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Effect of Phosphate Solubilizing Fungi from Rhizosphere soil of Medicinal plants on Growth and Phosphate uptake in *Raphanus sativus*

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ABSTRACT: This field experiment deals with the isolation and evaluation of phosphate solubilizing fungi of a total of 9 isolates isolated from 10 rhizosphere soil of medicinal plants by serial dilution method using Pikovaskaya's medium. After screening, 3 fungi were selected and evaluated, PSF 7 (*Talaromyces* sp.) showed good results in phosphate solubilization including solubilization index (3.08), reduced pH (3.2 from initial pH 6.8), titrable acidity (38.08), and phosphate present in culture broth (25µg/ml). The radish seeds showed 88% germination and growth, yield parameters include, plant height - 20.9cm, 40.8cm, and 71.5cm at 15 days, 30 days, and harvest respectively, several leaves at 9, 13, and 17 at 15 days, 30days and harvest respectively, root length (24.5cm), the weight of biomass (fresh weight -152 and dry weight – 15.7) and yield of root vegetable weight (39g). The maximum plant phosphorus uptake was recorded as 0.371% and the maximum P (Kg/ha) available in the rhizosphere soil was recorded as 344.29 Kg/ha. Due to the observation of good results in phosphate solubilization, they improve the growth and yield of radish. The selected PSF were recommended as phosphate bio inoculums in the agricultural field to improve plant growth and yield in radish and to maintain soil fertility.

KEY WORDS: Plant growth, Phosphate uptake, Radish, Solubilization index, Titrable acidity.

INTRODUCTION

Vegetables are important agricultural products, they are very much required for people's lives and the vegetable industry is an important industry related to the national economy and people's livelihood (Yousaf *et al.,* 2021). Radish (*Raphanus sativus* L.), is a root vegetable that belongs to the family Brassicaceae and is the most popular and widely root vegetable grown for its tender fleshy edible roots in both tropical and temperate regions (Lavanya *et al.,* 2014). It is one of the most ancient root vegetables rich in carbohydrates, protein, crude fiber, vitamin C, calcium, potassium, iron, and manganese (Yousaf *et al.,* 2021). It also has many health benefits and also a good source of antioxidants, so it reduces the risk of heart disease, reduces the risk of diabetes, and enhances liver function. The present area under radish in India is 2.84 lakh/ha with a production of 35.21 lakh tones and productivity of 12,390 kg/ha (Lavanya *et al.,* 2014).

Radish being a quick-growing crop required proper fertilization for sustaining better economic productivity. NPK is considered important for maximizing the production of radishes; therefore, a need for a proper supply of NPK increases the growth and yield of radishes (Ndang and Senna 1999). Phosphorus plays a significant role in plant metabolism and is important for the functioning of key enzymes that regulate metabolic pathways (Nisha *et al.,* 2014). Soil microorganisms play a key role in soil P dynamics and the subsequent availability of phosphate to plants (Anand *et al.,* 2016). Many types of microorganisms are known to inhabit soil, especially the rhizosphere and play an important role in phosphate solubilization. Microorganisms play an important role in the solubilization of insoluble phosphate in soil and making them available to plants is a well-known mechanism (Bhattacharya and Jain 2000) and such organisms are called Phosphate Solubilizers.

The major mechanism of mineral phosphate solubilization is the action of organic acid and phosphatase enzymes synthesized by soil microorganisms. Production of these organic acids resulted in the acidification of the microbial cell and its surroundings (Nisha *et al.,* 2014). Phosphate solubilizers not only provide phosphate to the plants but also facilitate the growth of plants by stimulating the efficiency of Nitrogen fixation, accelerating the accessibility of other trace elements, and synthesizing important growth-promoting substances including siderophore and antibiotics, and providing protection to plants against soil-borne pathogens. Hence in the present study different phosphate solubilizing fungi were isolated from the rhizosphere of different medicinal plants and the inoculants were used in field application to improve the growth and yield of radish.

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MATERIALS AND METHODS

Collection of rhizosphere soil samples

The rhizosphere soil samples at 10 – 15cm depth around the roots of medicinal plants were collected from the Malnad regions of the Shivamogga district and were placed in sterile polythene bags to avoid external contamination. These soil samples were brought to the laboratory and stored in the refrigerator at 4℃ until they were used for the isolation of phosphate-solubilizing fungi (Chatli *et al.*, 2008).

Isolation and Screening of Phosphate Solubilizing Fungi

Phosphate solubilizing fungi were isolated followed by the serial dilution method. In this method, about 1g of rhizosphere soil was suspended in 9 ml of sterilized 0.85% saline. Then serially diluted samples (0.1ml of each 10^{-4}, 10^{-5}, and 10^{-6} dilutions) were plated on Pikovskaya's agar plates (Tri-calcium phosphate 5g, Glucose 10g, Ammonium sulfate 0.5g, Potassium chloride 0.2g, $MgSO_4$ $7H_2O$ 0.1g, $MnSO_4$ $7H_20$ trace, Ferrous sulfate trace, Yeast extract 0.5g, Distilled water 1000ml, Agar 20g and pH - 7.2) and incubated for 7 days at room temperature. After incubation plates were examined for solubilization zone around fungal colonies and colonies showing solubilization zone were selected and sub-cultured on fresh media for further use (Nelofer *et al.*, 2015). The fungal cultures were point inoculated on Pikovskaya's agar medium and incubated at room temperature for 7 days. Then the solubilization index was calculated by using the following formula (Tomer *et al.*, 2017; Elias *et al.*, 2016).

$$SI = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

Characterization of Phosphate Solubilizing Fungi

Identification of PSF was done by lactophenol cotton-blue (LPCB) mounting technique (Aneja 2009). The specimen was stained with LPCB stain, the coverslip was placed above it and observed under the microscope at 40X magnification, and characters were noted by observing spore shape, spore size, spore arrangement, and arrangement of hyphae and identified by referring to the standard manuals (Booth 1971; Funder 1961).

Measurement of pH and Titrable acidity

Isolates were inoculated into Pikovskaya's broth and incubated at room temperature for 7 days. The sterile un-inoculated broth served as control. Initial pH and change in pH after incubation was recorded by digital pH meter (Jain and Singh 2015; Kumar *et al.*, 2014). For titrable acidity, about 50ml of culture supernatant was titrated against 0.1N NaOH solution with 2-3 drops of phenolphthalein indicator. The titrable acidity was expressed in g/L (Wang *et al.*, 2018; Khan and Gupta 2015).

Quantification of Phosphate

Phosphate solubilizing fungi were inoculated in 100ml of Pikovskaya's broth in a 250ml conical flask and incubated at room temperature for 7 days at 100rpm in an orbital shaker incubator. After incubation culture filtrate of PSF was collected and centrifuged at 3000rpm for 30min. Estimation of phosphate in the supernatant was done by the Vanadomolybdate yellow colour method and it was expressed in µg/ml. The amount of phosphate was calculated from a standard curve of KH_2PO_4. The absorbance of the developing yellow colour was measured at 420nm (Verma and Ekka 2015; Kumar *et al.*, 2014).

Seed germination and seedling vigor

The percentage of seed germination and seedling vigor of radish seeds were tested before field application. Seed germination was tested by the standard blotter method (Sane and Mehta 2015) and seedling vigor was determined by the paper towel method (Mahadevamurthy *et al.*, 2016) using the following formula.

$$\% \text{ Seed germination} = \frac{\text{No. of seed germinated}}{\text{Total no. of seeds placed}} \times 100$$

Seedling Vigor index = [Mean Shoot Length + Mean root Length] × Seed germination

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Preparation of bioinoculum

The inoculants were prepared by mixing the spore suspension with the carrier material (Lignite) in a ratio of 1:4 (spore suspension: carrier material). Radish (*Raphanus sativus*) seeds were treated with PSF inoculants, PSF inoculants were mixed with sterile water, and the slurry was prepared, then the seeds were soaked in the slurry and mixed in such a way that each seed was coated with a layer of PSF inoculants and air-dried, these treated seeds were used for field application (Saxena *et al.,* 2015).

Plant growth and yield parameters

After screening and quantification of phosphate solubilization by PSF under laboratory conditions, the treated radish seeds were sown into the field**,** and evaluated the plant growth parameters which includes plant height, number of leaves, root length, fresh and dry weight, and yield parameters which include the root vegetable weight was (Randy 2016; Vedpathak and Chavan 2016).

Estimation of Plant Phosphorous

Phosphorous uptake of radish plants was determined by the Vanado-molybdate phosphoric yellow colour method. 0.5g of powdered plant sample was digested in a triacid mixture comprising conc. Nitric acid, Perchloric acid, and Sulphuric acid (7:3:1 v/v). The digested residue was made up to 100ml. 10ml of digested residual aliquot and 10ml Vanadomolybate reagent and volume made up to 50ml. The intensity of the yellow colour developed due to the Phosphovanadomolybdate complex. The phosphorous uptake was determined by measuring the absorbance at 410nm. Plant phosphorus was measured by the following formula (Abbas *et al.,* 2013; Malviya *et al.,* 2011).

$$\text{P\% in Plant} = \frac{\text{P (ppm in plant) x Volume of Digest X 100}}{\text{Weight of plant}}$$

Soil Analysis for Available P (Kg/ha)

The available phosphorus (Kg/ha) in the rhizosphere soil of radish after harvesting the crop was extracted using Olsen's method. Here sodium bicarbonate (0.5M $NaHCO_3$) was used for the extraction process. The sodium bicarbonate solution extracts some exchangeable or surface-absorbed Al-P, Fe-P, Ca-P, and other forms of Phosphates. The extracted phosphorus was estimated by Olsen's reagent, available phosphate was determined using the ascorbic acid method, and the intensity of the blue colour was read by using a spectrophotometer at 730nm (Hefnawy *et al.,* 2017).

RESULTS AND DISCUSSION

Isolation, screening, and characterization of Phosphate Solubilizing Fungi

About 10 medicinal plant rhizosphere soil samples were collected from the Malnad regions of the Shivamogga district, from them 9 phosphate solubilizing fungi were isolated and they were labeled as PSF 1 to PSF 9 listed below in Table 1. The solubilization index (SI) of isolated 9 fungal colonies was measured by inoculating them onto Pikovasky's media in the range of 1.29 – 3.08. Among them, 3 phosphate solubilizing fungi showed the maximum solubilization indices i.e., *Penicillium* sp. as 3.01, *Talaromyces* sp. as 3.08, and *Aspergillus* sp. as 3.02. The maximum SI was recorded in *Talaromyces* sp. (Fig 1).

The results obtained were correlated with earlier findings of Chatli *et al.,* (2008) they collected the soil samples at 15cm depth of rhizosphere and non-rhizosphere of *Salix alba* for isolation and characterization of phosphate solubilizing microorganisms. Meanwhile, Nelofer *et al.,* (2016) serially diluted the soil samples and inoculated them in Pikovskaya's agar by pour plate method. Among the 45 soil samples, 11 were given colonies with clear zones that were considered P solubilizing strains. As that the results were highlighted by Tomer *et al.,* (2017) who studied the solubilization index of three bacterial isolates ranged from 7.2 to 62mm, and Elias *et al.,* (2016) obtained SI of 359 fungal isolates ranged from 1.10 to 3.05.

Table 1: Collection of rhizosphere soil sample from medicinal plants and isolation of PSF

Sl. No	Medicinal plants	Culture code	Culture	Solubilization Index
1	*Daturafastuosa*	PSF 1	*Aspergillus* sp.	2.47
2	*Moringaoleifera*	-	-	-
3	*Leucusaspera*	PSF2	*Aspergillus* sp.	2.70

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4	*Phyllanthusacidus*	PSF 3	*Alternaria*sp.	2.16
5	*Argemonemexicana*	PSF 4	*Aspergillus* sp.	1.29
6	*Achyranthusaspera*	PSF 5	*Penicillium* sp.	3.01
7	*Centellaasiatica*	PSF 6	*Penicillium* sp.	2.51
8	*Asparagus racemosus*	PSF 7	*Talaromyces* sp.	3.08
9	*Gymnemasylvestres*	PSF 8	*Aspergillus*sp.	3.02
10	*Tinosporacordifolia*	PSF 9	*Penicillium* sp.	1.60

Fig 1: Pure culture and microscopic observation (40X) of ***Talaromyces* sp.**

Measurement of pH and Titrable acidity

Various organic acids produced by the PSF lead to the acidification of the microbial cell and its surroundings for P solubilization. These organic acids reduce the pH in the culture media and decreased pH was observed in Pikovskaya's broth. The selected 3 PSF reduce the pH of the broth was recorded ranging from 3.2 to 4.2 from initial pH of 6.89. The amount of acid present in the culture broth of selected 3 PSF ranged from 30.01g/L to 38.08g/L (Table 2). Hence the results obtained correlated with earlier reports of Kumar *et al.*, (2014) they observed a significant change in the pH of broth (5.5 and 4.8) was shown by *B. megaterium* over control followed by *A. chlorophenolicus* respectively. And also Khan and Gupta (2015) have checked the ability of acid production of 29 acidophilic fungal isolates which were isolated and among them, 5 isolates LAK-2, BS-1.6, CM-2, DR-1 and DR-2 showed good acid production.

Quantification of Phosphate

The concentration of phosphate present in the culture filtrate of selected 3 PSF ranged from 60µg to 25µg was estimated by the vanadomolybdate yellow colour method using the standard curve of KH_2PO_4 (Table 2). Estimation of phosphate by the Vanadomolybdate method was adopted and the results were correlated with earlier findings of Verma and Ekka (2015) were reported the concentration of phosphate in culture broth ranged from 219.16µg/ml to 59.17µg/ml.

Table 2: Phosphate solubilization parameters

Sl no.	**Plant name**	**Culture code**	**Culture**	**P^H**	**TA**	**Conc. of P in µg**
1	*Achyranthusaspera*	PSF 5	*Penicillium* sp.	4.3	30.01	60
2	*Asparagus racemosus*	**PSF 7**	***Talaromyces* sp.**	**3.2**	**38.08**	**25**
3	*Gymnemasylvestres*	PSF 8	*Aspergillus*sp.	4.0	32.9	45

Seed germination and seedling vigor

The percentage of seed germination of the radish was recorded as 88% and seedling vigor as 1263.68 and 1466.96 on the 7th and 14th day after incubation respectively. The seeds were treated with PSF inoculants such as PSF 5, PSF 7, and PSF 8, seeds were directly sowed in the field. The results are contradictory to the findings of Sane and Mehta (2015) have isolated and identified *Aspergillus* and *Penicillium* spp. and checked the rock P solubilization and their seed germination in bajra seeds. Mahadevamurthy *et al.*, (2016) have isolated 22 rhizospheric fungi from different rhizosphere soil of healthy crop plants and checked the seed germination and seedling vigor. Maximum of 85.75 %, 80 %, and 83 % of seed germination and seedling vigor 985.25, 523 and 673.5 was recorded in pearl millet, brinjal, and tomato, respectively.

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Plant growth and yield parameters of radish

Plant growth parameters include plant height at 15 days of regular intervals recorded. Among the selected 3 PSF inoculants, PSF 7 (*Talaromyces* sp.) showed maximum plant growth and yield in radish. Plant height and number of leaves were, 20.9cm, and 9 leaves were recorded at 15 days after sowing, 40.8cm and 13 leaves were recorded at 30th days after sowing and 71.5cm and 17 leaves were recorded at harvest. The root length was measured as 24. 5cm. The fresh and dry weight of the radish was 152g and 15.7g respectively. After harvesting the crop, the maximum yield of root vegetables was 39g (Table 3) (Fig 2). The fresh and dry weight of the plant is one of the important growth parameters. The results obtained were correlated with earlier findings of Vedpathak and Chavan (2016) have studied the effect of organic and chemical fertilizers on the growth and yield of radish compared with the control. While Randy (2016) has evaluated the effect of different varying levels of vermicasts on the growth and yield of the radish.

Table 3: Plant growth and yield parameters of Radish

Sl No.	Code No.	Plant growth parameters									Root vegetable weight (in g)
		Plant height (in cm)			No. of leaves			Root length (in cm)	Weight of biomass		
		15 days	30 days	At harvest	15 days	30 days	At harvest		Fresh weight	Dry weight	
1	Control	15.2	31.5	53.1	5	8	11	17.3	52	5.6	**23**
2	PSF 5	17.1	35.9	62.7	7	10	12	21.3	89	9.1	**28**
3	PSF 7	**20.9**	**40.8**	**71.5**	**9**	**13**	**17**	**24.5**	**152**	**15.7**	**39**
4	PSF 8	18.2	37.3	63.3	7	11	15	22.6	94	9.8	**31**

Fig 2: Root vegetable of Radish after harvesting the crop

Estimation of Plant Phosphorous

The influence of the selected 3 PSF inoculants on phosphate uptake (%) in the radish plants is represented in table 4. The plant P uptake (%) by the crops was estimated and the maximum plant phosphorus uptake was recorded in PSF 7 (*Talaromyces*sp.) treated radish plants was 0.371% compared to the control plants. Plant Phosphate uptake was determined by the Vanado-molybdate phosphoric yellow colour method while Abbas et al. (2013) followed the same method for determining the plant P uptake and results showed that higher plant P contents (0.29) were observed in treatment having the combination of IpleIple (II) + PSB + Recommended K + ¾ N + ¾ P followed by 0.24. The minimum plant phosphorous content (0.10) was recorded in the control.

Soil analysis for available Phosphate (Kg/ha)

The available Phosphate (Kg/ha) in the rhizosphere soil of crop plants after harvesting were represented in table 4. The maximum Phosphate (Kg/ha) available in the rhizosphere soil of radish crop was 344.29Kg/ha treated PSF 7 (*Talaromyces* sp.) in radish plants compared to the control plants. While Olsen extracts of air-dried soil for analysis of available phosphorus (Kg/ha) were estimated using the ascorbic acid method as followed by earlier findings of Hefnawy *et al.*, (2017) who obtained similar results while using *Aspergillus niger* and *Aspergillus fumigates* as PSF.

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Table 4: Effect of PSF inoculants on plant Phosphorus uptake (%) and available Phosphorus in rhizosphere soil of radish after harvest the crop

Sl No.	Culture code	Plant phosphorus uptake (%)	Available P in soil (Kg/ha)	
			P^H of the soil	Phosphate
1	Control	0.178	7.35	198.15
2	PSF 5	0.289	7.49	213.98
3	PSF 7	0.371	7.41	344.29
4	PSF 8	0.265	7.58	239.11

CONCLUSION

Phosphorus (P) is the second most important nutrient next to nitrogen. P is an essential element for plant growth and development, making up about 0.2% of plant dry weight. In nature, several different bacterial and fungal species majorly solubilize inorganic and organic forms of the P compound. Therefore primary approach in the agronomic management of phosphate is to scavenge the native/fixed P and also to overcome the fixation of applied P fertilizer. The low-cost practice to activate this objective is to inoculate the soil with phosphate-solubilizing fungi. The application of phosphate solubilizing bioinoculum to soil significantly increased the plant height, number of leaves, root length, and yield of root vegetables. Among the various isolates, PSF 7 (*Talaromyces* sp.) was found more beneficial phosphate solubilizer and significantly improved the growth and yield of radish crops. So the *Talaromyces* sp. can be recommended as a P solubilizing biofertilizer.

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