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# PHOSPHATE-SOLUBILIZING BACTERIA AND THEIR IMPACT ON THE GROWTH AND METABOLISM OF PISUM SATIVUM L. IN KODAIKANAL HILLS, TAMIL NADU.

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## ABSTRACT

Compared with the other major nutrients, phosphorus is by far the least mobile and available to plants in most soil conditions. Although phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major or even the prime limiting factor for plant growth. To circumvent phosphorus deficiency, phosphate-solubilising bacteria (PSB) would play an important role in supplying phosphate to plants in a more environmentally-friendly and sustainable manner. The organisms with phosphate-solubilising potential will increase the availability of soluble phosphate and will enhance plant growth. In this present study, the phosphate solubilising microorganisms isolated from different soil samples from Kodaikanal Hills were identified on Pikovskaya agar plates with clear zone around the colonies. From the observation the strain-I and strain-II identified as Psedumonas aureginosa and Bacillus megaterium. Both strains showed higher than 180 (phosphate solubilisation efficiency), and reduced PH from 8.0 to below 6.0 indicates high phosphate solubilising efficiency, increased organic acid production. The influence of this phosphate solubilises on the growth and metabolism of Pea Plants were analysed. According to the results obtained from comparative field trial conducted in Kodaikanal Hills, use of PSB bio fertilizer shows marked difference in soil fertility, better performance in yield and yield related characteristics observed in the field of pea plants. (Pisum sativum L)

**Keywords**: PSB, Pea plants, Pseudomonas aeruginosa, Bacillus megaterium

## 1. INTRODUCTION

Phosphorus is present in the soils both in organic and inorganic forms. Of these, organic forms, as found in humus and other organic materials including decayed plant, animal and microbial tissues, is an important reservoir of immobilized P accounting for about 20–80% of total soil P. (Richardson,1994). Chemical fertilizers, e.g., manufactured water-soluble phosphatic (WSP) fertilizers (Superphosphates) have played a significant role in the green revolution and are commonly recommended to correct phosphorus deficiencies. (Mohammad Saghir Khan et al., 2007). Most of the developing countries, however, import these fertilizers, which are often in limited supply and represent a major outlay for resource-poor farmers. (Ritika Bhattacharjee et al., 2014). Due to insufficient uptake of these chemical fertilizers by plants, they

reach into water bodies through rain water, cause eutrophication in water bodies and affect living beings including growth inhabiting microorganism. (Pratibha Rawat et al., 2020). The excess uses of chemical fertilizers in agriculture are costly and also have various adverse effects on soils as depletion of water holding capacity, soil fertility and disparity in soil nutrients. (Panhwar, Q.A et al.,2017)

Phosphate solubilising microorganisms (PSMs) are organisms that offer an ecologically acceptable mean for converting insoluble phosphate to soluble forms making them available for plants to absorb. (Walpola and M. Yoon 2012). Phosphate solubilising microorganisms can improve the growth and yield of a wide variety of crops. (Chala Dandessa et al., 2018). Inoculating seeds/crops/soil with Phosphate Solubilising Microorganisms (PSM) is a promising strategy to improve world food production without causing any environmental hazard. (Alori, 2017). Pea (Pisum sativum L.) belongs to family Fabaceae. This crop was grown by Greeks and Romans as an important vegetable crop in 11th century. (Khan et al., 2013). It is herbaceous, annual in habit and self-pollinated vegetable crop. The crop is grown for its green pods and seeds. The immature green seeds are consumed fresh, canned or in dehydrated jars and is leading frozen vegetable food. It is one of the most important vegetables in the world and ranks among top ten vegetable crops. (Singh et al., 2006).

Pea is an important vegetable crop of India generally cultivated for its green pods. It is highly nutritive and rich in protein. It is used as a vegetable or in soup, canned frozen or dehydrated besides being cooked as a vegetable. Being a cool season crop, pea performs best in the temperature range of $10^{O}$C to $18^{O}$C. However, ideal temperature for pea cultivation is $10^{O}$C to $30^{O}$C. Pea is generally sown in India during the Rabi season from the beginning of October to mid of November in the plains and from middle of March to end of May in the hills. The aim of our investigation was to isolate phosphate solubilising bacteria on selective medium from the field near Kodaikanal Hills, Dindigul. They were inoculated in the sterile soil containing Tricalcium phosphate. The influence of these phosphate solubilisers on the growth and metabolism of pea plants (Pisum sativum L.) were analysed. (Suresh, K. and Sankaranarayanan,C 2009).

## 2. MATERIALS AND METHODS

### STUDY AREA

The present research work was carried out in Poondi, Koodaikanal, which is situated in Dindigul district of Tamil Nadu, India. The biogeographically province of the Western Ghats covers 16,000 sq.km2 of which about 100,000 from mountains terrain. The Western Ghats traverses the states of Tamil Nadu, Kerala, Karnataka, Goa, Maharastra and Southern Gujarat.

The Palani Hills are an Eastward spur of the Western Ghats, with maximum (East-West) length of 65 km, a maximum width of 40 km (mean with 24 km), and cover area of 2068 km2. The boundaries are the following: Palani taluk in North, Dindigul and Nilakottai taluk in the East, Periyakulam taluk in the South and the West partly in Coimbatore district of Tamil Nadu and partly Kerala State.

The hills run into two clear zones i.e., the upper and lower Palani, separated by the neutral saddle, a ravine running from Palani is the North to Periyakulam in the South. The upper Palani is the western block, is a plateau that has an area about 385 km2 of an averaging altitude by about 2200 m, with bare grasslands interspersed with wooded valleys called Sholas. Palani hills are one of the important global.

## 3. SITE DESCRIPTION

### GEOGRAPHICAL LOCATION

Princess of Hills (Kodaikanal) is situated in the Palani Hills. It was established in the year of 1845 Kodaikanal is a part of the upper Palani Hills of the Western Ghats and is located at 2145 km2 (8.28 sq mi) latitudes and 2133m (6,998 ft) longitudes. The greater Kodaikanal is the TamilNadu state and also the border of the Dindigul district.

Batlagundu is the nearest town of Kodaikanal. Sex ratio is about 51% of male and 49% of female. The literacy rate is 89.50%. The temperature is alternatively changed during the summer and winter season. Average temperature of summer is about 19.8°C (67.6°F) and the winter is about 8.3°C (46.9°F). Kodaikanal was established as a refuge from the high temperature and tropical diseases.

The field study was carried out in Tamil Nadu, India's Kodai Hills. The Kodai Hills experimental site was situated 296 meters above mean sea level (Site 1- 10.187645, 77.323256; Site 2- 10.193142, 77.312963) in terms of geographical regions.

### METEOROLOGICAL DATA OF THE STUDY AREA

The temperatures of the study area ranged from 8 oC to 24 oC. The annual rainfall reaches 1200 mm. The major soil types of the study sites are sandy loam, sandy clay loam soil and sandy clay. Loamy clay and clay soils also occupying in the study sites. The natural vegetation has two main types of vegetation such as ridge forests and sholas.

## EXPERIMENTAL PROTOCOL

The test experiment was conducted using randomized complete block design and it was done with the following treatments.

- Treatment Test for plant (1-5): Sterile soil mixed with water (BLANK)
- Treatment Test for plant (6-10): Sterile soil mixed with Tri Calcium Phosphate and water (CONTROL)
- Treatment Test for plant (11-15): Sterile soil mixed with Tri Calcium Phosphate, Phosphate Solubilising Bacteria and water. (TEST)

## SOIL CHARACTERISTICS

- The colours and textures of the soils were reddish brown and sandy clay loam, respectively. The macro- and micronutrient levels in agricultural soil were found to be greater than in samples of forest soil, indicating that the soil in Kodaikanal is nutrient-rich.

## IRRIGATION

- Total of three irrigations were provided through the cultivation period.
- 1st = 30 Days after sowing (DAS) (Crown root initiation)
- 2nd =60 Days after sowing (DAS) (Tillering stage)
- 3rd = 90 Days after sowing (DAS) (Harvesting stage)

Fertilizer Application

Nitrogen was administered at 120 kg ha-1via three separate doses, half as a baseline and the other half in two equal doses at 30 and 50 days following transplantation. Potassium and phosphorus were administered at a baseline level of 60 kg ha-1.

## AMPLIFICATION OF 16S RRNA

Primers are employed to amplify the 1.5-kb 16S rRNA gene. Amplification of DNA was carried out using the Genamp PCR equipment (Applied Biosystem, USA). The mixture used for the PCR reaction contains 1x PCR buffer, 200 M dNTPs, 1.5 mM MgCl2, 0.1 M primers and 2.5 u of Taq DNA polymerase (Fermentas, USA) in a final volume of 100 liters. The PCR conditions for amplification of a 1.5 kb segment of 16S rRNA were as follows; Preheating at 94 °C for three minute, followed by 36 cycles of 94°C for 2 min, 54°C for 1 minute, and 72°C for 2 minute, with a last extension at 72 °C for a total of seven minute. The technique of verifying amplified DNA involved electrophoresizing aliquots of the PCR result (5 µl) on a 0.8% agarose gel in 0.5% TE buffer, following the instructions provided by (Girden 1992).

## GROWTH PARAMETERS

The purpose of the study was to investigate whether PSB affected the yield and growth of vegetables. The study period, site description, soil and climate conditions of the experimental area, crop or material for planting, treatments, experimental planning and arrangement, the crop growing process, intercultural operations, gathering data and statistical evaluation are all briefly covered in this chapter. Below is a description of the experimental details and procedures.

## ISOLATION OF PHOSPHATE SOLUBILISING BACTERIA FROM FIELD OF PEA PLANTS

Soil samples are collected from the field of pea plants from Kodaikanal Hills, Tamil Nadu. 10g of soil sample is dissolved in the 100 ml of distilled water and the sample is mixed well, and by dilution making the sample 10-1. Then the soil sample in sterilized water is serially diluted up to 10-7 dilution. Then 10-5, 10-6, 10-7 dilution is taken into Spread-plate technique. (Sandhimita Mondal et.al., 2017)

The selective media prepared for isolation and growth of Phosphate Solubilising Bacteria had the following constituents. (Chen, Y. P et.al., 2006) Yeast extract, Dextrose, Tricalcium Phosphate, Ammonium Sulphate, Potassium Chloride, Magnesium Sulphate, Manganese Sulphate and Ferrous Sulphate, Agar and Distilled water. (Walpola, B.C et.al., 2012). The media was sterilized by autoclaving at 1210celsius for 15 minutes. The media was poured into petri plates and allowed to solidify. The soil solution of about 0.1 ml was spread on to the plate by Spread-plate technique. The plates were incubated for 24 hours at 370 Celsius. (Walpola and Yoon 2013). After incubation for 24 hours, the plates were taken out and growth of microorganisms was seen on the plates. Plates of dilutions 10-2, 10-3, 10-4, 10-6, and 10-7 were chosen for further screening and formation of halo zone around the selected colonies. (Girmay Kalayu, 2019)

## IDENTIFICATION AND CHARACTERIZATION OF THE BACTERIAL ISOLATES

All the selected isolates were examined for the colony morphology, cell shape, colony type, motility, pH, and Halo zone test.

## FIELD TRIAL OF THE PSB ON PEA PLANTS: (PISUM SATIVUM L.)

A comparative field trial was carried out on the field of pea plants on Kodaikanal Hills, Dindigul, Tamil Nadu.

## TRIAL-I: (CONTROL)

In this field of pea plants, 10 kg of pea plant seeds were grown normally without using PSB.

## TRIAL-II: (PSB)

In this field of pea plants, 200 g of PSB were used for 10 kg of pea plant seeds. PSB were sown into the fields of pea plant. (Soil Application) (Anonymous, 2010).

## MEASUREMENT OF PLANT GROWTH AND METABOLIC PARAMETERS

The following parameters affecting plant growth and metabolism were measured after few days of growth. Shoot length, Root length, Number of pods per plant, Number of seeds per plant, Pod yield per plant, Weight of seeds per plant, Fresh plant weight, Dry plant weight, Days to first flowering, Days to first pod picking, and Shelling percentage were measured for plant growth and chlorophyll content and protein content were measured by using plant leaf extract for measuring plant metabolism.

# 4. RESULTS AND DISCUSSION

In this present study, the phosphate solubilising microorganisms isolated from different soil samples from Kodaikanal Hills were identified on Pikovskaya agar plates with clear zone around the colonies. (Krishnananda Pralhad Ingle 2017).Two bacterial isolated strains showed formation of solubilisation halo in Pikovskaya medium. (10-4 & 10-6 dilution). They were undergone morphological, macroscopic and microscopic observation. They revealed that, 10-4 dilution (strain-I) was Rod shaped, Opaque, Motile, Bluish green, Flat colonies and 10-6 dilution (strain-II) was Rod shaped, Opaque, Motile, Milk white, Concave and smooth colonies. Their gram reaction revealed that strain-I (10-4 dilution) was gram negative and strain-II (10-6 dilution) was gram positive.

From the observation the strain-I and strain-II identified as Psedumonas aureginosa and Bacillus megaterium. Their pH test showed that both were above 8.0, after incubation 5-7 days, their pH decreased for 5.3, 5.5 for strain-I and strain-II respectively, indicates increased organic acid production. Their phosphate solubilisation efficiency was 185, 190 for strain-I and strain-II. Inoculation of strains with soil showed increased solubilisation of fixed soil phosphorous which will results in increased crop yield. (Youssef M.M.A1 et al., 2014) Both strains showed higher than 180 phosphate solubilisation efficiency, indicates high phosphate solubilising efficiency.

A comparative field trail was carried out on the field of pea plants, cultivated on Kodaikanal Hills, using trial-I as control (without PSB) and trail-II as PSB (with PSB) with three replications. In pea plants, maximum pod length, number of pods, weight of seeds, pod yield, shelling percentage, ascorbic acid and total soluble solids were recorded with and without application of PSB and labelled Control and PSB as Trial-I and Trial-II respectively.

Pod length is directly correlated with the yield. Long pods have more number of seeds and give more yields and consumer also prefers long pods. (Saharan et al., 2011) Increased root and shoot length and increased weight of pea plants, may be due to greater availability of nitrogen and phosphorus which leads to more vegetative growth. (Khan, K. S 2009) The more vegetative growth may be due to inoculation in pea rhizosphere through soil application probably through soil application induced more amount of nitrogen and phosphorus fixation in nodules of pea leading to solubilisation of fixed nitrogen and phosphorus from non- available to exchangeable pool which imparts more vegetative growth (De et al., 2006); (Awal et al., 2016).

Number of pods is directly correlated with yield. It is a major yield contributing character as more the number of pods more will be the yield. Maximum number of pods per plant was observed in saw dust due to maximum soil moisture conservation, nutrient uptake, water holding capacity and increased aeration of soil (Khan et al., 2013). These results are in conformity with the findings of (Awal et al.,2016). Maximum number of pods, seeds and weight of seeds is a parameter which determines yield of plant. The increase in weight of seeds/plant may be due to the mulch cover which increased soil water storage (Awal et al., 2016). The aim of growing crop is to have maximum yield for better returns Another possible reason for the increase in yield can be related to increase in rate of photosynthesis, enhanced rate of protein accumulation, resulting in better flowering (60 days), fruiting and pod formation (90 days). (Khan et al., 2013); (Qureshi et al., 2015).

## MOLECULAR IDENTIFICATION OF PHOSPHATE SOLUBILISING BACTERIAL STRAINS GENOMIC DNA ISOLATION

The genomic data was collected from colonies with solubilisation capacity. Isolated DNA was stored in eppendorf tubes at -20 °C for up to six months for future analysis. The DNA was of sufficient quality to proceed with PCR.

## BACTERIAL IDENTIFICATION USING 16S RRNA SEQUENCES PCR AMPLIFICATION

The results of DNA sequencing were obtained and to confirm Pseudomonas putida the sequences were compared with BLASTn analysis for 16S rRNA gene homology along with sequences from type strains retrieved from Ez Taxon- database 122. The nucleotide sequences were deposited in NCBI Genbank database under the accession numbers PP275108.1.
Amplification of 16s rRNA gene was carried out for the samples using universal primers 27 F: AGAGTTTGATCMTGGCTCAG and 1492 R: TACGGYTACCTTGTTACGACTT were used for amplification. Expected band was amplified in all the samples. Polymerase Chain Reaction (PCR)-generated amplicon was confirmed and purified using (Thermo Scientific, EU-Lithuania) Gene JET PCR purification kit.
Shelling percentage is an important character in pea which determines the yield of pea plants. (Deepa Sharma et.al.2020) More the length of pods more will be the number of seeds per pods and ultimately shelling percentage is higher. (Table I-V, Fig 1-4)

**Table 1: Bacteriological Profile of Isolated PSB Strain**

| S.no | Isolate characterization | Results | |
|---|---|---|---|
| | | **Strain-I ($10^{-4}$ dilution)** | **Strain-II ($10^{-6}$ dilution)** |
| 1. | Gram's reaction | Gram negative | Gram positive |
| 2. | Shape | Rod shaped | Rod shaped |
| 3. | Size | Flat colonies | Concave and smooth colonies |
| 4. | Opacity | Opaque | Opaque |
| 5. | pH | 5.3 | 5.5 |
| 6. | Colour | Bluish green | Milk white |
| 7. | Motility | Motile | Motile |
| 8. | Phosphate Solubilising index (PSI) | 185 | 190 |

**Table 2: Influence of PSB on Growth of Pea plants**

| S.No. | Shoot length (cm) | | Root length (cm) | | Number of pods / plant | | Number of seeds / plant | |
|---|---|---|---|---|---|---|---|---|
| | Trial-I (Control) | Trial-II (PSB) | Trial-I (Control) | Trial-II (PSB) | Trial-I (Control) | Trial-II (PSB) | Trial-I (Control) | Trial-II (PSB) |
| 1. | 67.056 | 91.44 | 5.334 | 9.398 | 10 | 14 | 11 | 12 |
| 2. | 64.008 | 88.392 | 5.842 | 8.128 | 8 | 12 | 8 | 10 |
| 3. | 60.96 | 82.296 | 5.08 | 7.62 | 6 | 10 | 7 | 9 |

**Table 3: Influence of PSB on Yield of Pea plants**

| S.No. | Pod length (cm) | | Pod yield /plant (g) | | Fresh plant weight (g) | | Dry plant weight (g) | |
|---|---|---|---|---|---|---|---|---|
| | Trial-I (Control) | Trial-II (PSB) | Trial-I (Control) | Trial-II (PSB) | Trial-I (Control) | Trial-II (PSB) | Trial-I (Control) | Trial-II (PSB) |
| 1. | 10.4 | 11.3 | 26 | 30 | 21 | 28 | 10 | 12 |
| 2. | 9.2 | 10.3 | 22 | 46 | 17 | 25 | 8 | 9 |
| 3. | 7.2 | 9.9 | 18 | 48 | 15 | 22 | 6 | 7 |

**Table 4: Influence of PSB on Metabolism of Pea plants**

| S.No. | Weight of seeds/plant (g) | | Total chlorophyll content (mg g-1 FW) | | Protein content (mg g-1 FW) | |
|---|---|---|---|---|---|---|
| | Trial-I (Control) | Trial-I (PSB) | Trial-I (Control) | Trial-II (PSB) | Trial-I (Control) | Trial-II (PSB) |
| 1. | 24 | 36 | 1.62 | 1.91 | 3.10 | 4.52 |
| 2. | 21 | 38 | 1.57 | 1.80 | 3.25 | 4.64 |
| 3. | 18 | 33 | 1.70 | 1.85 | 3.53 | 4.68 |

**Table 5: Percentage Increase of Parameters Influencing Plant Growth**

| S.No | Parameters influencing Plant Growth | Percentage increase after inoculation with PSB (%) |
|---|---|---|
| 1. | Root length | 54.69 |
| 2. | Shoot length | 36.50 |
| 3. | Number of pods per plant | 50.00 |
| 4. | Number of seeds per plant | 19.23 |
| 5. | Pod length | 17.53 |
| 6. | Pod yield per plant | 87.88 |
| 7. | Weight of seeds per plant | 69.84 |
| 8. | Fresh plant weight | 41.50 |
| 9. | Dry plant weight | 16.67 |
| 10. | Total chlorophyll content | 13.70 |
| 11. | Protein content | 40.09 |
| 12. | Shelling percentage | 60.00 |

## INFLUENCE OF PSB ON THE GR OWTH AND METABOLISM OF PEA PLANT

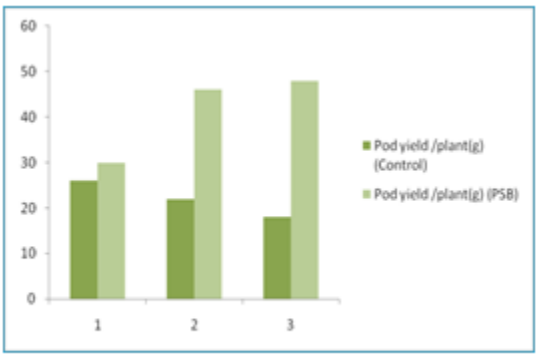


**Fig 1: Pod yield / plant**

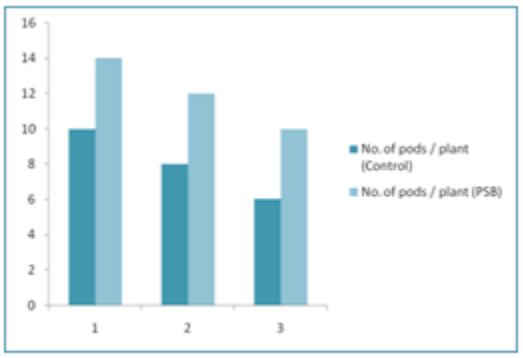


**Fig 2: No. of pods / plant**

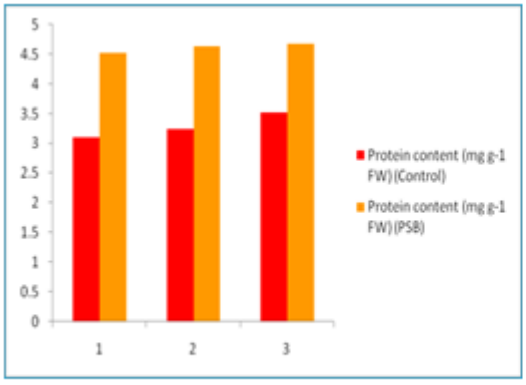


**Fig 3: Protein content**

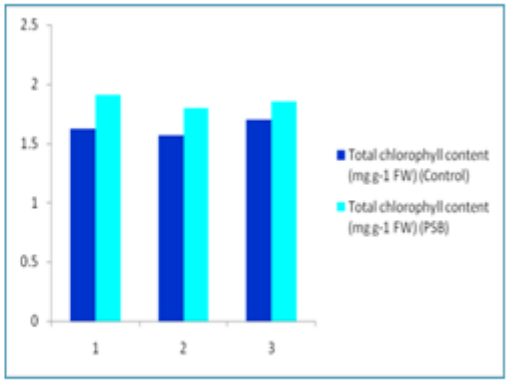


**Fig 4: Total chlorophyll content**

## CONCLUSION

Inoculation of PSB with sterile soil, after inoculation the potential candidates were isolated according to its capability to produce Halo zone in the Pikovskaya medium. The isolated strains of microorganisms 10-4 Strain-I (Psedumonas aureginosa) and 10-6 Strain-II (Bacillus megaterium) showed significant phosphate solubilising activity. The decrease

in pH levels of the media shows the production of organic acids, enzymes by the microorganisms to help with the solubilisation of the phosphate provided in the medium. According to the results obtained from comparative field trial conducted in Kodai Hills, use of PSB bio fertilizer shows marked difference in soil fertility, better performance in yield and yield related characteristics like Shoot length, Root length, Number of pods per plant, Number of seeds per plant, Pod yield per plant, Weight of seeds per plant, Fresh plant weight, Dry plant weight, Days to first flowering, Days to first pod picking, Shelling percentage, Total chlorophyll content and Protein content observed in the field of pea plants. It was also observed that plants growing with the help of PSB bio fertilizer had better quantity and quality of pods and flowers, than those that grew on the control.

## CONFLICT OF INTERESTS

None.

## ACKNOWLEDGMENTS

None.

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