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Effect of *Pseudomonas fluorescens* on various Growth Parameters of Lentil (*Lens* sp.): A Healthy Nutrition for Rural India

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ABSTRACT: Dal (Pulse) is one of the most important part of our diet and also a chief source of protein in the rural areas. The present study was done with the main objective of enhancing the cultivation and growth of Lentil (*Lens* sp.), an important pulse belongs to fabaceae and one of the chief source important proteins. The whole experiment was design and done in pots. *Pseudomonas fluorescens* was selected on the basis of its plant growth promoting activity by dissolve the nutrients into their organic form. The results were observed in 0, 10 and 25 days, reflected in comparison to control, treatments with used plant growth promoting bacterium (PGPB) ultimately increases root, shoot, total lengths and no. of leaves in plant. Moreover, such improved results have been found in the measurement taken after 10 days and these more enhancement after 25 days respectively. Furthermore, it can be concluded that use of *P. fluorescens* can enhance growth and development of Lentil to meet the current demands of protein in rural as well as urban India.

Keywords: *Lentil*, PGPB, Pulse, medicinal etc.

I. INTRODUCTION

There are 3 basic needs of human beings since prehistoric periods: bread, clothing and shelter. These requirements were fulfilled during the 1900 when the population was in control. But as the population increased with urbanization, people felt more requirements other than basic needs. Most of the rural population is vegetarian and greatly depends pulses and vegetable to fulfill the daily need of proteins. India is one of the immense producer and consumer of several pulses. Now days there is great demand of pulses are accomplished by imports because of fix arable lands. Pulses are one of the chief food materials and can serve as a good source of dietary protein constituents for human consumption; it is rich source of protein in our diet. Pulses intake fulfill the protein requirement specially those people who does not depend on animal or fish sources. Various types of pulses are used in our diet [1]. The current requirements will be achieved by maximum production with improving the agriculture practices specially soil. The aforesaid conditions can be attained by one group of rhizospheric bacteria called plant growth promoting rhizobacteria (PGPR). These microorganisms improves the soil quality and helps in utmost production in complex mechanisms [2].

These rhizospheric microbes are increasing the quality and quantity of crop plants [3] which have been found to be modified by various farming practices [4]. Some important PGPRs are *Pseudomonas fluorescens*, *Bacillus subtilis*, *Pseudomonas putida, Paenibacillus elgii* and *Paenibacillus polymyxa* etc [5].

These reported bio-inoculants diversely improve the crop yield [6, 7] through mechanisms such as establishment of endophytic populations [8, 9] on interactions to root interior for the benefits and better adaptations with the host plants.

Lentil (Lens sp.)-
Classification/taxonomical position:
Kingdom: Plantae
Class: Dicotyledons
Sub class: Polypetalae
Order: Rosales
Family: Fabaceae
Genus: *Lens* sp.

Fig. 1. *Lens sp.* L.

In Indian sub continent, it is mostly consumed as 'Dal'. Whole pulse grain as 'dal' and snack preparation and soup preparation is also served, in restaurant of mega cities. It is easily digestible with high biological value, hence, also referred to patients also. Dry leaves, stems, empty pods and broken pods are used as valuable cattle feed. This is the only crop among the pulses which provides highest natural nitrogen fixation in the range of 60-147 kg /ha besides 30-35 quintals of crop residues.

II. MATERIALS AND METHODS

A. Soil sampling and site of Experiment conduction

Soil samples for these experiments were collected randomly from the ROXBURGH GARDEN, Department of Botany, University of Allahabad by digging depth of about 5 cm in pots. All experiments were conducted in the Biological Product Lab. The study was done from January 2015 to March 2015.

B. Procurement of bacterial culture, plant seeds and inoculum preparation

The selected culture, *Pseudomonas fluorescens* was procured from Microbial Type Culture Collection (MTCC-9768), Chandigarh, India and the plant *Lens* sp. seed from Gullu Mal Girdhari lal, Gumat Bajar, Jammu, J&K, India. Seeds were surface sterilized with 0.02% (w/v) $HgCl_2$ and washed three to five times with sterile distilled water to remove traces of $HgCl_2$. Inoculum was prepared according to CLSI recommendations [10].

C. Indole acetic acid (IAA) production

IAA was measured by inoculating the 24hrs old culture in pre-sterilized Peptone broth containing 1 % of trypton for 48 hrs at 37°C. Then after, 1 ml of Kovac's reagent was mixed in the culture tube of *P. fluorescens* and shake after 15 minutes. The appearance of red ring at the top confirmed the indication of IAA.

D. Phosphate solubilisation Test

P solubilising activity was observed in Pikovskaya plates [11]. The components of aforesaid medium (in gms/ltr) with 0.5 yeast extract, 10.00 dextrose, 5.00 calcium phosphate, 0.5 ammonium sulphate, 0.2 potassium chloride, 0.1 magnesium sulphate, 0.0001 manganese sulphate, 0.0001 ferrous sulphate, 15.00 agar were taken in 1000 ml deionised water. Freshly prepared medium was autoclaved at 121°C and 15 lbs pressure for 15 minutes. Then, streaked plates were kept in BOD at temp of 35±2 for 24 hrs.

E. Experimental setup

A pot study was designed for the present study. The seeds were washed with detergent and sown in pots which were already filled with autoclaved soil, according to the agronomic practices to determine the effect of *P. fluorescens* on *Lens* sp. The dose of *P. fluorescens* was 50 ml per pods. The seeds were sown directly on seed beds and placed in dark place at room temperature. After germination of seeds the treatment of *P. fluorescens* was given as a proper dose and then put the pods in the light as a ratio of 16:8 hrs. The observations were made in three parts such as same day of sowing, after 10 days and finally after 25 days. The parameters of the observation which were taken: Shoot length, Root length, total length, and no of leaves. Readings were also taken in triplicates.

III. RESULTS AND DISCUSSION

A. Phosphate Solubilizing activity

The *P. fluorescens* was found to be a very good phosphate solublizer as shown in the Fig. 2 (A-B). The clear growth rings are seen in the medium plate because of the efficiency of *P. fluorescens* to insoluble inorganic phosphorus into the solubilized form of phosphate which can be used by the plant. Phosphorus (P) is one of the most important macronutrient present in the soil which is required for the better growth and development of plants [11]. Its least availability also makes it significant when compared to nitrogen which is most abundant in environment.

The zone of clearance is considered as a result of release of some low molecular weight organic acids by the experimented strain. Lactic acid is produced during the solubilization process of several inorganic phosphates by efficient phosphate solubilizer *i.e. Azotobacter rhizospherae* and *P. putida* [1].

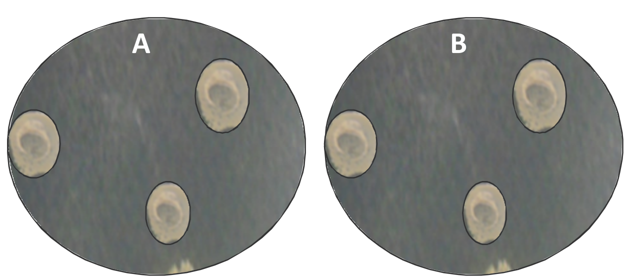

Fig. 2. The phosphate solubilization activity of *P. fluorescens* on Pikovskaya agar plates (A-B).

Phosphorus which is very important for the plant growth as it is taken as a macro-nutrient is a known fact. In the soil, heterogeneously distributed microbe communities together with dependent rhizobia and different plant growth promoting rhizobacteria perform a dynamic role in plant nutrition by remodeling nutrients in soils that is useful to plant growth through a method referred to as biogeochemical athletics, and directly transporting these nutrients to plants. Soil testing is one of the most important instruments for analysis of macro and micro nutrient. The odd availability of nutrients also makes it interesting [1].

B. Indole acetic acid production

The *P. fluorescens* was found to be a very good source of IAA production (Table 1). The availability of precursors and uptake of microbial IAA by plants are the two main factors that affect the ability of bacteria to produce IAA in the rhizosphere. The causal mechanism of growth promotion is due to the production of plant growth promoting hormones in the rhizosphere and other plant growth promoting activities.

C. Impact of P. fluorescens on growth parameters of Lentil plant

Lens sp., a highly medicinal plant majorly found in all the areas of India The observations were made as a measurement of 4 growth parameters such as Shoot length, Root length, total length and no. of leaves in the interval of zero days, ten (10) days and twenty five (25) days in the form of triplicates (Fig. 3). In the same day no significant changes were made. New changes were observed after 10 days and found the increased growth in the all parameters as compared to control (Fig. 3). These parameters clearly shows the signal of positive growth effect reflected by the plant after treatment; *lens* sp. is increased due to production of hormones especially auxin [12] (Wu *et al.* 2005) results in increased efficiency of treated plant. The final measurement shows excellent growth and development in each treatment.

Table 1: Biochemical activities of used bacterial culture.

S. No.	Characteristics/Tests	Results
1.	Grams stain	Gram-negative
2.	Shape	Rod
3.	Potassium Solubilization test	+++
4.	IAA Production	+++

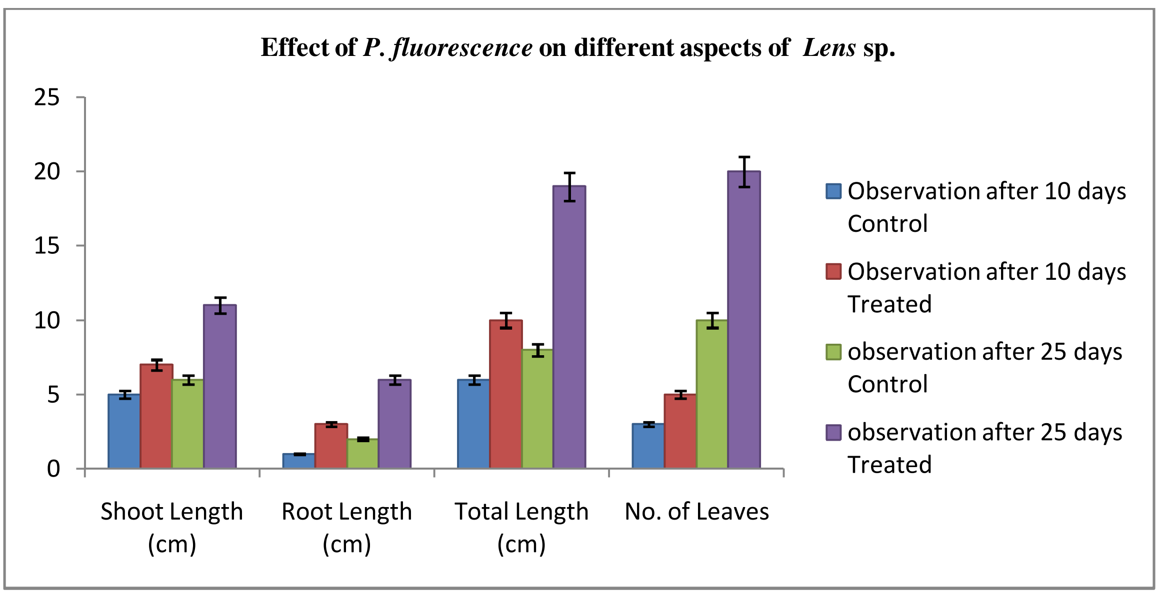

Fig. 3. Impact of *P. fluorescens* on different aspects of *Lens* sp.

A good amount of enhancement was obtained and all the differences were found to be just double as shoot length was found 11 cm but 6 cm in control, root length was 4 cm greater as compared to the controlled one, and so on in case of total length (Fig. 3).

It was also noteworthy that the enhancement in root and shoot length is due to the production of phytohormones [12] as well as more nutrients were absorbed from rhizosphere of root as induced by *P. fluorescens* which is a good P and K solubilizer. Leaves number also increased parallelley with the increased shoot length. This increased growth in shoot length and number of leaves led to the maximum photosynthesis resulting in formation of ample food which is essential for plant growth [13].

IV. CONCLUSION

Lentil is one of the most important pulses growing in the dry regions of the country. In the present study, it was clearly shown the possible ample cultivation by using *P. fluorescence*. The application of aforementioned soil bacterium made effective improvement in the growth of the plant, as a result of excellent plant nutrient uptake. Finally it was found that there was significant change in the growth of treated plant as compared with the controlled ones. Approximately 72.8% increase in the growth of treated plant have been observed; while there was 50 % and 133% growth enhancement in shoot and root length respectively. In the similar manner, it can solve the foremost global problem of environmental pollution and also fetch a premium in the agricultural market.

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