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(RESEARCH ARTICLE)

Screening of phosphate solubilizing *Aspergillus spp.* from soil samples

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Abstract

Phosphorus (P) is a naturally existing element crucial for plant growth among the 17 essential elements. It's integral to various biomolecules like nucleic acids, coenzymes, phosphoproteins, and phospholipids, playing key roles in cellular processes and structural integrity. Its presence is fundamental for sustaining healthy plant growth and ecosystem balance. Rhizosphere soil samples from medicinal plants at Shree Shivaji College Akola were collected aseptically. Phosphate Solubilizing Fungi (PSF) were isolated using Pikovskya's agar medium, characterized microscopically, and screened for phosphate solubilization potentials. % Solubilization Efficiency was determined, and quantitative estimation of phosphate solubilization was conducted by ammonium molybdate method by using KH2PO4 as standard for determination. Fourteen *Aspergillus* spp. isolates from soil were screened for phosphate solubilization on PVK medium. Results showed varied solubilization indices and efficiencies across different time intervals, with pH changes observed during solubilization. Optical density measurements provided insights into concentration variations among isolates.

Keywords: Phosporous (P); Phosphate Solubilizing Fungi (PSF); *Aspergillus* spp.; Pikovskya's agar; Rhizosphere

1. Introduction

Phosphorus (p) is a naturally occurring element and is one of 17 elements that are essential for plant growth (Nisha *et al*., 2014). It is also an important constituent of biomolecules such as nucleic acids, coenzyme, phosphoproteins, and phospholipid (Fontes and Weed 1996). High proportion of phosphate solubilizing microorganism is concentrated in rhizosphere and they are metabolically more active than microorganisms from other sources (Vazquez *et al*., 2000). Fungi have been reported to possess greater ability to solubilized insoluble phosphate than bacteria (Nahas 1996). Most of the researchers employed the Pikovskaya's medium (Pikovskaya *et al.,* 1948). The basic method used for the screening of PSM was determination of solubilization index (S1) and solubilized P (Banik *et al*.,1989) Filamentous fungi are widely used as producers of organic acids and particularly black *Aspergilli* (Matty, 1992).

The most common groups of organisms are those of fungal (*Aspergillus*) (Wakelin *et al*., 2004). Inoculation of soil with microbes to improve soil fertility began in the late 19th century when microbes were sold for the purpose of producing fertilizers (kilian *et al.,*2000). Production of organic yids results inacidification of the microbial cell and its surroundings PSMs are a low-cost solution that enriches the soil giving a thrust to economic development without distrubing ecological balance (Reyes *et al*., 2002). Microorganisms are very important components of biofertilizers as their use can improve soil fertility and proliferation of beneficial bacteria and fungi (Pal *et al*., 2015).

Phosphate-solubilizing microorganisms are recognized as a solution to the challenges in P fertilization management due to their abilities to mobilize P from recalcitrant sources (Mendes GDO *et al*., 2014). To date, most representative strains of *Aspergillus spp*. and *Penicillium spp*. have been widely reported as P solubilizers (Mendes GDO, Sahoo HR *et al*., 2014). Species of *Aspergillus, Penicillium* and yeast have been widely reported to solubilize various forms of inorganic

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phosphates (Whitelaw, 2000) Fungi have been reported to possess greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996).

The fungi and probably all living organisms, synthesize a number of phosphatases which are necessary to scavenge phosphates (Pi) from medium containing bound phosphorus (Naik *et al.,* 2013). *Aspergillus niger*, an organism that is generally recognized as safe, has been widely used in the food industry (Meyer. 2008). To optimize enzyme fermentation and investigate they regulatory mechanisms of secondary metabolite biosynthesis, classical genome editing methods are required to construct mutants (Meyer, 2008). P-solubilizing microorganisms can be also applied directly to soils to improve the efficiency of P fertilization. (Asea *et al*., 1988; Mittal *et al.*, 2008; Jain *et al*., 2015).

2. Materials and methods

2.1. Collection of rhizosphere soil samples

The rhizosphere soil samples were collected from different medicinal plants available in different areas of Shree Shivaji College Akola. These samples were collected at 10-15cm depth near to roots of the medicinal plants and the collected samples were placed in sterile polythene bags and then brought into the laboratory through the aseptic condition. In the laboratory, the soil samples were maintained at 4°C until further use of samples.

2.2. Isolation of Phosphate Solubilizing Fungi

Phosphate Solubilizing Fungi (PSF) were isolated from the sample by serial diluted method using Pikovskya's agar medium (containing Tri-calcium Phosphate 1g, Glucose 0.1g, Ammonium sulphate 0.1g, Potassium chloride 0.04g, $MnSO_4$ 0.02g, $MgSO_4$ 0.003g, Ferrous sulphate 0.003g, yeast extract 0.1g. Dextrose 2g, Agar 5g, Distilled water 200ml, pH-7.2) and incubated at room temperature for 7 days. After incubation, plates were examined for solubilization zone around fungal colonies, and they were subcultured for further use.

2.3. Microscopic characteristics

Identification of PSF was done by a drop of lactophenol cotton blue place on glass slide and observed under microscope. The specimen was stained with LPCB stain, a coverslip was placed above it and observed under the microscope at 40X magnification, and characters were noted by observing spore shape, spore size and identified by referring to the standard manuals.

2.4. Screening of phosphate solubilization potentials of isolates On PVK agar medium.

The fungal colonies showing a clear zone of solubilization, and their growth were subcultured on Pikovskaya's agar slants. Clear zone around the colonies indicated the capacity of Phosphate Solubilization. The isolates previously grown on PVK agar were screened to quantify the amount of phosphate solubilized in the solid medium. The samples were then incubated at 37°C for 4 to 7 days. The solubilization index was measured based on colony diameter and solubilization zone diameter formed around the colony of phosphate solubilizing fungi as follows.

$$\text{Solubilization Index (SI)} = \frac{\text{olony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

2.5. % Solubilization Efficiency

The isolates were point inoculated on Pikovskya's agar plates and incubated at room temperature for 7 days. % Solubilization efficiency was measured by using the following formula (Joseph and Jisha 2008).

$$\%\ \text{SE} = \frac{\text{Solubilization zone}}{\text{Diameter of the colony}} \times 100$$

2.6. Quantitative Estimation of phosphate solubilization.

Pikovskaya's broth medium (100 ml) was prepared and sterilized: 1 ml of each isolate was inoculated into the PKV broth medium. Then the inoculated sample were incubated for 7 days on room temperature 37°C. Uninoculated broth served as control. The quantitative estimation of phosphate solubilization was done by ammonium molybdate method by using KH_2PO_4 as standard for determination.

3. Results

The total 14 isolates were isolated from soil samples. The isolates were primarily identified by cultural and morphological characteristic to *Aspergillus spp.* In the present study, fourteen *Aspergillus spp.* were isolated and screened for phosphate solubilization ability on PVK medium. The formation of clear zone around the colony, colony diameter for each PSM isolate is presented in figure -1. Results showed that highest zone of hydrolysis was obtain by isolate PA5 which was 6.8cm.

On different time intervals solubilization index was determined (Figure 2). On Day 7, the solubilization index of the selection 14 PSM cultures was observed to range from 2.01 to 2.45. Notably PA9 exhibited the highest solubilization index of 2.45.

On Day 14, it was found that the PA10 showed a maximum solubilization index of 3.16. The results showed that PSM on Day 14, PA10 is the highest solubilization index.

On Day 21, phosphate solubilization of all the selected isolates were found to be potent phosphate solubilizers showing clear zone around their colonies. All 14 microbial isolates showed highest phosphate solubilization index (PSI) ranged from 2.21- 3.74 were selected for further studies. The PSI measurement was shown in the (Figure 3). The results showed that PSM 21, PA5 is the highest solubilization index at 3.74. The amount of phosphate solubilized by *Aspergillus spp.* On a solid medium (PVK agar) supplement with calcium phosphate is reported in (Figure 2). As it can be seen from the figure, *Aspergillus spp.* had the highest solubilization index (3.74 SI).

The qualitative analysis of the phosphate solubilization potential of *Aspergillus spp.* was measured *in vitro*. On Day 7, selected to the solubilization efficiency PA4 showed a maximum solubilization efficiency (SE) of 98.57. The solubilization efficiency based on solubilization zone and colony diameter for each PSM isolate is presented in (Figure 3). Results showed that highest solubilization efficiency was obtain by isolate PA4.

On Day 14, phosphate solubilization efficiency PA1 showed a maximum solubilization efficiency of 84.41 (figure 3). The results showed that PSM on Day 14, PAI is the highest solubilization efficiency.

On Day 21, The PSM isolates showed highest phosphate solubilization efficiency (PSE) ranged from 42.85 - 86.60 in the studies. The PSE measurement was shown in the (Figure 3). The results showed that PSM on Day 21, PA8 is the highest solubilization efficiency at 86.60.

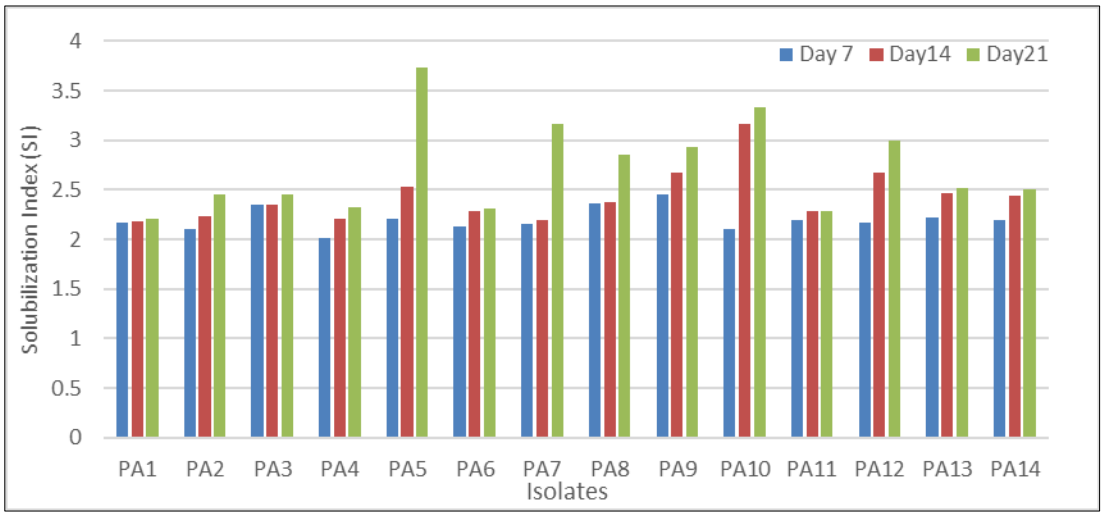

Figure 1 Determination of solubilization index of isolates**.**

In the present study pH change during phosphate solubilization was record. Most phosphate solubilizing microorganisms studied lowered the pH of PVK medium (Table 3). Fungi were found to be equally active in lowering the pH Drop in pH by PSM ranged from 7.0 to 30. Decreased pH of 6.0 to 3.0 was recorded on Day 21 from the initial pH of 7.0. In phi measured by Day 7. Day 14 and Day 21 the results showed that PSM on Day 21 changes to pH is 6.0 and lowest pH range 3.0 which was acidic.

The table presents a concise summary of optical density (O.D) measurements at 880 nm alongside corresponding concentrations (mg/lit) for various isolates. Notably, Isolate Al demonstrated an O.D of 0.965 at 0.19~mg/lit, followed closely by Isolate A2 with an O.D of 0.807 at 0.16 mg/lit. Conversely. Isolate A4 exhibited the highest O.D among the isolates, recording 1.384 at 1.12 mg/lit. Isolate A9 recorded the second-highest O.D at 1.408, observed at a concentration of 0.93 mg/lit. In contrast, Isolate A12 displayed the lowest O.D at 0.429, noted at 0.12 mg/lit. These findings provide valuable insights into the varying optical densities across different isolates and concentrations.

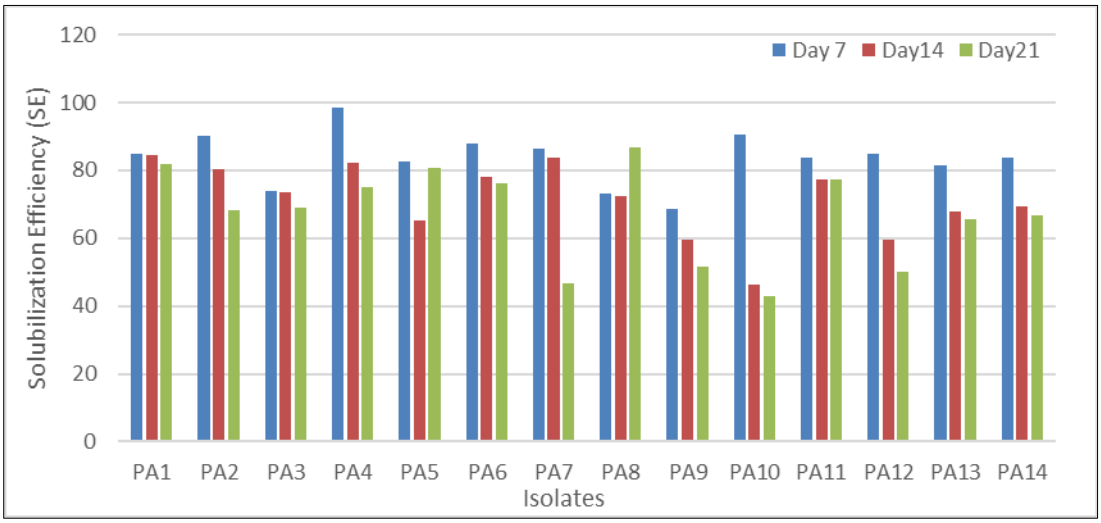

Figure 2 Determination of solubilization efficiency of isolates

Table 1 Determination of change in pH of medium

Sr No	Isolate	Change in pH		
		7th Day	14th Day	21th Day
1	PA1	7.0	6.1	6.0
2	PA2	6.9	6.3	6.0
3	PA3	6.0	6.0	6.0
4	PA4	6.0	6.0	6.0
5	PA5	6.7	6.2	6.0
6	PA6	6.7	6.2	6.0
7	PA7	7.0	6.0	6.0
8	PA8	6.8	6.1	6.0
9	PA9	6.0	6.0	6.0
10	PA10	7.0	7.0	6.0
11	PA11	6.9	6.3	5.5
12	PA12	6.7	5.6	3.0
13	PA13	6.5	3.0	3.0
14	PA14	7.0	6.6	3.0

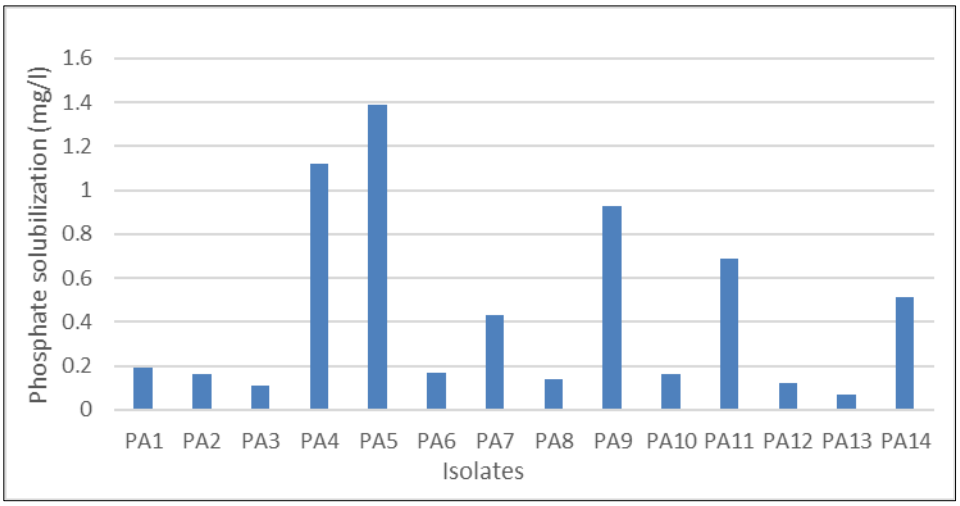

Figure 3 Phosphate solubilization by *Aspergillus* isolates

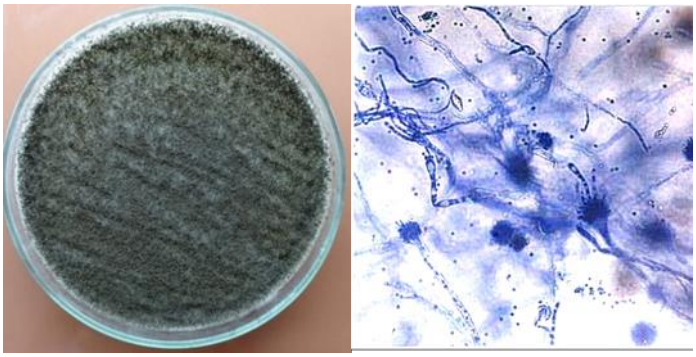

Figure 4 Isolation of Aspergillus spp. from soil sample

Figure 5 Screening of phosphate solubilizing *Aspergillus spp*. on Pikovskaya agar medium.

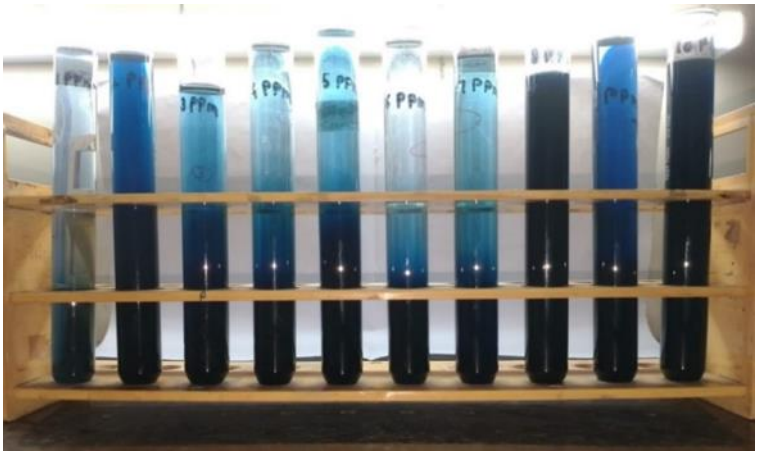

Figure 6 Quantitative Estimation by Ammonium molybdate method

4. Discussion

In the present study 14 *Aspergillus spp.* Were screened for their P solubilization ability. It was found that PAS showed highest zone on PVK medium. Similarly Karpagam and Nagalakshmi (2014), reported 2.3 mm zone on phosphate solubilization. Thimmappa *et al*., (2016), showed *Aspergillus niger* show 13 ±1.0 mm of halo zone on phosphate solubilization medium.

It was observed that PA5 exhibited the highest phosphate solubilization index of 3.74, while PSE demonstrated the highest phosphate solubilization efficiency of 86.60 on PVK medium. Previous research by Verma and Ekka (2015), reported solubilization indices ranging from 1.06 to 3.56 among 18 fungal culture strains, whereas Joseph and Jisha (2008), documented solubilization efficiencies ranging from 100 to 575. Our study adopted a similar methodology to assess solubilization index and efficiency.

During the study effect of Change in pH of medium was studied. A consistent trend of pH reduction is observed across most isolates as time advances. Initially, p11 levels are relatively high (6.0 to 7.0) on the 7th Day, but by the 21st Day, there's a significant decline, with many isolates reaching pH values of 6.0 or lower. Similarly, Jain and also reported Singh (2015), have also reported the inoculation of phosphate solubilizing fungi, decrease in pH was observed in a liquid medium ranging from 4.0 to 3.2 from initial pH of 7.5±0.2.

Daring the study Estimation of phosphate solubilization is done by ammonium molybdate method, optical density (O.D) readings at 880 nm and corresponding concentrations (mg/l) of various isolates. Isolates show diverse O.D values, ranging from 0.429 to 408, reflecting differences in biomass or metabolic activity. Concentrations range from 0.07 to 139 mg/1, indicating variations in the production of metabolites or biomass accumulation among isolates. Similarly. Verma and Ekka (2015), have recorded with Vanadomolybdate method that the phosphate solubilization gradually increased ranging from 219.17µg/ml to 59.17µg/ml.

5. Conclusion

In the present study it was found that isolate PA5, PA4, PA9, PA11, PA14 and PA7 showed prominent P. solubilization ability. The isolate PA5 showed highest phosphate solubilization index (3.74) and solubilization efficiency (80.64%), and 1.135 mg/lit of phosphate solubilization. The phosphate solubilization was found to be increased with time from 7 to 21 days and also decreases pH of medium from 7-3 by varying isolates. These isolates could be better natural source of biofertilizers for solubilization of inorganic phosphate into organic P which will improve soil fertility, enhance plant growth and reduce the risk of environmental pollution and diminish the accumulation of phosphorus.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have no conflict of interest.

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