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INFLUENCE OF PHOSPHATE SOLUBILIZING BACTERIA ON THE METABOLIC PARAMETERS AND GROWTH OF CARROT PLANTS (DAUCUS CAROTA L.) IN KODAI HILLS OF TAMILNADU, INDIA

U. Muthuvel[1], B. Harinathan[2], K. Suresh[3]

[1]PG and Research Department of Botany, Saraswathi Narayanan College, (Autonomous), Institution Affiliated to Madurai Kamaraj University, Madurai - 625 022, Tamil Nadu, India.
[2]PG and Research Department of Microbiology, V. H. N. Senthi Kumara Nadar College, Virudhunagar (Autonomous), Institution Affiliated to Madurai Kamaraj University, Madurai - 625 022, Tamil Nadu, India

Corresponding Author
K. Suresh,
sureshdeanresearch@gmail.com

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ABSTRACT

Daucus carota is an important vegetable which is ranked third among the succulent vegetables in world production. Excessive amount of inorganic fertilizer results in soil acidification, increased greenhouse gas (GHG) emissions, and increased eutrophication of water bodies, can be mitigated by soil amendment using PSB bio-fertilizers, which resulted in improved plant growth and productivity. In this present study, the chosen potential PSB strains Micrococcus luteus, Paenibacillus polymyxa after characterization were selected for inoculation, and the field experiment was conducted using randomized complete block design with three replications, and the influence of these phosphate solubilizers on the growth, yield and metabolism parameters were evaluated after growth of carrot plants. Both PSB 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, when treated with plants Carrot Test (CT) - (11-15), they tend to enhance the vegetative growth, yield and qualitative parameters of carrot plants when compared to other treatments Carrot Test (CT) - (1-5), Carrot Test (CT) - (6-10). The application of Phosphate-Solubilizing Bacteria (PSB) bio-fertilizers Carrot Test (CT) - (11-15) in combination with calcium phosphate would aid uptake of phosphorus for better crop growth and yield and under a long run would aid to substantially sustainable soil fertility.

Keywords: Carrot, Phosphate Solubilizing Bacteria, Micrococcus Luteus, Paenibacillus Polymyxa

1. INTRODUCTION

Carrot is one of the most cultivated plants of Kodaikanal Hills farmers due to its economic potential and its maximum nutritive value which is well-known by the urban health conscious people for decades. The crop is tolerant to soil pH of 5.5 to 6.5 and it requires a deep and well-drained loamy soil with high amount of organic matter. Carrots are relatively tolerant to a wide variety of temperatures but prefer cooler agro-climatic conditions where temperature varies between 15.6 and 21.1°C. They grow in well drained alluvial and sandy loam soils with pH of 6.6-7.1 but not in heavy clay and water-logged soils. Apart from its high potential for agricultural products import and export in continental trade, it is one of the exotic vegetables with high nutritive and economic value and of great demand in urban centres of the country. [1] The beta-carotene of the carrot is proved to be most useful for ocular diseases and beneficial for eye problems. Due to its economic potential and high nutritional content, which has long been recognised by urban health-conscious people, carrots are one of the vegetables that Kodaikanal Hills farmers most frequently produce. Due to its poor availability in the soil, phosphorus is a crucial component for carrot plants' development, production, and metabolism, especially in monsoon-influenced subtropical highland climatic areas. Either chemical fertilisers or biological fertilisers can be used to solve this issue. But the first one is very harmful to the environment, dangerous for water bodies, pricey for farmers, and energy-draining. The mostly undiscovered When applied to crops, Phosphate Solubilising Bacteria, which are culture preparations with carrier material, promote plant growth by converting insoluble phosphates into soluble forms through solubilisation, mineralization, and immobilisation. It also makes it easier for plants to absorb phosphorous from the soil. When compared to chemical fertilisers, they are more efficient in application, cost-effective, and boost crop output. Phosphorus is the essential nutrient for crop growth, yield and metabolism for carrot plants, particularly in monsoon-influenced subtropical highland climate area, due to its low availability in the soil. [2] This problem can be averted by either using chemical fertilizers or bio-fertilizers. But, the former one is quite hostile to the environment, hazardous to the water bodies, energy draining and expensive to the farmers. [3] So, the mostly unexplored Phosphate Solubilising Bacteria are the culture preparations with carrier material, possessing plant growth promoting effects and helping to convert insoluble phosphates into soluble forms through solubilisation, mineralisation and immobilisation and it facilitates uptake of phosphorous from soil when applied to the crops. [4] They are eco-friendly, cost-effective, increased crop-productivity, and efficient in application when compared with chemical fertilizers. [5] The crop responds favourably to both organic and inorganic fertilizers. [6] However, excessive amount of inorganic fertilizer results in soil acidification, increased greenhouse gas (GHG) emissions, and increased eutrophication of water bodies. [7] These are detrimental to production and loss of nutritional qualities of most crops. As a way to mitigate the environmental pressure resulting from inorganic fertilizers and simultaneously improve carrot quality and yield, soil amendment using bio-fertilizers has been recommended. Various hills regions have conducted this research of their locally based plant. These various researches where conducted under the premise that a different percentage of phytochemicals present due to nutrimental exposure of the plant during its cultivation. These phytochemicals were shown to be the basis for the antimicrobial effects of this plant. [8-10] 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. [11] The influence of these phosphate solubilizers on the growth, yield and metabolism of carrot plants (*Daucus carota* L.) were analysed.

2. MATERIALS AND METHODS

Phosphate solubilising bacteria were isolated from the field of Kodaikanal Hills, Tamilnadu using *Pikovskaya* Agar medium by standard isolation method and isolates were identified using appropriate techniques. Microbial characterization was performed for the isolates, and from this chosen isolate used for Treatment (Tt) - (11-15) test conduct.

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)**

3. CLIMATE CHARACTERISTICS

Kodaikanal has a year-round, extremely pleasant, chilly climate. The hill station has a moderate subtropical climate because of its high elevation. Kodaikanal experiences a range of temperatures, from mild to extremely cold. The summer time temperature ranges from 11 to 20 degrees celsius. Being there in summer time is quite calming. It becomes really chilly at Kodaikanal throughout the winter. These months have a range of surface temperatures from 8 to 17 degrees. The outside temperature can occasionally drop to below freezing during these months. Between June and September, rainfall occurs in Kodaikanal. The major cause of the rainy season in Kodaikanal is the monsoon's northward retreat via this mountainous area. About 1650 mm of rainfall occurs here on average each year. The experimental location was located in a subtropical highland climate that was impacted by the monsoon. Because of the high elevation, the weather is cool all year round.

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.

FIELD PREPARATION

First of all, the field of 79 m low in Phosphorus (P) content was ploughed at a depth of 15–20 cm thoroughly for proper aeration, and indigenous spores were allowed to sun sanitize by placing a plastic sheet over the ploughed field for 2 days. Then, an almost 3 cm layer of sterilized soil sand mixture was evenly distributed. Farmyard manure and organic waste were added composing 25% of water content, 7.5 pH, 1.05% Nitrogen (N), 0.22% P, 0.59% K to make the land loam- clayey measured by [12]. Plant-beds/plots of 1.5 m with 15 cm alleyways were tilled, as shown in Furrows of 20–30 cm were made on which cut tubers were placed at a depth of 5–7 cm at the centre of the ridge, keeping them at 15– 20 cm apart. Each plant - beds were having 3 furrows and 15 plant samples out of which 10 random plants of each treatment were selected for morphological and biochemical analysis after 90 days. Drip irrigation was installed for irrigation.

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)

PLANT CHARACTERIZATION AND DATA ANALYSIS

Plant characterization was performed after 80 days of sowing. Root and shoot length of all the plants in replication were measured at maturity. The total chlorophyll and carotenoid content were estimated to Arnon's method. [13] Arbuscular mycorrhizal fungi (AMF) and AM root colonization (%) were determined based on the methods defined elsewhere. Some pots were intentionally remained undisturbed so that they develop seeds, and that were collected for the next round of experimentation under open field conditions.

FERTILIZER APPLICATION

Nitrogen was administered at 120 kg ha^{-1}via 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}.

PLANT PROTECTION MEASURES

At 30 Days after sowing (DAS), diathane M-45 was administered as a preventative strategy for preventing fungal infection.

ROOT LENGTH AND AVERAGE DIAMETER

Root length and average diameter were measured using a Hewelett Packard scanner controlled by Win-RHIZO Programme V. 2002C Software (Regent Instruments Inc. Ltd., Quebec, Canada). Here roots were placed in the plexiglas tray (200 mm 300 mm) with 4–10 mm deep water layer, depending on root size. Roots were spread on the tray before scanning to minimize overlapping. After scanning, the analysis of the image was done by the programme itself. After scanning, root samples were oven-dried to determine dry weight.

SPECIFIC ROOT LENGTH

Specific root length (SRL) is probably the most commonly measured morphological parameter of fine roots because it is believed to characterize the economic aspects of root systems. SRL is the length-to mass ratio of a root fragment.

$$\text{Specific root length (SRL, m/g)} = \frac{\text{Root length (RL)}}{\text{Root dry weight } (Root_w)}$$

4. FIELD EXPERIMENT

Field experiment was conducted at Kodaikanal Hills, Tamil Nadu, India in the field of carrot plants. (**Fig. 1**) The experiment was conducted in plot based randomized complete block design with three replications. Seeds of Carrot (*Daucus carota.*L) were sown in different plots labelled as CT-(1-15). The growth and metabolic parameters Plant height, Root length, Root diameter, Root girth, Fresh root weight, Dry root weight, Acid phosphatase activity [14] Amino acid content [15] Ascorbic acid content [16] Indole-3-acetic acid content Phosphorus content [17] Protein content [18] Reducing sugar content [19] Starch content [20] Total phenolic content [21] Total soluble sugar content [22] Anthocyanin content [23] Chlorophyll and carotenoid content [24] were evaluated after growth of carrot plants.

MORPHOLOGICAL CHARACTERIZATION

Out of fifteen plants in each plot, five plants were selected randomly for examination. Morphological characters and yield parameters like plant biomass, leaf number and weight, moisture content, number of nodes per plant were measured, considering. [25] Plant biomass was calculated by subtracting fresh weight with dry weight (oven-dry at 55 °C for 2 days) of harvested plants and expressed in grams. The number of carrot leaves was counted upon harvesting, and five healthiest carrots were selected for the accounting number of nodes and weight. The weighed carrots were kept for oven-dry (60 °C for 3 days) and noted the dry weight. After this moisture content in percentage was calculated by the given formula.

$$\text{Moisture content} = \frac{\text{Fresh weight - Dry Weight}}{\text{Fresh weight}} \times 100$$

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTIC OF PSB

The morphological and biochemical features of the bacterial culture allowed for genus-level identification. Morphologically, isolates were categorized according to the size, shape and color of the colonies. A milliliter of the culture was added to peptone water and nutritional broth, resulting in the identification of the majority of the bacterial isolates by biochemical and physiological testing. The evaluations for Indole, Methyl Red, Voges Proskauer, Citrate Utilization, Carbohydrate Fermentation, Urease, Catalase, and Oxidase were conducted using a loop full of culture. The isolated bacterial strains have been identified using conventional biochemical tests and bacteriological manuals, as specified in Bergey's Manual of Determinative Bacteriology, following a 48–72 hour incubation period.

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 $MgCl_2$, 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 minutes, followed by 36 cycles of 94°C for 2 minutes, 54°C for 1 minute, and 72°C for 2 minutes, with a last extension at 72 °C for a total of seven minutes. 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 [26].

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.

STATISTICAL ANALYSIS

ANOVA was used to analyze differences in Data are expressed on a dry weight except for moisture content. Treatment (Tt), Days after swing (DAS) Data are mean ± Standard Deviation (SD = ±2). Different letters within one column denote statistically significant differences by ANOVA. Homogeneity of variance and normality were assessed,

and log transformations were applied as needed to meet these assumptions. A probability level of p = 0.05 was considered statistically significant [27].

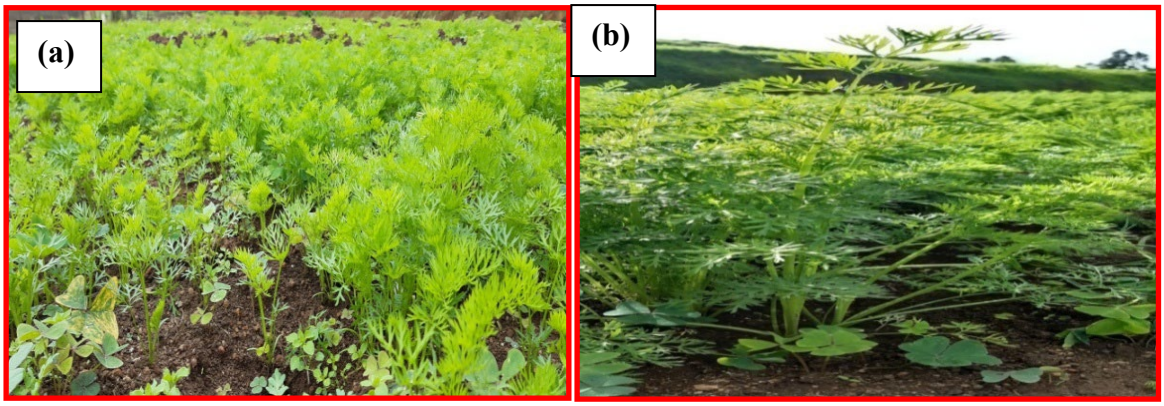

Fig.1 Field Experiment on Kodaikanal Hills in the Field of Carrot Plants (a) 30 Days After Sowing (DAS), and (b) 90 Days After Sowing (DAS)

5. RESULTS AND DISCUSSION

The experimental findings showed potential two phosphate solubilising microbial strains provided by a detailed account of characterization of the strains. [28] From the detailed characterization, strain-I was Gram positive, circular shaped, opaque, non-motile, bright yellow, convex and smooth colonies and strain-II was Gram positive, rod shaped, opaque, milky white and flat colonies. [29] From the observed findings strain-I and strain-II identified as *Micrococcus luteus* and *Paenibacillus polymyxa*. Their pH values were above 7.0, after incubation their pH reduced to 5.9 and 5.75 respectively, indicates increased organic acid production. Their phosphate solubilisation efficiency was higher than 180, (182 and 245 for strain-I and strain-II) indicates high phosphate solubilization efficiency. [**Table 1**, **Fig. 3**] The test samples set Tt - (1-15) was tested in the field on a field of carrot plants in the Kodaikanal Hills using a randomised full block design with three replications. They were assessed for carrot plant vegetative growth, yield, and metabolism parameters. The treatment with PSB strain Tt - (11-15) resulted more appeasing in carrot vegetative development, yield, and quality characteristics, according to the study. [30] During the growing phase, those PSB treated crop performed well, and there was a substantial difference between the PSB treatments and their combinations. [31] The data can be found in **(Table 1, 5) (Fig. 4)**

Table 1. Treatment and inoculums of under open field conditions.

Sl. No.	Treatment Combinations for Carrot Test (CT)	Treatment Details
	Tt -(1-5)	Sterile water + Soil **(Blank)**
	Tt -(6-10)	Soil + Tricalcium Phosphate + Sterile water **(Control)**
	Tt -(11-15)	Soil + Tricalcium Phosphate + Sterile water + Phosphate Solubilizing Bacteria (PSB) **(Test)**

Table 2. Microbial Characterization of Isolated PSB Strains

S.no	Isolate characterization	Results	
		Strain-I	Strain-II
1.	Gram's reaction	Gram positive	Gram positive
2.	Shape	Circular shaped	Rod shaped
3.	Size	Convex and smooth	Flat
4.	Opacity	Opaque	Opaque
5.	pH	5.9	5.75
6.	Colour	Bright yellow	Milk white
7.	Motility	Non-Motile	Motile
8.	Phosphate Solubilizing index (PSI)	182	245

BIOCHEMICAL CHARACTERISTICS OF SELECTED PSB ISOLATES

The bacterial isolate namely T-10-6 demonstrated the highest P solubilisation in tricalcium phosphate supplemented PKV broth, was chosen for further biochemical analysis. The isolates were rod-shaped gram-negative bacteria with motility Catalase and gelatin liquefaction tests revealed that the isolates were positive. The bacterial isolate tested negative for indole, methyl red, voges-Proskauer, urea utilization, citrate utilization, hydrolysis of starch, nitrate reduction and hydrogen sulfide test Based on their morphological features and biochemical analysis, the isolate was identified as *Pseudomonas* sp.

Table 3. Morphological characterization of the efficient phosphate solubilising bacteria

Character		*Pseudomonas putida*
Configuration, margin, surface	-	Round Entire, Smooth
Cell shape arrangement	-	Rods, Moderate, Single
Pigment	-	Fluorescence
Gram staining	-	Negative
Motility	-	Motile
Growth temperature	-	15-38^{o}C

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. **Fig. 2**

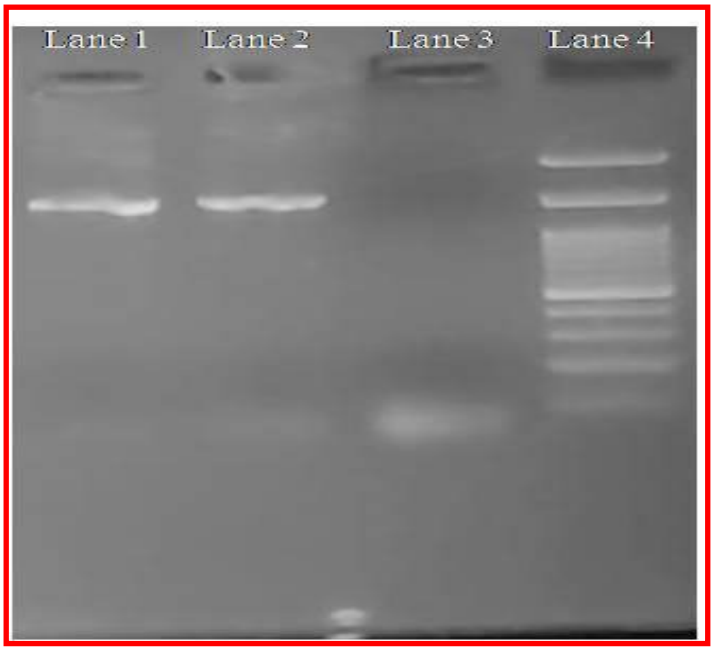

Fig. 2. Gene JET PCR Purification Kit

Table 4. Effect of PSB on Vegetative Growth of Carrot Plants-I

Treatments	Plant Height (cm)			Number of Leaves Per Plant		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
Tt-1	9.47±0.19	22.33±0.22	56.88±0.77	2	6	9
Tt -2	8.48±0.33	20.10±0.45	67.82±0.72	3	5	10
Tt -3	8.00±0.61	21.18±0.54	64.43±0.36	3	5	9
Tt -4	9.11±0.73	22.04±0.82	63.87±0.69	2	6	10
Tt -5	9.63±0.28	19.43±0.31	67.62±0.65	3	6	9
Tt -6	10.53±0.43	24.78±0.78	72.87±0.77	5	7	11
Tt -7	12.80±0.57	27.82±0.73	73.95±0.62	4	8	12
Tt -8	12.09±0.94	26.71±0.71	72.63±0.71	5	8	11
Tt -9	10.88±0.66	27.67±0.86	72.06±0.77	4	7	12
Tt -10	12.02±0.80	25.63±0.72	74.61±0.86	5	8	11
Tt -11	15.18±0.71	35.78±0.52	79.78±0.81	5	10	15
Tt -12	16.99±0.89	36.68±0.75	77.96±0.87	5	10	14
Tt -13	14.83±0.63	33.80±0.90	77.78±0.63	6	9	13
Tt -14	17.67±0.85	35.82±0.88	81.73±0.62	6	9	14
Tt -15	18.44±0.67	36.36±0.33	80.69±0.52	5	10	15

Effect of Bio inoculants on Morphological and food storage parameters of *Daucus carota* in the pot experiment.

Data are expressed on a dry weight except for moisture content. Treatment (Tt), Days after swing (DAS) Data are mean ± Standard Deviation (SD ± 2 cm). Different letters within one column denote statistically significant differences by ANOVA.

Table 5. Effect of PSB on Vegetative Growth of Carrot Plants-II

Treatments Plant	Leaf Length (cm)	Fresh Weight of Leaves (cm)	Shoot Length (cm)	Whole Plant Fresh Weight (g)	Whole Plant Dry Weight(g)
	90 DAS	90 DAS	90 DAS	90 DAS	90 DAS
Tt -1	19.38±0.18	34.75±0.58	45.31±0.28	67.48±0.36	41.12±0.63
Tt -2	21.69±0.75	37.69±0.56	56.69±0.59	65.10±0.71	45.69±0.69
Tt -3	24.85±0.72	38.95±0.66	53.59±0.65	61.94±0.67	41.24±0.79
Tt -4	26.58±0.38	40.35±0.40	52.26±0.20	62.93±0.68	44.52±0.87
Tt -5	23.87±0.58	45.75±0.63	56.92±0.56	69.15±0.27	47.78±0.70
Tt -6	27.98±0.60	46.62±0.82	58.74±0.74	76.88±0.80	32.51±0.73
Tt -7	29.06±0.61	48.78±0.74	60.84±0.90	75.72±0.92	36.26±0.66
Tt -8	31.77±0.92	52.59±0.77	59.76±0.94	72.62±0.72	32.63±0.61
Tt -9	31.29±0.68	55.67±0.73	60.00±0.87	89.20±0.61	40.01±0.68
Tt -10	32.72±0.75	54.67±0.85	60.80±0.82	87.10±0.57	37.54±0.86
Tt -11	35.37±0.80	63.63±0.71	59.94±0.93	101.2±0.94	22.20±0.73
Tt -12	38.01±0.79	65.68±0.69	63.13±0.47	114.6±0.79	23.26±0.73
Tt -13	39.61±0.80	66.49±0.52	61.03±0.85	117.7±0.83	29.12±0.91
Tt -14	37.74±0.64	64.64±0.90	60.88±0.55	112.1±0.88	21.88±0.92
Tt -15	36.86±0.47	63.71±0.81	65.09±0.71	113.8±0.93	24.22±0.58

Effect of Bio inoculants on Morphological and food storage parameters of *Daucus carota* in the pot experiment.

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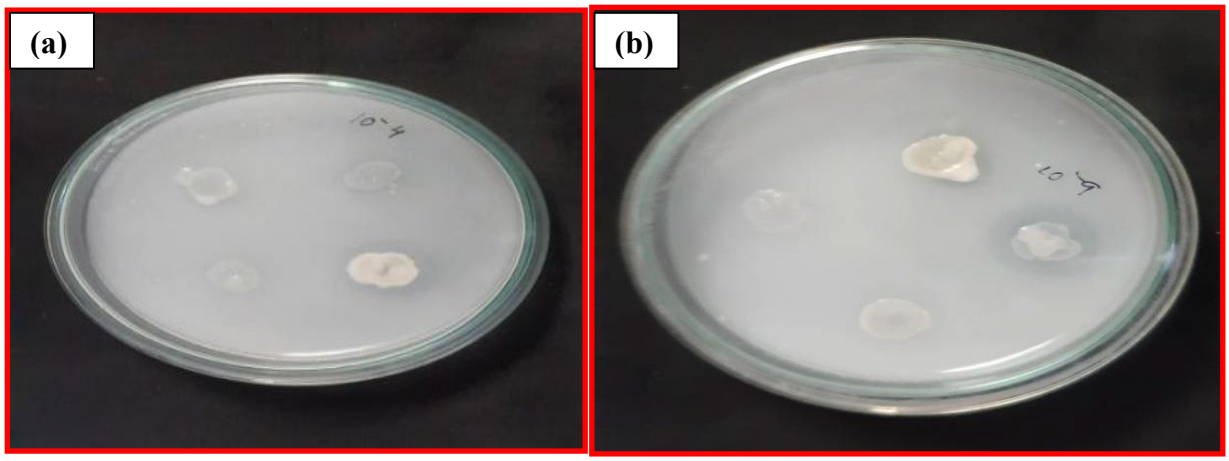

Fig. 3 Phosphate Solubilisation Efficiency of PSB strains

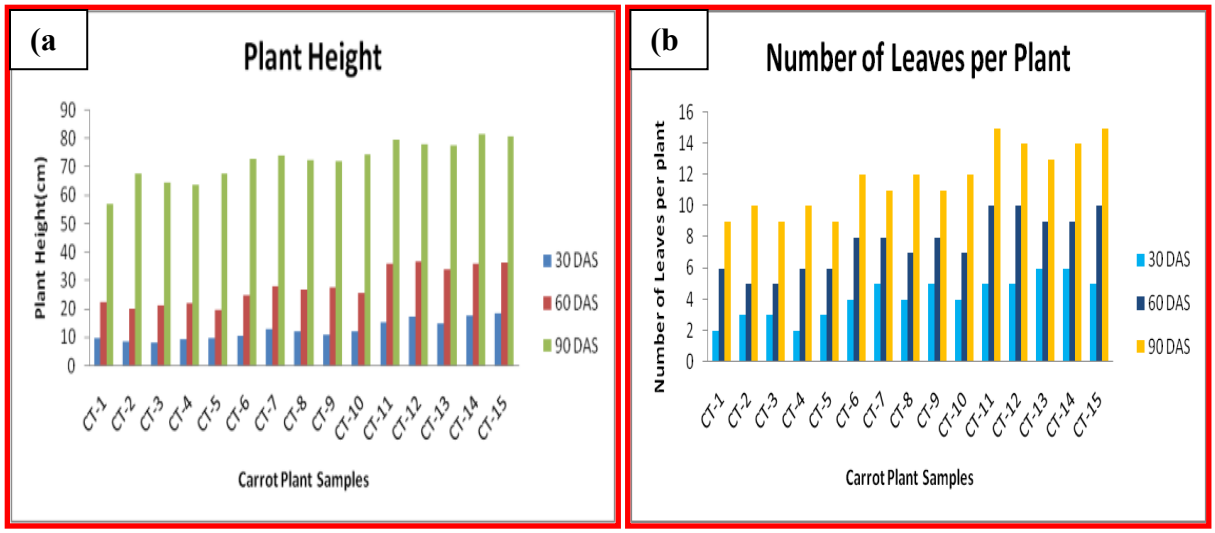

Fig. 4 Effect of PSB on Vegetative Growth of Carrot Plants (a) Plant height (b) Number of leaves per plant

The PSB treated plants had the highest plant height, yielded maximum number of leaves, prominent vegetative growth in leaf length, shoot length and in quantitative manners like leaf weight, plant weight when compared to other treatments of plants. The treatments with PSB showed highest yield and its related characters. [32] The PSB treated plants had the highest root length, root girth, root diameter and quantitatively in root weight when compared to other treatments of plants. (**Table 6, Fig. 5**)

Table 6. Effect of PSB on Yield of Carrot Plants

Treatments Plant	Root Length(cm)	Root Diameter(cm)	Root Girth(cm)	Fresh Root Weight(g)	Dry Root Weight(g)
Tt -1	10.62±0.88	1.24±0.23	3.78±0.56	26.06±0.77	3.57±0.29
Tt -2	10.13±0.75	2.18±0.21	4.33±0.27	30.53±0.15	3.26±0.10
Tt -3	10.39±0.32	2.43±0.18	5.46±0.41	33.32±0.57	3.13±0.73
Tt -4	10.36±0.14	1.95±0.70	5.35±0.16	35.08±0.60	4.65±0.62
Tt -5	10.21±0.71	2.32±0.30	5.18±0.56	35.89±0.84	4.63±0.40
Tt -6	11.37±0.30	2.90±0.61	6.77±0.77	38.88±0.65	5.87±0.80
Tt -7	11.61±0.53	2.61±0.83	6.55±0.65	42.02±0.54	5.70±0.71
Tt -8	11.27±0.11	3.06±0.81	6.90±0.92	48.99±0.83	6.37±0.42
Tt -9	12.10±0.58	2.71±0.54	6.73±0.60	52.68±0.67	7.75±0.59
Tt -10	12.12±0.69	2.56±0.69	6.13±0.69	53.25±0.84	7.68±0.82
Tt -11	13.26±0.69	3.69±0.93	7.85±0.65	76.61±0.73	7.95±0.87
Tt -12	13.53±0.45	3.57±0.76	8.89±0.88	76.89±0.39	10.72±0.81
Tt -13	15.76±0.62	3.55±0.68	8.66±0.82	85.94±0.87	12.17±0.69
Tt -14	16.00±0.81	4.06±0.87	8.76±0.87	92.09±0.75	11.91±0.57
Tt -15	17.58±0.42	3.97±0.68	9.26±0.83	97.76±0.58	13.68±0.79

Effect of Bio inoculants on Morphological and Food storage parameters of *Daucus carota* in open field condition. **Data are expressed on a dry weight except for moisture content. Treatment (Tt), Days after swing (DAS) Data are mean ± Standard Deviation (SD). Different letters within one column denote statistically significant differences by ANOVA.**

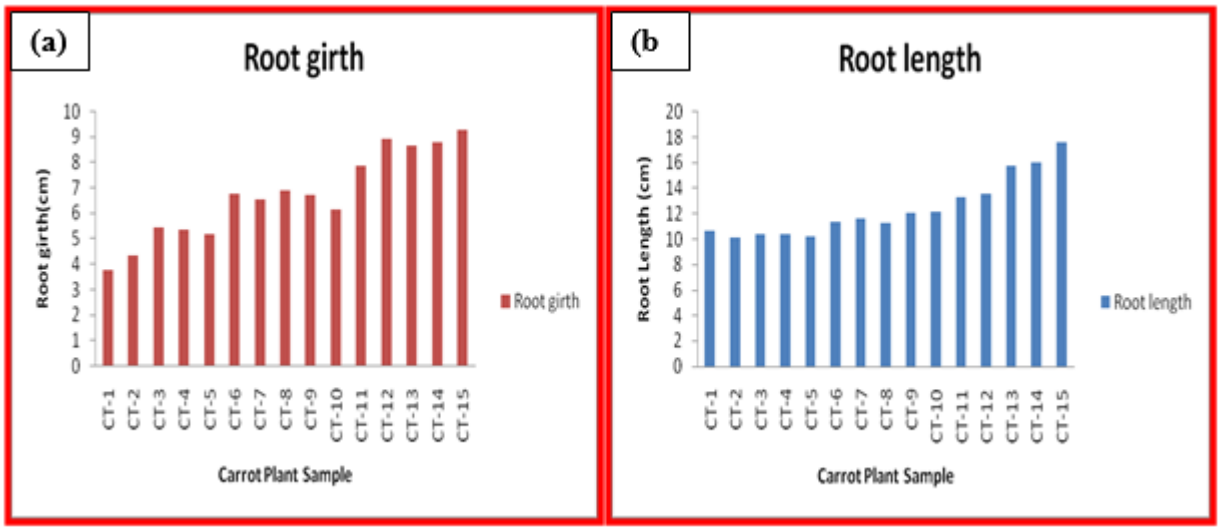

Fig. 5 Effect of PSB on Yield of Carrot Plants (a) Carrot Root girth parameters, and (b) Carrot Root length Parameters

There was increased plant productivity for certain metabolites which acts on plants, increase phosphorus content and elicit positive growth response, and the decreased plant productivity observed for the metabolites which act on plants, decrease phosphorus content and elicit negative growth response. (**Table 7 to 8, 9, Fig 6, 7, 8, 9**)

Table 7. Effect of PSB on Metabolic Characteristics of Carrot Plants-I

Treatments Plant	Reducing Sugar content (mg/g of leaves)	Total Sugar content (mg/g of leaves)	Non-reducing Sugar content (mg/g of leaves)	Starch content (mg/g of leaves)	Protein content (mg/g of leaves)	Amino Acid content (mg/g)of leaves of Leucine Equivalent
Tt -(1-5)	14.35	39.54	25.38	10.54	6.41	0.68
Tt -(6-10)	18.78	42.72	27.01	11.84	5.52	0.57
Tt -(11-15)	21.2	48.09	29.13	14.22	4.08	0.43

Treatments Plant	Reducing Sugar content (mg/g of leaves)	Non-reducing Sugar content (mg/g of leaves)	Total Sugar content (mg/g of leaves)	Starch content (mg/g of leaves)	Protein content (mg/g of leaves)	Amino Acid content (mg/g)of leaves of Leucine Equivalent
Tt -1-5	11.64±3.57	20.98±6.33	34.41±7.92	7.80±3.54	4.38±2.75	0.50±0.23
Tt -6-10	14.35±5.87	24.28±4.96	37.53±7.11	9.11±4.03	3.76±2.75	0.42±0.21
Tt -11-15	16.69±6.18	26.45±3.97	42.59±8.21	11.37±4.03	2.91±2.24	0.34±0.16

Effect of Bio inoculants on Biochemical and Physiological attributes of Daucus carota in pot experiment Data are expressed on a dry weight except for moisture content. Treatment (Tt), Days after swing (DAS) Data are mean ± Standard Deviation (SD). Different letters within one column denote statistically significant differences by ANOVA.

Table 8. Effect of PSB on Metabolic Characteristics of Carrot Plants-II

Treatments	Cholorophyll-a	Cholorophyll –b	Total Cholorophyll (mg/g of leaves)	Total Carotenoid content (mg/g of leaves)	Anthocyanin content (mg/g of leaves)
Tt -(1-5)	0.69	0.39	0.92	0.14	2.18
Tt -(6-10)	0.61	0.52	1.2	0.19	1.56
Tt -(11-15)	0.66	0.82	1.7	0.36	1.47

Treatments	Cholorophyll-a	Cholorophyll –b	Total Cholorophyll (mg/g of leaves)	Total Carotenoid content (mg/g of leaves)	Anthocyanin content (mg/g of leaves)
Tt -1-5	0.57+0.16	0.29+0.15	0.77±0.20	0.21±0.23	1.92±0.52
Tt -6-10	0.49±0.19	0.42±0.21	0.93±0.33	0.41±0.59	1.41±0.21
Tt -11-15	0.53±0.18	0.34±0.16	1.14±0.69	0.26±0.12	1.29±0.22

Effect of Bio inoculants on Biochemical and Physiological attributes of *Daucus carota* in pot experiment. Data are expressed on a dry weight except for moisture content. Treatment (Tt), Days after swing (DAS) Data are mean ± Standard Deviation (SD). Different letters within one column denote statistically significant differences by ANOVA.

Table 9. Effect of PSB on Metabolic Characteristics of Carrot Plants-III

Treatments	Total Phenolics content (mg/g of leaves)	Ascorbic Acid content (mg/g of leaves)	Indole-3-Acetic Acid content (mg/g of leaves)	Shoot Phosphorus content (mg/g of DW of shoot)	Acid Phosphatase activity(mU/ml)
Tt -(1-5)	96.53	1.51	0.33	4.124	35.52
Tt -(6-10)	89.56	1.91	0.64	5.967	41.38
Tt -(11-15)	76.57	2.46	0.83	8.034	60.03

Treatments	Total Phenolics content (mg/g of leaves)	Ascorbic Acid content (mg/g of leaves)	Indole-3-Acetic Acid content (mg/g of leaves)	Shoot Phosphorus content (mg/g of DW of shoot)	Acid Phosphatase activity(mU/ml)
Tt -1-5	82.50±20.40	1.43±0.12	0.24±0.13	3.11±1.42	27.92±11.04
Tt -6-10	75.65±24.59	1.75±0.24	0.54±0.14	4.71±1.66	35.74±12.18
Tt -11-15	62.44±19.94	2.12±0.65	0.69±0.21	6.83±1.92	52.20±13.81

Effect of Bio inoculants on Biochemical and Physiological attributes of *Daucus carota* in pot experiment. Data are expressed on a dry weight except for moisture content. Treatment (Tt), Days after swing (DAS) Data are mean ± Standard Deviation (SD). Different letters within one column denote statistically significant differences by ANOVA.

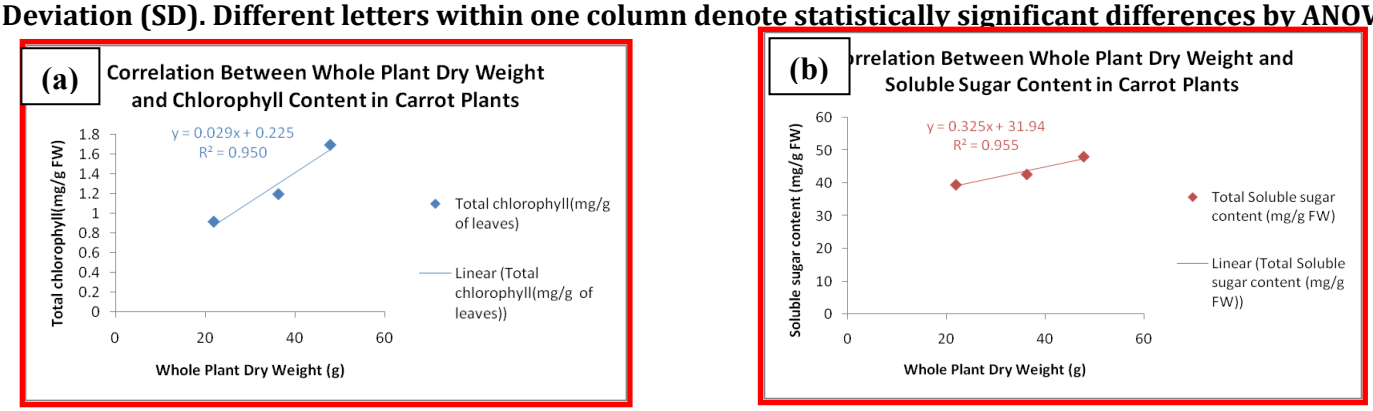

Fig. 6 Effect of PSB on Metabolic Characteristics of Carrot Plants (a) Correlation between whole plant dry weight and Chlorophyll content, and (b) Correlation between whole plant dry weight and soluble sugar content

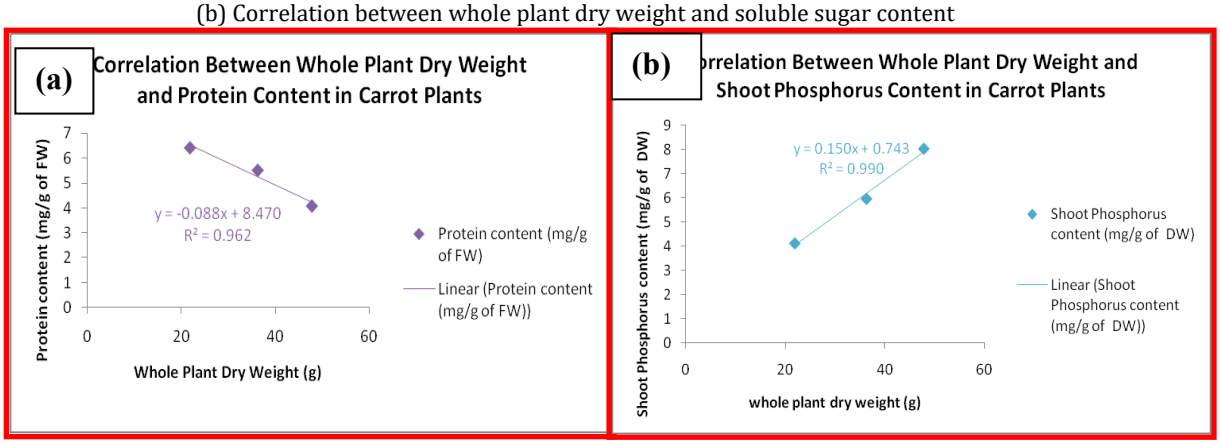

Fig. 7 Effect of PSB on Metabolic Characteristics of Carrot Plants (a) Correlation between whole plant dry weight and protein content (b) Correlation between whole plant dry weight and shoot phosphorus content

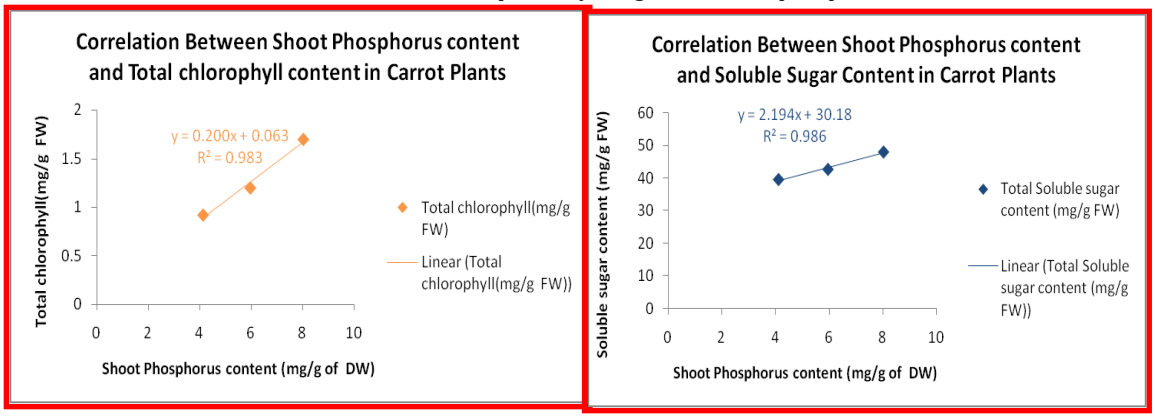

Fig. 8 Effect of PSB on Metabolic Characteristics of Carrot Plants (a) Correlation between shoot phosphorus content and total Chlorophyll content (b) Correlation between shoot phosphorus content and soluble sugar content

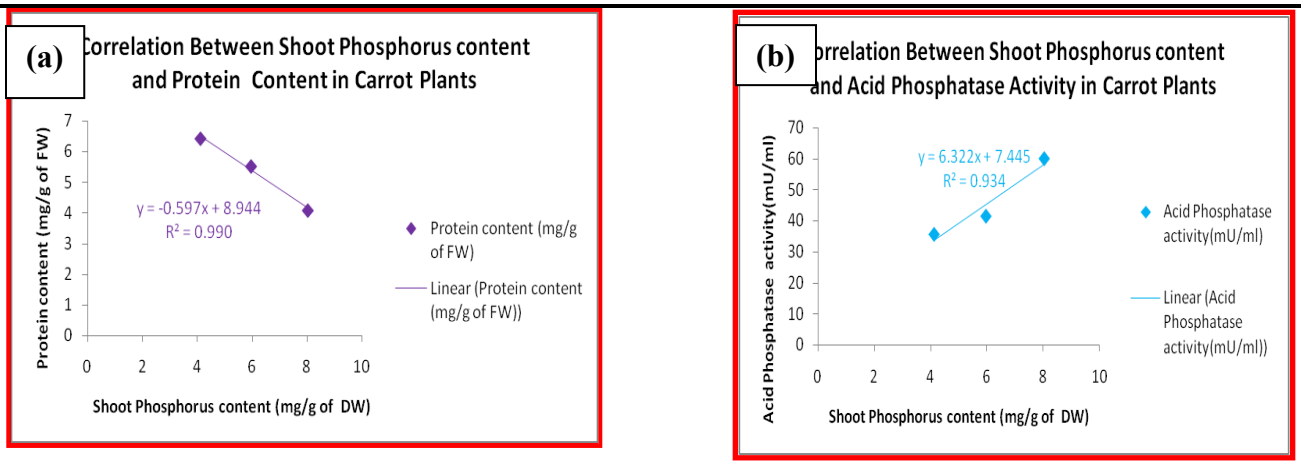

Fig. 9 Effect of PSB on Metabolic Characteristics of Carrot Plants (a) Correlation between shoot phosphorus and protein content (b) Correlation between shoot phosphorus content and acid phosphatase activity.

The application of Phosphate Solubilisation Bacteria (PSB) treatments to the carrot plants, increased production of growth hormones resulted in increased vegetative growth of the plants.The application of PSB bio-fertilizers might have triggered the maximum potential of photosynthesis in the plants and ultimately producing the higher values in terms of yield related attributes. The phosphate solubilising capacity of the *Micrococcuss luteus, Paenibacillus polymyxa* increased uptake of phosphorus might have helped in increasing the nutrient uptake efficiency by exerting their synergistic effect with inorganic fertilizers like calcium phosphate. This could also have accelerated cell division and elongation as well as greater chlorophyll synthesis and higher metabolic activity. The obtained results were in accordance with in carrot and in carrot. [33]

6. CONCLUSION

PSB treatment and the Pseudomonas appear to be promising for the carrot in nutrient procurement and maintaining ionic homeostasis. In this direction, the PSB seems to be in a appropriate inoculum to improve plant development, yield, and quality especially in the case of carrots. Although, an optimum values for the biochemical and the morphological traits have been based on the treatment with P. fluorescens, and *P.pudita*. Overall, this work highlights microbial bacterial inoculum possess an efficacy in attaining a profitable carrot production by improving water uptake, maintaining osmotic balance, enhancing photosynthetic efficiency, and modulating phytohormones profiling. Results of this study also established that PSB application enhance the quality of carrots. It is conducted that the application of PSB along with different levels of inorganic phosphorus give the best results for yield and yield component. Hundred percent of inorganic phosphorus along with PSB application significantly increased yield (69%) and yield component of wheat crop. Hence, 100% of P from inorganic source along with PSB for maximum productivity. A critical next step is about observing the various changes occurring in soil concerning their interaction with microbes at different points of crop growth in a season. From the results, it is suggested that rhizospheric microbes form strong interaction with roots for nutrient and water uptake and revealed that they influence water use efficiency, nutrient cycling and yield. From the present experiment, the potential candidates of PSB strains were micrococcus luteus, paenibacillus polymyxa indicated by their reduced pH below 6.0 after incubation, and phosphate solubilising efficiency above 180, resulted increased organic acid production and high phosphate solubilising efficiency. Both tested strains isolated from the field of carrot plants in Kodaikanal Hills and treated with plants Treatment (Tt) -(11-15), tend to enhance the vegetative growth, yield and qualitative parameters of carrot plants when compared to other treatments Treatment (Tt) (1-5), Treatment (Tt) (6-10). Based on the statistical analysis conducted it was found that the crude extract of the stems and leaves of the *Daucus carota* plants exhibit 16s rRNA activity and produces a larger mean for the zones of inhibition when compared to the fresh leaf juice of the plant. The application of PSB bio-fertilizers in combination with chemical fertilizers like calcium phosphate would aid uptake of phosphorus for better crop growth, yield and metabolism under a long run would aid to substantially sustainable soil fertility.

CONFLICT OF INTERESTS

None.

ACKNOWLEDGMENTS

None.

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