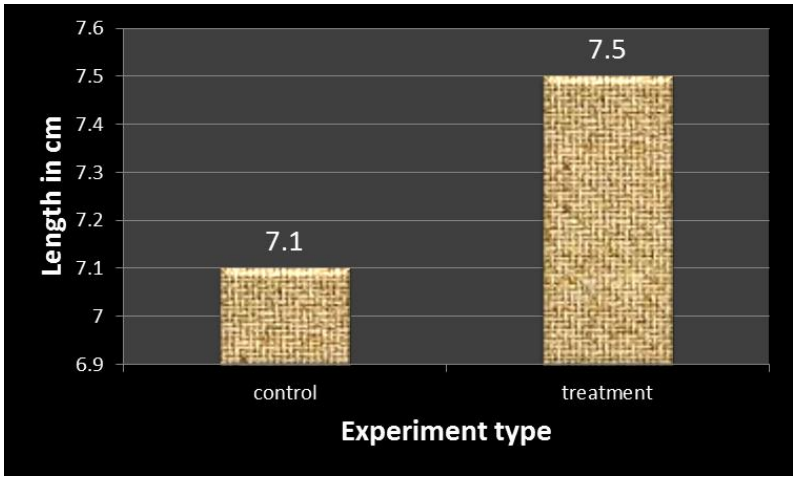
S. no.	Experiment design	Plant dry weight	Vigour index	Soil pH	Available p (Kg/Hac.)	Stem strength	Root Hairs	Nodulation
1	Control	3.46±0.7	2217. 2	6.4	10.6±0.7	Weak and thin	few root hairs	No
2	PSB	6.2±0.98	2606.8	5.3	31.3±4.2	Strong and thick	Multiple root hairs	yes



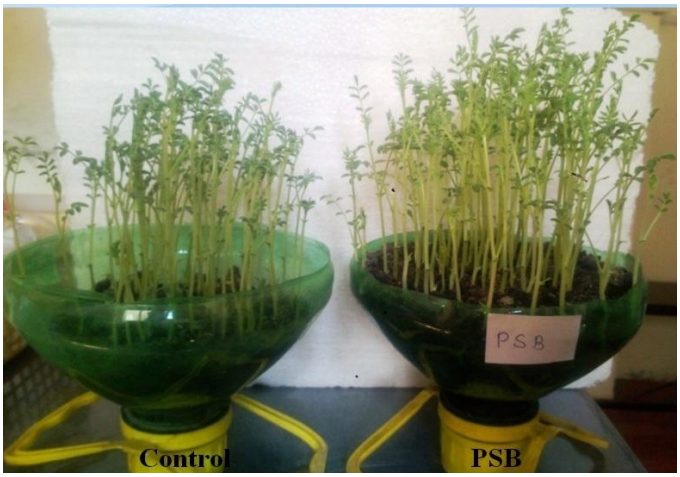
Graph 1 Comparision of seed germination of control and test pot



Graph 2 Comparison of shoot length with control and test Plant



Graph 3 Comparison of root length with control and test Plant



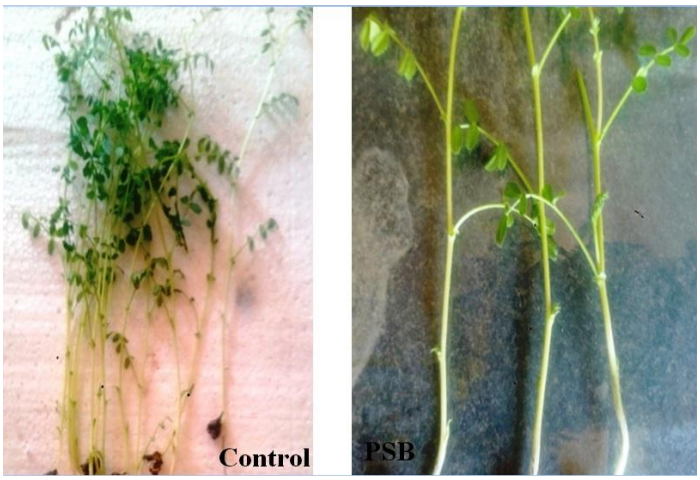


Figure 2 Shoots length & stems thickness of control and test plant

Figure 3 Comparison of roots of control and test plants

The microorganisms have enormous potential in providing soil P for plant growth. Use of PSMs can increase crop yields up to 70 percent [16]. The phosphate solubilizing bacteria as inoculants simultaneously increases P uptake by the plant and crop yield. The PSB exhibiting multiple plant growth promoting traits on soil-plant system is needed to uncover their efficacy as effective bio inoculants. The inoculation of PSB and plant growth-promoting rhizobacteria (PGPR) together could reduce 50% of P fertilizer application without any significant decrease of crop yield [17]. Combined inoculation of arbuscular mycorrhiza and PSBs give better uptake of both native P from the soil and P coming from the phosphatic rock and enhance plant growth by solublizing P from different fractions of soil [18]. The PSBs are able to synthesize phyto hormones like Indole acetic acid (IAA), Gibberellic acid (GA_3) and siderophore [19]. PSBs are also enhances plant growth by increasing the efficiency of biological nitrogen fixation or enhancing the availability of other trace elements such as iron, zinc, etc. These Bacteria enhance the growth and grain yield of different plants as reported in Zea mays [20] and wheat [21]. Moreover, PSB and NPK had a positive effect on germination and seed quality of some plants like radish that directly improved vigour index [22].

Conclusion:

It is concluded that plant height and biological yield have been affected significantly by PSM inoculation because this biofertilizer can enhance phosphate solubilization for plant. Thus, it can be said that PSM biofertilizer is suitable for obtaining maximum growth of plants. In the present investigation, even though the experimental plant belongs to a legume crop, PSM influenced much for the maximum growth and yield than the other treatments which may be due to the efficient interaction between them. In most parameters, the biofertilizer were higher than control or nearly equal. The high growth of root and shoot was obtained in the presence of PSM as compared to control yield. The length was also higher in shoot and root of plant inoculated with PSM. From the above results it is concluded that potentials of PSM could be effectively exploited in the future for the production of eco-friendly phosphate solubilizing biofertilizer for sustainable agriculture.

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