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### RESEARCH ARTICLE

## BIODIVERSITY OF PHOSPHATE SOLUBILIZING FUNGI ISOLATED FROM AGRICULTURAL FIELDS OF MARATHWADA REGION

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### Abstract

Fungi are the core components of soil microorganisms, accounting for more of the soil biomass than bacteria, depending on soil depth and nutritional requirements. In the current study, phosphate solubilizing fungi were isolated from the soil of Maharashtra's Marathwada area, and all fungal isolates were examined for their ability to solubilize phosphate. Only 11 fungal isolates out of a total of 40 were found to have P-solubilizing activity. After 48 hours of incubation, the fungal isolates *Aspergillus niger* (PQ9), *Trichoderma* spp (PQ36), and *Penicillium* spp (PQ19) demonstrated a considerable zone of solubilization with 34, 31 to 30 mm on selective agar medium. The potent phosphate solubilizing fungi were identified in 18S rRNA analysis. The study, therefore, proposed that these fungal species have strong phosphate solubilizing properties and can be used for excellent crop productivity as a biofertilizer.

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### Introduction:-

Phosphate-solubilizing microorganisms have been studied in soils, mangroves, and the rhizosphere by a number of researchers [1,2]. The phosphate solubilizing microorganism has the ability to release metabolites such as organic acids, which chelate into the soil due to their hydroxyl and carboxyl groups and are then transformed to soluble forms. Different microbial reactions, including organic acid synthesis and proton extrusion, are used to solubilize phosphate. Different microbial phosphate solubilization routes are present in nature in the cycling of insoluble organic and inorganic soil phosphates [3].

Fungi have been demonstrated to have a better ability to solubilize insoluble phosphate than bacteria [4]. Insoluble phosphorous species have been reported to be solubilized by soil fungi such as *Aspergillus niger* and *Penicillium*, which are the most prevalent fungi capable of solubilizing phosphate [5]. Many soil researchers have performed research into phosphate solubilizing microorganisms [6,7], mangrove [8], and rhizosphere [9,10]. From such exploration's different forms of phosphate solubilizing microorganisms have been successfully described. In recent decades, phosphate has been identified in a broad range of rhizosphere bacteria and fungi, including *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Pantoea agglomerans*, *Enterobacter aeruginosa*, *Penicillium*, and *Azotobacter chroococcum* [11].

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*Aspergillus* spp. and *Penicillium* the dominant filamentous phosphate solubilizing fungi in the rhizosphere are [12]. As sources of organic acid and other biotechnological uses, such as biocontrol, biodegradation, and mobilization of phosphate, they are widely used [13,14].

Fertilisers are now widely used in the Marathwada region in place of manures. As a result, crop production is increasing at a rapid rate, while soil quality and microbial diversity are declining. However, little is known about the diversity of phosphate-solubilizing fungus present in the rhizospheres in this region. The aim of this research was to look into the diversity of phosphate solubilization efficiency of isolated fungi from the Marathwada region that have the potential to promote plant growth in nutrient-deficient soils.

## Materials And Methods:-

### Sample Collection:

A total of 160 rhizosphere soil samples were collected from various locations namely Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad, and Parbhani districts of Marathwada, Maharashtra (India). The study's main focus was on analyzing soil samples obtained from every tehsil in the Marathwada district.

### Physicochemical analysis of Soil Samples:

Soil samples were collected in a sterilized bottle and thoroughly mixed on a clean cloth before being broken up with a wooden pestle and mortar and air-dried [15]. The air-dried samples were sieved into ten mesh sizes, placed in glass bottles, and labelled for analysis. After collection, a portion of each sample was immediately transferred to the laboratory and stored at 4°C for microbial analysis. Physicochemical parameters like pH, Electrical Conductivity (EC), Total Organic Carbon, Available Nitrogen (N), Available Phosphorus (P<sub>2</sub>O<sub>5</sub>), and available potassium (K<sub>2</sub>O) of soil samples were analyzed as per the methods recommended by APHA [16].

### Primary Screening of Phosphate Solubilizing Fungi

Soil samples were prepared for microbial analysis, which included the isolation of phosphate solubilizing fungi on Pikovskaya's (PKV) agar medium supplemented with 25 g/mL chloramphenicol to prevent bacterial growth [17]. The existence of a transparent halo zone around the fungal colony suggested that the fungus was capable of phosphate solubilization, and the zone's diameter was measured in millimetres.

### Characterization of Phosphate Solubilizing Fungi

After Screening of Phosphate Solubilizing all fungal isolates were transferred on Potato Dextrose Agar to accelerate the growth rate and the production of enough conidia (Pandey *et al.*, 2008). Isolates were compared to mycological identification keys and taxonomic descriptions to classify the isolated fungi to the genus level [17]. Such as color of the colonies both from the upper and lower side, surface appearance, and texture. Microscopy was also used to establish conidia, conidiophores, spore arrangement, and vegetative structures [18]. For further analysis, the detected fungi were held on Potato Dextrose Agar (PDA) slant at 4°C.

### Qualitative analysis of Phosphate solubilization

In the centre of Pikovskaya's plate, spot inoculated isolates with phosphate solubilizing capability were placed and incubated at 37°C. The diameter of the clearance zone was measured every 24 hours for up to 7 days.

### Quantitative analysis of Phosphate solubilization

Pikovskaya's broth medium with Tricalcium phosphate (0.3g/100ml) was prepared and sterilised for quantitative analysis of phosphate; 1ml of each isolate was inoculated into the broth medium. The sterilised sample was then incubated for 5 days on a rotatory shaker at 37°C, after which the culture broth was centrifuged for 30 minutes at 10,000rpm. As a control, uninoculated broth was used. Calorimetrically at 410nm with standard KH<sub>2</sub>PO<sub>4</sub>, the usable phosphorus was calculated.

### Assessment of the biodiversity of isolates

The diversity of the isolates was made from the microscopic, cultural/morphological description of the fungal isolates. This description allowed highlighting the similarities and the differences between isolates.

### Molecular identification of fungal isolates

The molecular techniques are being very much employed for the classification and characterization of various fungal species [19]. For species-level identification, genomic DNA of the potent phosphate solubilizing fungi was extracted

by a standard protocol and analyzed on 0.8% agarose electrophoresis. Further, PCR was carried out to amplify the 18S rRNA gene of the extracted genomic DNA of fungi using Gene Amp PCR with the forward and reverse primers. The genus of the strain was determined based on the sequence of 601bp of the 18S rRNA gene. The obtained 18S rRNA gene sequences were assembled and exploited for phylogenetic analysis. The 18S rRNA gene sequence-related taxa were acquired from the GenBank database at the National Center for Biotechnology Information. The report (sent online) includes NCBI-BLASTn (<http://blast.ncbi.nlm.nih.gov>) result for fungi showing closest VALID neighbor of the organism in the TYPE database along with percent similarity.

### Results and Discussion:-

The pH values of the soil samples vary from 7.7 to 8.7, while the ideal pH range is 7.5 to 7.8, suggesting that the soil of Marathwada is slightly alkaline due to excessive evaporation of water in dry areas, which brings salts to the surface. Alkaline medium restricts plant growth, and pH may affect nutrient availability in the soil [20] (Table 1). This is most likely due to the fact that these soils were formed from basaltic parent material with a high concentration of basic cations. Padole and Mahajan found similar results [21].

**Table 1:-** Number of soil samples taken from various parts of the Marathwada region(Properties of soil in Range).

SN	Location	No. Sample	pH	E.C m/s.	P (k/hect)	N (Kgs. /Ha)	O.C (%)	K (k/hect)
1.	Aurangabad	27	7.2-8.2	15.9-18.4	6.50-20.45	80.40-250.30	0.15-0.95	100-320
2.	Beed	24	7.1-7.8	16.1-18.7	5.78-20.58	75.60-237.56	0.18-0.89	110-310
3.	Hingoli	15	7.1-8.5	16.-18.4	5.20-19.21	78.30-180.90	0.20-0.82	98-290
4.	Jalna	27	7.0-7.8	16.1-17.8	5.50-20.90	90.70-240.20	0.17-0.86	105-355
5.	Latur	15	7.3-8.2	16.-18.3	7.90-16.20	100.50-219.50	0.15-0.73	100-280
6.	Nanded	16	7.0 -8.5	15.7-18.1	6.11-18.30	90.50-170.70	0.25-0.83	110-260
7.	Osmanabad	15	7.1-8.6	15.8-17.5	5.25-17.80	95.50-210.50	0.17-0.79	100-340
8.	Parbhani	21	7.1-8.8	16.2-18.7	7.50-18.30	100.50-200.30	0.25-0.86	95-355
<b>Total Total Sample</b>		<b>160</b>	<b>7.0-8.8</b>	<b>15.7-18.7</b>	<b>5.20-20.90</b>	<b>75.60-250.30</b>	<b>0.15-0.95</b>	<b>95-355</b>

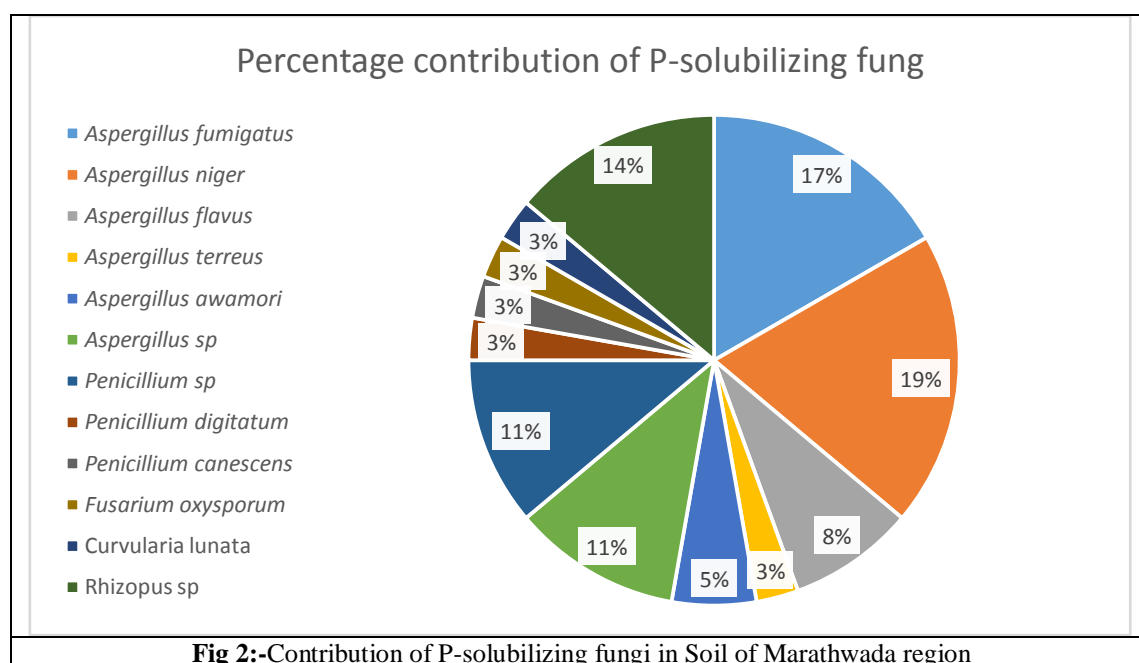
**Table 2:-**The fungal isolates' colony morphology and microscopic characteristics.

Sr.No	Culture number	Colony morphology	Microscopic observations	Name of isolates
1.	PQ3, PQ11, PQ21	Initially white floccose mycelium colonies on PDA. Through the development of black spores, colonies easily turn black in colour.	Conidia were thin, brownish-black, and green conidia. Conidiophores are rough brown and smooth colourless on septate hyphae.	<i>Aspergillus</i> species
2.	PQ7, PQ24	Colonies began as white and evolved into a yellowish-green to light green colour.	Tree or fan like branching, septate, hyaline, acute angle branching.	<i>Aspergillus fumigatus</i>
3.	PQ13, PQ40, PQ14	On potato dextrose agar, colonies were lime green in colour. The texture ranged from woolly to cottony to granular.	Phialides are born on cylinder branches and arranged in a brush-like head, septate distinct and bearing a cluster of branches.	<i>Aspergillus flavus</i>
4.	PQ19, PQ37	Colonies start out white, then turn brownish-red, and eventually turn	Tree- or fan-like branching, septate, hyaline, acute angle branching	<i>Penicillium</i> spp.

		green or bluish-green.		
5.	PQ36	On PDA, viride tends to be a little granular, with green conidia strewn around. Around the inoculum, there was an unusual yellow zone without conidia.	Irregularly branched, not verticillate, single-celled, and not forming a single chain.	<i>Trichoderma spp.</i>

### Contribution of P-solubilizing fungi in Soil of Marathwada region

At different locations, the percentage contribution of different fungal species to the overall fungal population varied. Since they were described by a large number of species, the genus *Aspergillus flavus* contributed the most to all of the samples. The percentage contribution of fungal species resulted in significant differences. *Penicillium sp* ranked first of all P-solubilizers, contributing the highest percentage of 9.15 percent to the total fungal population. *Aspergillus flavus* contributed 3.26 percent of the total, *Penicillium sp* contributed 2.61 percent, and *Trichoderma spp* contributed 5.22 percent. Together, these species account for 24.81 percent of the total fungal population (Fig.2).



A total of 347 fungal isolates were isolated in this study, with 40 of them proving to be effective phosphate solubilizers. PQ13, PQ19, PQ36, PQ40, PQ67, PQ88, PQ135, PQ153, PQ207, PQ233, and PQ277, among the best 11 PSF isolates, developed a maximum zone of solubilization. PQ153 was found to be a potent phosphorus solubilizing bacterium, with a 42 mm zone of solubilization, relative to other isolates. Phosphorus deficiency is a natural phenomenon on soil all over the world, and it is one of the factors that limits crop production. Fertilizers containing phosphorus account for a substantial portion of agricultural production costs. Many bacteria, fungi, and a few actinomycetes can solubilize bound phosphates in the soil, making them soluble and thus available to plants. Nitrogen, phosphorous, and potassium are the most essential soil nutrients for normal plant germination, development, and maturity.

Based on the phosphate solubilizing ability, 11 highly efficient PSF isolates were further analyzed for their biodiversity study. Based on the analysis, *Aspergillus niger* isolates were recovered from Parbhani and Latur district Marathwada region. PQ67, PQ88, PQ135, and PQ153 were the isolates with the highest P solubilization compared to the other isolates studied. These isolates were collected in the Marathwada districts of Jalna, Latur, Beed, and Parbhani. PQ207, PQ233, and PQ277 isolate recovered from Nanded, Osmanabad, and Hingoli district. The isolates PQ13, PQ19, PQ36, PQ40 obtained from Aurangabad and Jalna district

In the present study, genomic DNA was extracted by a standard protocol and analyzed on 0.8% agarose electrophoresis. Further, PCR was carried out to amplify the 18S rRNA gene of the extracted genomic DNA of UK-2 fungi using Gene Amp PCR with the forward and reverse primers (Forward Primer 5'-GAGTTTGATCCTGGCTCAG-3' and Reverse primer 5'-GAAAGGA GGTGATCCAGCC-3'). The genus of the strain was determined based on the sequence of 356 bp of the 18S rRNA gene. The obtained 18S rRNA gene sequences were assembled. All fungal species *Penicillium canescens*, *Penicillium sp.*, *Penicillium digitatum*, *Curvularialunata*, *Fusarium oxysporum*, *Rhizopus* sp., and *Trichoderma* sp., *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus awamori* and *Aspergillus terreus*, *Aspergillus sp.*, evaluated on Pikovskaya (PVK) selective media, they tested phosphate solubilization capacity, and some of them discovered excellent phosphorus solubilizing properties. The successful isolates from the sample were evaluated for their plant growth promotion activities such as antagonism, phytohormone production, siderophores, and biopesticide function.

### Conclusion:-

The biodiversity of fungal species from the rhizosphere soils of various locations in the Marathwada area was investigated in terms of morphological characterization and phosphate solubilizing potential in the current study. *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium spp.*, and *Trichoderma spp.* were evaluated in the study. These could be used to create inoculum, and the effects of inoculation on plant growth should be tested in vitro, in greenhouses, and in field trials.

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