

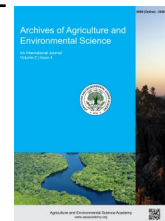


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ORIGINAL RESEARCH ARTICLE



## Plant growth and phosphate uptake of two leafy vegetables (*Trigonella* sp. and *Spinacia* sp.) as influenced by phosphate solubilizing fungi

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### ABSTRACT

The present work was focused on improving the growth and phosphate uptake in leafy vegetables by supplying phosphate bioinoculants. A total of 41 isolates from 50 rhizospheric soil samples were isolated through the serial dilution method by Pikovskaya's medium. Among them 6 PSF with maximum solubilization index (3.08 to 3.86) selected and further evaluated. They showed good results in decreased pH (4.0 to 3.1 from an initial pH of 6.89), titrable acidity (30.16g/L to 38.08g/L), and phosphate concentration in culture broth (60µg to 25µg). Isolate PSF 39 showed maximum plant height (7.8cm at 15 days after sowing and 10.1cm at harvest), root length (5.5cm), biomass (2.11g of fresh weight and 1.01g of dry weight), leafy vegetable weight (2.01g), plant phosphorus uptake (0.393%) and available P in rhizosphere soil (346.96Kg/ha) in *Trigonella* sp. PSF 38 produced the most plant height (15.7cm at 15 days after sowing and 30.6cm at harvest), root length (10.9cm), biomass (11.23g fresh weight and 3.82g dry weight), leafy vegetable weight (10.32g), plant phosphorus uptake (0.369%) and available P in rhizosphere soil (346.29 Kg/ha) in *Spinacia* sp. From the results, the PSF inoculants can be recommended as phosphate solubilizers in the agricultural field as able to promote plant growth in leafy vegetables.

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### INTRODUCTION

India is the world's largest producers, consumers and exporter of seed spices. Among the broad group of vegetables, leafy greens are of paramount importance in providing succulent leaves and stem throughout the year. These are often termed as 'nutritional powerhouses' being rich sources of proteins, minerals, vitamins, essential fatty acids and dietary fiber (Sunitha *et al.*, 2025). Fenugreek (*Trigonella foenum-graecum* L.) popularly known by its vernacular name 'methi' is an important vegetable and condiment crop grown in Northern India (Boori *et al.*, 2017). Fenugreek is considered as spice as well as legume crop, is annual herbaceous and autogamous crop belonging to family Fabaceae (Rathod *et al.*, 2023). Its fresh tender leaves and pods are eaten as fried vegetables being rich in iron, calcium, protein and vitamins.

Its grains are used to form a concentrate feed for animals. Besides this, it has immense medicinal utility. Fenugreek seeds have high nutritive values containing protein (9.5%), fat (10%), crude fiber (18.5%), carbohydrate (42.3%) and many other minor nutrients and vitamins. It also contains good percentage of gums (23.06%), mucilage (28%), trigonelline (0.13-30%), saponine (1.7%) and calorific values (370 calories per 100 gm seed). Palak (*Spinacia* sp.) a member of the family Chenopodiaceae, is a cost-effective source of vitamin-A, which helps to improve eyesight, and also rich in vitamin-C, iron, calcium, phosphorus, and amino acids (Khedkar *et al.*, 2023). Palak contains 268.60mg of nitrogen, 49.68mg of phosphorus, 141.68mg of potash, 368.00mg of calcium, 42.32mg of iron, 50.24mg of ascorbic acid and 52.00µg of carotene content per 100 g of edible portion. Due to its high mineral content, it is often referred to as the "Mines of Minerals".

Phosphorus is the kingpin in Indian agriculture and is one essential nutrient after the Nitrogen required for plant growth and yield (Amit et al., 2021; KarunaiSelvi et al., 2011). The necessity of phosphorus throughout the life cycle of the crop involves energy expenditure by the plant (Mayadunna et al., 2023). Fertilizers that are used in the agricultural field are the process of expensive (Silva et al., 2023). In India, the average soil P content is 0.05%, total of P (0.5%) only 0.1% is plantable (Padmavathi & Usha, 2012). Soil contains the majority insoluble form of phosphate, so the plants are not easily uptake it (Khan et al., 2010). Phosphate deficiency severely restricts growth and yields in plants like slow growth, weak stems, and chlorosis (Mahantesh & Patil, 2011). Stunting, purpling, or browning appearance on the lower leaves and stem base are the major symptoms observed in severe case deficiency of phosphate, specifically in cereal crops. Phosphate deficiency symptoms are evident first on the tips of leaves, and later towards the base (Nisha et al., 2014). Leaf tips are dies for many reasons, but also it is very difficult to phosphate deficiency diagnosis by visual, these can be confirmed by soil analysis and plant tissue analysis. Phosphate deficiency problems are alleviated by the applications of phosphate bioinoculants through field practitioners; these achieve good plant productivity (Khan et al., 2010).

The bacteria and fungi are diverse in soil those have the capacity for phosphate solubilization which is shown to develop the growth and yield of plants in agriculturally important crops by various processes like fixation of nitrogen into the soil, solubilization of insoluble phosphate, plant growth hormone production, siderophore production, and biocontrol agents against soil-borne pathogens (Kinjal & Pandya, 2022). The exploitation of microorganisms in soil phosphate acts on insoluble phosphate, releasing organic acids or producing enzymes. The function of phosphate-solubilizing microorganisms (PSM) in the soil is to solubilize the insoluble phosphate has been attributed mainly to the ability in reducing the pH by producing the organic acids in their surroundings (Mayadunna et al., 2023).

Many microbes influence their beneficial effect on plant growth enhancement not only in biological nitrogen fixation improvement but also in phosphate solubilization potential (Ponmurugan & Gopi, 2006). Plants required 0.3 – 0.5% Phosphate for optimum growth in the dry matter during their vegetative growth. Less leaf expansion and reduced leaf surface area are visually noticed in phosphate deficient plants, these highlights the required for adequate phosphate nutrition in the sustainable increase and development of plant growth (Doilom et al., 2020). Green leafy vegetables are popular foodstuffs around the world and constitute an important part of the human diet (Pandey & Bala, 2015). Leafy vegetables are the leaves of herbs eaten as a vegetable with rich nutrients and high fiber content. Nearly a thousand species of leafy vegetable plants are known, the majority of these come under short-lived herbaceous plants. Leafy vegetables are rich in protein with low calories and fat, hence, a daily vegetable intake of 400-600g is recommended by the Food and Agriculture Organization of the United Nations (Pandey & Bala, 2015). With these highlights, the present work is focused on improving the growth and

development of leafy vegetables such as fenugreek and spinach by providing phosphate-solubilizing fungi as phosphate bio inoculums.

## MATERIALS AND METHODS

### Rhizosphere soil samples collection

Rhizosphere soil samples of medicinal plants (10–15 cm depth around the roots) were collected from the Malnad regions of the Shivamogga district and were placed in sterile polythene covers to keep away from external contamination. The collected soil samples after brought into laboratory and kept in a refrigerator for isolation at 4°C (Amit et al., 2021; Nasr et al., 2021).

### Isolation, screening, and identification

Phosphate solubilizing fungi were isolated by the serial dilution technique using Pikovskaya's agar medium through the pour-plate method and incubated at room temperature for up to 7 days. After incubation, plate examination was done for solubilizing zones around fungal colonies, and such colonies were selected and subcultured for further use (Elfiati et al., 2021). The solubilization zone that appeared surrounding the fungal colony was an indication of phosphate solubilization and the index of phosphate solubilization was calculated by using the formula mentioned below (Mayadunna et al., 2023; Tomer et al., 2017). Identification was done by the lactophenol cotton-blue (LPCB) mounting technique (Aneja, 2009). The specimen was stained with LPCB stain, observed under the microscope at 40X magnification, and identified by referring to the standard manuals (Booth, 1971; Funder, 1961).

### Measurement of pH and titrable acidity

Sterile Pikovskaya's broth was inoculated by isolates and subjected to room temperature incubation for 7 days, meanwhile, the sterilized uninoculated broth acts as a control. The pH of the broth at the initial stage (before inoculation) and the change in pH after incubation were recorded by a digital pH meter (Mayadunna et al., 2023). For titrable acidity, about 50ml of culture supernatant was titrated against a 0.1N NaOH solution with a few drops of phenolphthalein indicator. The titrated acidity was expressed in g/L (Wang et al., 2018; Khan & Gupta, 2015).

### Quantification of phosphate

Isolates were inoculated into 250ml of a conical flask containing 100ml of sterilized Pikovskaya's broth and incubated at room temperature for 7 days at 100rpm in an orbital shaker incubator. The culture filtrate was collected after the period of incubation and then centrifuged these filtrate at 3000rpm for 30 min. Estimation of phosphate in the supernatant was done through the Vanado-molybdate yellow colour method at 420nm and was expressed in µg/ml. The phosphate amount in broth was calculated regarding the standard curve of  $\text{KH}_2\text{PO}_4$  (Mayadunna et al., 2023; Verma & Ekka, 2015).

### Seed germination and seedling vigour

Seed germination was done by the standard blotter method. The Petri plates with moist blotter sheets for both upper and lower lids were placed with seeds (16 replicates of 25 seeds each)

(Sharmila & Rebecca, 2021; Sane & Mehta, 2015) and for detection of seedling vigour, the paper towel method (50 seeds were placed in moist blotting paper towels of 8 replicates) was followed using the formula mentioned below (Mahadevamurthy et al., 2016; Pant & Agrawal, 2014).

### Preparation of bio inoculum

The inoculants were prepared by mixing the spore suspension with the carrier material (Lignite) in a ratio of 1:4 (spore suspension: carrier material). The Methi (*Trigonella foenum-graecum*, Family-Fabaceae) and Palak (*Spinacia oleracea*) seeds were treated with selected 6 PSF inoculants, PSF inoculants were mixed with sterile water separately, and the slurry was prepared. The seeds were soaked in the slurry for about 30 min in such a way that each seed was coated with a layer of PSF inoculants and air-dried. These air-dried seeds were used for field application (Aadesh et al., 2024).

### Plant growth and yield parameters

After screening and quantification of phosphate solubilization by PSF under laboratory conditions, the inoculums were amended in soils and evaluated on Methi and Palak. The parameters of plant growth include the length of a plant during growth (Sunitha et al., 2025), leaves count, root length (Sharmila & Rebecca, 2021), fresh-dry weight (Khedkar et al., 2023; Nayak et al., 2017), and yield parameters like the leafy vegetable weight of Methi and Palak were measured (Aadesh et al., 2024; Sunitha et al., 2025).

### Estimation of phosphorous uptake

The uptake of phosphorous by the tissues of Methi and Palak was estimated by the Vanado-molybdate phosphoric yellow color method. A triacid mixture of conc. Nitric acid, perchloric acid, and sulfuric acid (7:3:1 v/v) were used to digest 0.5g of

powdered plant sample. The digested residue made up to 100ml. 10ml of digested residual aliquot and 10ml of vanadium-molybdate reagent volume was made up to 50ml. The intensity of the yellow colour developed was due to the PhosphoVanado-molybdate complex. The absorbance of phosphorous was taken at 410nm. Phosphorus uptake was measured by the following formula (Khedkar et al., 2023; Rathod et al., 2023).

### Soil testing for available phosphate

The phosphorus (Kg/ha) present in the rhizosphere soil after harvesting the crop was extracted using Olsen's method. In this method sodium bicarbonate (0.5M NaHCO<sub>3</sub>) was used for the extraction process and extracted phosphorus was estimated by Olsen's reagent. Phosphate available in the rhizosphere soil was determined colorimetrically through the ascorbic acid method. The intensity of the blue color was read by a spectrophotometer at 730nm (Khedkar et al., 2023; Hefnawy et al., 2017).

## RESULTS AND DISCUSSION

### Isolation of phosphate solubilizing fungi

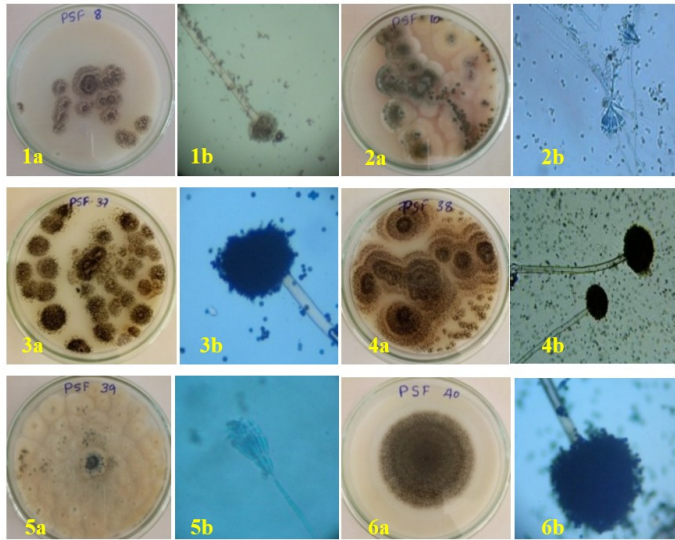
Totally 50 soil samples were collected from the different rhizosphere of medicinal plants located in Malnad regions of Shivamogga district, from them 41 fungal colonies were isolated and make revealed a solubilization zone all over the colonies on Pikovskaya's agar medium and were labeled as PSF1 to PSF41 listed below in Table 1. The present work was highlighted by earlier findings of Nasr et al. (2021) have isolate 137 fungi from rhizosphere soil cultivated with different plants, a total of 9 phosphate solubilizing fungal cultures having potential of phosphate solubilization were selected with SI of *A. niger*, *A. carbonaceous*, *A. japonicus* 3, *P. expansum* and *P. purpurgenum* was 1 while SI of *A. japonicus* 2 was 1.5.

**Table 1.** Isolation of phosphate solubilizing fungi from rhizosphere of medicinal plants.

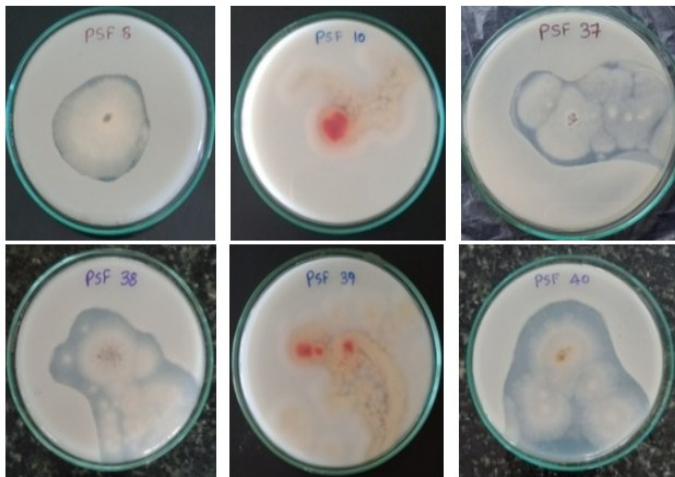
S. No.	Plant name	Code No.	S. No.	Plant name	Code No.
1.	<i>Datura fastuosa</i>	PSF 1	26.	<i>Vinca rosea</i>	PSF 21
2.	<i>Moringa oleifera</i>	-	27.	<i>Ocimum sanctum</i>	PSF 22
3.	<i>Leucus aspera</i>	PSF2	28.	<i>Solanum nigrum</i>	-
4.	<i>Phyllanthus acidus</i>	PSF 3	29.	<i>Phyllanthus emblica</i>	PSF 23
5.	<i>Argemone mexicana</i>	PSF 4	30.	<i>Amaranthus viridis</i>	PSF 24
6.	<i>Achyranthus aspera</i>	PSF 5	31.	<i>Amaranthus spinosus</i>	-
7.	<i>Centella asiatica</i>	PSF 6	32.	<i>Alternanthera sessilis</i>	PSF 25
8.	<i>Asparagus racemosus</i>	PSF 7	33.	<i>Euphorbia hirta</i>	PSF 26
9.	<i>Gymnema sylvestres</i>	PSF 8	34.	<i>Euphorbia heterophylla</i>	PSF 27
10.	<i>Tinospora cordifolia</i>	PSF 9	35.	<i>Ixora coccinea</i>	PSF 28
11.	<i>Costus ingneus</i>	PSF 10	36.	<i>Mimosa pudica</i>	PSF 29
12.	<i>Saraca asoca</i>	PSF 11	37.	<i>Cassia occidentalis</i>	PSF 30
13.	<i>Calotropis procera</i>	-	38.	<i>Punica granatum</i>	-
14.	<i>Calotropis gigantea</i>	PSF 12	39.	<i>Asclepias curassavica</i>	PSF 31
15.	<i>Vitex nigundo</i>	PSF 13	40.	<i>Bauhinia purpurea</i>	PSF 32
16.	<i>Holorrhena antidiysenterica</i>	PSF 14	41.	<i>Momordica charantia</i>	PSF 33
17.	<i>Eugenia jambolina</i>	-	42.	<i>Solanum xanthocarpum</i>	PSF 34
18.	<i>Clitoria ternatea</i>	PSF 15	43.	<i>Eclipta prostrata</i>	PSF 35
19.	<i>Wrightia tinctoria</i>	PSF 16	44.	<i>Brassica sp.</i>	PSF 37
20.	<i>Phyllanthus amarus</i>	-	45.	<i>Anacardium occidentale</i>	-
21.	<i>Santalum album</i>	PSF 17	46.	<i>Phyllanthus niruri</i>	PSF 38
22.	<i>Azadirachta indica</i>	PSF 18	47.	<i>Phyllanthus sp.</i>	PSF 39
23.	<i>Aegle marvelous</i>	-	48.	<i>Acalypha indica</i>	PSF 40
24.	<i>Eucalyptus sp.</i>	PSF 19	49.	<i>Ricinus communis</i>	PSF 41
25.	<i>Pongamia glabra</i>	PSF 20	50.	<i>Tabernaemontana divaricate</i>	-

### Screening and identification of phosphate solubilizing fungi

The solubilization index of those 41 isolates was measured by inoculating them onto Pikovskaya's media. Among them, 6 fungi with a maximum solubilization index were selected and further evaluated for their phosphate solubilization under laboratory and field applications. The selected six PSF were identified as PSF 8 (*Aspergillus aculeatus*), PSF 10 (*Talaromyces amestolkiae*), PSF 37 (*Aspergillus carbonarius*), PSF 38 (*Aspergillus japonicus*), PSF 39 (*Penicillium oxalicum*) and PSF 40 (*Aspergillus niger*) based on their cultural characteristics (Figure 1). The selected 6 phosphate solubilizing fungi showed solubilization indices from 3.08



**Figure 1.** Pure culture and microscopic view of PSF 8 - (1a and 1b), PSF 10 (2a and 2b), PSF 37 (3a and 3b), PSF 38 (4a and 4b), PSF 39 (5a and 5b) and PSF 40 (6a and 6b).



**Figure 2.** Solubilization index of PSF 8 (A), PSF 10 (B), PSF 37 (C), PSF 38 (D), PSF 39 (E) and PSF 40 (F).

to 3.86 (Table 2 and Figure 2). Solubilization of phosphate was observed and correlated with previous reports by Elfiati *et al.* (2021) has isolate 12 and 8 phosphate solubilizing and cellulolytic fungi respectively. Whereas Mayadunna *et al.* (2023) have identified 12 bacterial isolates (B1– B12), 11 fungal isolates (F1–F11), and an actinomycete (B13). Tomer *et al.* (2017), who studied the solubilization index of three bacterial isolates ranging from 7.2 to 62 mm.

### Measurement of pH and titrable acidity

The selected 6 fungal cultures reduced the pH of the broth and values ranged from 4.0 to 3.1 from an initial pH of 6.89. The amount of acid present in the culture broth of selected 6 PSF was measured titrimetrically using strong alkali and ranged from 30.16g/L to 38.08g/L (Table 2). Organic acids produced by the PSF cultures reduce the pH in the culture media. Meanwhile, the results obtained in our work were correlated with the earlier reports of Mayadunna *et al.* (2023) have observed significantly dropped pH compared to the controls. The pH value of the controls was  $6.20 \pm 0.01$  in the TCP-enriched PK broth and ERP-enriched PK broth. The lowest pH was observed in F10 as  $3.07 \pm 0.05$  in ERP-enriched media and  $3.68 \pm 0.02$  in TCP-enriched media. F1 recorded the highest pH in the ERP enriched medium as  $6.27 \pm 0.09$ . It was slightly higher than the pH of the control. The same was observed in F11, where pH increased more than the control, in the TCP-enriched broth.

### Quantification of phosphate

The concentration of phosphate in selected 6 PSF culture filtrates was estimated using the Vanado-molybdate yellow color method and a standard curve of  $\text{KH}_2\text{PO}_4$  ranging from 60g to 25g (Table 2). Estimation of Phosphate by the Vanado-molybdate method was adopted in our work and the results were correlated with earlier findings of Mayadunna *et al.* (2023) have checked available P concentration, the highest significant improvement was shown by F10 as  $655.55 \pm 10.41$  mg/L. F7,  $626.98 \pm 17.88$  mg/L and B4,  $620.63 \pm 7.94$  mg/L have also shown higher improvements than other cultures. The lowest improvement was shown by B6 as  $138.09 \pm 17.17$  mg/L. The available P concentrations in the culture broths F1, F2, and F4 and B13 had reduced the available P concentration by more than that of the control. Meanwhile Verma & Ekka (2015) where the concentration of phosphate in culture broth ranged from  $219.16 \mu\text{g/ml}$  to  $59.17 \mu\text{g/ml}$ .

**Table 2.** Phosphate solubilization parameters of selected isolates.

S. No.	Code No.	Culture	SI	pH	TA	Conc. of P in $\mu\text{g}$
1.	PSF 8	<i>Aspergillus aculeatus</i>	3.08	4.0	30.16	60
2.	PSF 10	<i>Talaromyces amestolkiae</i>	3.21	3.8	32.80	45
3.	PSF 37	<i>Aspergillus carbonarius</i>	3.54	3.6	37.76	30
4.	PSF 38	<i>Aspergillus japonicus</i>	3.61	3.4	37.54	30
5.	PSF 39	<i>Penicillium oxalicum</i>	3.86	3.1	38.08	25
6.	PSF 40	<i>Aspergillus niger</i>	3.45	3.7	37.6	30

### Seed germination and seedling vigor

The % germination of seeds in Fenugreek and Spinach was recorded as 100% and 100% respectively, and the seedling vigor of the selected Fenugreek and Spinach seeds was calculated as 1211, 1299 on the 7<sup>th</sup> day after incubation, respectively and 1495, 1564 on the 14<sup>th</sup> day after incubation, respectively. Phosphate fertilizers are required for crop production, and inoculation of PSF to the plants increases the growth and yield in plants. Obtained results in our work contradictory to the findings of Mahadevamurthy *et al.* (2016), who isolated 22 fungi from different rhizosphere soils of healthy crop plants, and good seed germination and seedling vigour were recorded in pearl millet, brinjal, and tomato.

### Growth and yield parameters of fenugreek and spinach

Among the selected 6 PSF inoculants, PSF 39 (*Penicillium oxalicum*) showed the highest plant growth and yield in Fenugreek. Length of Plant and leaves count was 7.8cm and 15 leaves 15 days after sowing, and 10.1cm and 21 leaves at harvest. Root length was measured as 5.5cm after harvesting the crop. The fresh-dry weight of the plant is one of the important growth parameters, fresh and dry weights were measured as 2.11g and 1.01g, respectively. After harvesting the crop, the maximum weight of yield, such as the weight of leaves of Fenugreek was 2.01 when contrasted to the control plants (1.01) (Table 3 and

Figure 3a, b, c). The results were observed in the present work was highlighted with earlier findings of Rathod *et al.* (2023) have indicated the results foliar application of GA3 at 50 ppm at both 25 and 50 DAS resulted in significantly higher values for various growth parameters, including primary and secondary branches per plant, as well as yield attributes such as the number of pods per plant, number of seeds per pod, pod length, number of seeds per plant, and test weight. This treatment also exhibited superior seed and stover yields, as well as biological yield, compared to the control (water spray). Notably, the performance of GA3 at 50 ppm was comparable and not significantly different from GA3 at 25 ppm and NAA at 50 ppm, especially concerning seed and stover yields.

Among the selected 6 PSF inoculants, PSF 38 (*Aspergillus japonicus*) showed the highest plant growth and yield in Spinach.



Figure 3a. Methi crop at field, b. harvesting and c. Yield of leafy vegetable fenugreek

Table 3. Plant growth and yield parameters of fenugreek.

S. No.	Code No.	Plant growth parameters					Yield parameters		
		Plant height (in cm)		No. of leaves		Root length (in cm)	Weight of biomass		Leafy vegetable weight (in g)
		15 days	At harvest	15 days	At harvest		Fresh weight	Dry weight	
1.	Control	4.1	6.3	3	9	3.8	1.47	0.14	1.01
2.	PSF 8	6.5	7.9	9	15	5.1	1.62	0.27	1.18
3.	PSF 10	7.1	9.1	12	18	4.9	1.98	0.96	1.51
4.	PSF 37	4.9	4.7	9	15	4.7	1.59	0.21	1.33
5.	PSF 38	6.9	9.4	12	18	5.2	1.92	0.87	1.74
6.	PSF 39	7.8	10.1	15	21	5.5	2.11	1.01	2.01
7.	PSF 40	4.7	7.2	9	15	4.1	1.52	0.19	1.29

Table 4. Plant growth and yield parameters of spinach.

S. No.	Code No.	Plant growth parameters					Yield parameters		
		Plant height (in cm)		No. of leaves		Root length (in cm)	Weight of biomass		Leafy vegetable weight (in g)
		15 days	At harvest	15 days	At harvest		Fresh weight	Dry weight	
1.	Control	9.7	19.5	3	5	7.3	5.36	1.27	5.16
2.	PSF 8	11.2	22.1	4	6	12.9	6.84	1.91	6.47
3.	PSF 10	13.5	27.1	6	9	10.1	8.65	2.99	9.02
4.	PSF 37	12.6	25.3	5	8	8.4	9.97	3.23	7.99
5.	PSF 38	15.7	30.6	8	11	10.9	11.23	3.82	10.32
6.	PSF 39	10.9	20.8	4	7	7.8	6.13	1.58	5.89
7.	PSF 40	11.4	22.3	5	7	7.5	67.56	2.26	7.15

Length of Plant and leaves count was 15.7cm and 8 leaves were recorded 15 days after sowing, and 30.6cm and 11 leaves were recorded at harvest. Root length was measured as 10.9cm after harvesting the crop (Figure 5). The fresh and dry weight of the plant is one of the important growth parameters, fresh and dry weights were measured as 11.23g and 3.82g, respectively. After harvesting the crop, the maximum weight of yield, such as the weight of leaves of Spinach was 10.32g compared to the control plants' 5.16g (Table 4 and Figure 4a, b, c). Hence the results were correlated with earlier findings of Sunitha et al. (2025) have noticed integrated nutrition containing treatment T4 (75% RDF + FYM 5 t/acre + Vermicompost 2.5 t/acre + Neem cake 125 Kg/acre) is superior over the all the treatments and suited in order to obtain higher growth of Spinach. Meanwhile Khedkar et al. (2023) have recorded among the nutrient levels, application of N6 (120:70:60 NPK kg/ha) recorded highest growth parameters, quality parameters and nutrient uptake. Though combined effect of varieties and nutrient levels was non-significant with respect to all the parameters of Spinach.

#### Estimation of plant phosphorus

The plant P uptake (%) by the crops was estimated and the maximum plant phosphorus uptake was recorded in fenugreek plants that were treated with PSF 39 (*Penicillium oxalicum*) as 0.396% and PSF 38 (*Aspergillus carbonarius*) in spinach, respectively (Table 6). Plant phosphorus uptake was correlated with earlier findings of Rathod et al. (2023) have reported the foliar application of GA3 at 50 ppm at 25 and 50 DAS resulted in a notable enhancement in nutrient content (N, P, and K) and their uptake by fenugreek seeds and stover. This improvement was more pronounced compared to the control and GA3 at 25 ppm.



Figure 4a. Palak crop at field, b. harvesting and c. Yield of leafy vegetable spinach

Meanwhile Khedkar et al. (2023) have recorded N6 (120:70:60 NPK kg/ha) was recorded maximum fresh weight of plant (24.67 g, 24.68 g and 27.06 g), maximum dry weight of plant (4.70 g, 5.11 g and 5.20 g) at 1<sup>st</sup>, 2nd and 3rd cuttings, respectively, maximum TSS content (8.16 °Brix), maximum iron content of leaf (14.90 mg/100 g) and maximum N (3.122%), P (0.862%) and K (3.743%) content in plant at harvest.

#### Soil analysis for available phosphorus (Kg/ha)

The maximum P (Kg/ha) available in the rhizosphere soil of the crop was recorded. The values for PSF 39 (*Penicillium oxalicum*) in Methi were 346.96Kg/ha and for PSF 38 (*Aspergillus japonicus*) in Palak were 346.29Kg/ha, respectively (Table 5). Olsen extracts of air-dried soil for analysis of available phosphorus (Kg/ha) were estimated colorimetrically using the ascorbic acid method as followed by earlier findings by Khedkar et al. (2023) have recorded maximum nitrogen (216.05 kg/ha), phosphorus content in soil (20.23 kg/ha) and potassium (418.42 kg/ha) content in soil after harvest was found with N6 (120:70:60 NPK kg/ha). Hefnawy et al. (2017) who obtained similar results while using *Aspergillus niger* and *Aspergillus fumigatus* as PSF.

Table 5. Influence of PSF inoculants in plant phosphorus estimation.

S. No.	Culture code	Plant phosphorus	
		Fenugreek	Spinach
1	Control	0.192	0.187
2	PSF 8	0.268	0.265
3	PSF 10	0.363	0.351
4	PSF 37	0.255	0.267
5	PSF 38	0.319	0.369
6	PSF 39	0.393	0.285
7	PSF 40	0.226	0.254

Table 6. Influence of PSF inoculants on available phosphorus of soil after harvest the crop.

S. No.	Culture code	Available P in soil (Kg/ha)			
		Fenugreek		Spinach	
		pH of the soil	Phosphate	pH of the soil	Phosphate
1	Control	7.01	208.06	7.33	193.51
2	PSF 8	7.31	234.75	7.51	248.32
3	PSF 10	7.28	304.18	7.50	303.12
4	PSF 37	7.17	218.29	7.68	204.67
5	PSF 38	7.08	247.32	7.31	346.29
6	PSF 39	7.51	346.96	7.67	234.74
7	PSF 40	7.45	214.67	7.50	239.10

## Conclusion

In conclusion, phosphorus is one of 17 nutrients considered key nutrient next to nitrogen. Phosphorus is standing most needed element for the growth and development of plants, it is making up about 0.2% of the plant's dry weight. In nature, several distinct species of bacteria and fungi majorly solubilize the insoluble forms of the Phosphate compound. The low-cost practice to achieve this objective is to inoculate the soil with PSF and bacteria. The present work aims to highlight major PSF, PSF 39 (*Penicillium oxalicum*) for *Trigonella* sp. and PSF 38 (*Aspergillus japonicus*) with maximum plant growth and yield which could be used for bio-inoculum preparation in an eco-friendly manner. Primarily, the preparation of bio inoculums will reduce dependency on chemical fertilizer and this will reduce the accumulation of phosphorus in reservoirs and will make the environment safe from pollution.

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## DECLARATIONS

**Author contribution statement:** Data collection, Methodology, Formal analysis and investigation, writing-original draft preparation V. J.; Software and validation, Writing review and editing: G. N.; Conceptualization. Resources, Visualization, Supervision, Project administration B. T.

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