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# Isolation And Characterization Of Some Rhizospheric Phosphate-Solubilizing Fungi From Paddy Fields Of Bilaspur, Chhattisgarh

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***Abstract:*** Phosphorus availability in soil is often limited due to its presence in insoluble forms, leading to reduced plant growth and productivity. Microbial phosphate solubilization presents a sustainable strategy to enhance phosphorus availability in soil. In this study, phosphate- solubilizing fungi were isolated from soil samples and evaluated using qualitative and quantitative assays. Fourteen fungal isolates were obtained and tentatively identified based on morphological characteristics, primarily belonging to the genera *Aspergillus, Penicillium,* and *Fusarium*. Screening on Pikovskaya's agar medium revealed phosphate solubilizing activity in only two isolates, which are identified as *Aspergillus flavus* and *Aspergillus niger*. Quantitative estimation in liquid medium supplemented with 0.5% tricalcium phosphate showed a time-dependent increase in soluble phosphate concentration, after seven days. Phosphate solubilization was associated with acidic pH conditions, suggesting organic acid-mediated dissolution. These findings highlight the strain-specific nature of phosphate solubilization and support the potential application of selected *Aspergillus* isolates in sustainable agriculture.

***Keywords*** **-** Phosphate solubilizing fungi, Qualitative and Quantitative assays, Tricalcium phosphate ,Biofertilizer, Sustainable agriculture.

## I. Introduction

Phosphorus (P) serves as the second most vital macronutrient which plants require to develop their growth and development (Majumder *et al*., 2019). Phosphorus boosts photosynthesis through its role in sugar production and nucleic acid formation and it enables legumes to fix nitrogen (Saber *et al*., 2005). Phosphorus in plants leads to stronger cereal straw and better flower and fruit development and it promotes root growth and is needed for seed development (Gizaw *et al*., 2017). Only 0.1% of the overall phosphorus content in the soil is accessible to plants in a soluble form, with the majority being fixed in an unavailable form. (Islam *et al*., 2019). The main chemical factor which restricts plant growth makes phosphorus deficiency a major problem so farmers apply chemical phosphatic fertilizers at full yield levels (Alam *et al*., 2002). Phosphorus in soluble fertilizers can be precipitated quickly and the immobile P forms can remain in the soil and bond with Al and Fe in acidic soils and with Ca and Mg in alkaline soils (Alam *et al*., 2002; Gizaw ., 2017). Excessive use of phosphate fertilizers leads to environmental problems due to soil erosion and runoff, resulting in phosphorus pollution in the environment (Wang et al., 2020).

Many soil fungi and bacteria possess the ability to solubilize inorganic phosphates, and are therefore classified as phosphate-solubilizing microorganisms (PSMs) (Mahadevamurthy *et al*., 2016). Various microorganisms, including fungi, bacteria, and actinomycetes, can significantly contribute to the solubilization of both fertilizer phosphorus and bound phosphorus in the soil, thereby promoting environmental friendliness and sustainability (Sundara *et al*., 2002; Khan *et al.*, 2009).

Fungi are essential members of soil microbiota, vital for biodiversity and ecological balance. Often comprising a significant portion of soil biomass, they outstrip bacteria in abundance. Their dominance varies with soil depth, nutrient availability, and environmental factors, favoring fungi in richer or deeper soils, and bacteria in poorer or surface soils. This interplay influences vital processes like nutrient cycling and organic matter decomposition, highlighting their critical role in soil health and ecosystem function (Mahadevamurthy *et al*., 2016). In this investigation, fungal subspecies exhibiting the ability to solubilize insoluble phosphates have been identified and isolated.

## II. MATERIAL AND METHODS

### 2.1 Study Area and Soil Sampling

Soil samples for the present study were collected from the rhizosphere (0–15 cm depth) of paddy fields located in Parsada Village, Bilaspur district, Chhattisgarh state. This area is primarily located in irrigated lowlands, where regular flooding occurs during the rainy season, leading to anaerobic conditions in the soil. Such conditions affect phosphorus availability and microbial activity, making this area suitable for studying phosphate-solubilizing fungi. Soil samples were collected during the active growth phase of the rice crop, i.e., from August to September. Soil was collected from the rhizosphere region at a depth of 0–15 cm in each selected field. A composite sample was prepared at each sampling site by collecting soil from five different points randomly selected within the field to ensure proper representation of the soil in the area.

The collected samples were thoroughly mixed, stored in sterile polyethylene bags, and immediately transported to the laboratory in an ice-cooled environment to maintain the viability of the microorganisms. In the laboratory, the samples were processed as soon as possible for further analysis.

### 2.2 Media Preparation for Fungal Isolation

Czapek's Dox Agar medium, which is considered suitable for the selective growth of fungi, was used to isolate fungal strains from the paddy field soil. The composition of this medium per liter was as follows: dextrose (10.0 g), $NaNO_3$ (6.0 g), KCl (0.52 g), $MgSO_4$ (0.52 g), $KH_2PO_4$ (1.52 g), trace amounts of $CuSO_4$, $FeSO_4$ and $ZnSO_4$ and agar (10.0 g). All components were completely dissolved in tap water, and the final volume was adjusted to 1 litre. The pH was maintained at 6.0–6.2 using 1N NaOH or HCl, which is considered optimal for fungal growth.

To further enhance fungal growth, additional nutrients were incorporated into the medium, including peptone 2.0 g, yeast extract 1.0 g, casamino acids 1.5 g, and a vitamin mixture 1.0 mL per liter. This vitamin mixture contained riboflavin, biotin, nicotinamide, para-aminobenzoic acid, pyridoxine hydrochloride, and thiamine hydrochloride**.** After pH adjustment, the vitamin solution was added to the medium, and the medium was sterilized by autoclaving before use**.**

### 2.3 Isolation of Fungi

Soil samples were air-dried for 24 hours and sieved through a 2 mm sieve to remove debris. Serial dilutions (10-1 to 10-5) were prepared using sterile distilled water. One millilitre of each dilution was plated onto Czapek's Dox agar medium supplemented with 1% streptomycin to prevent bacterial contamination. The plates were incubated at 28–30°C for 3–5 days. Fungal growth was observed after 96 hours and morphologically distinct colonies were purified using the single-colony isolation technique. One hundred

microliters (100 µL) of each dilution were spread in triplicate onto PVK agar plates and incubated at 28–30°C for 5–7 days.

### 2.4 Identification of the fungal species

For purification and identification, the fungal isolates were transferred to sterilized media plates. The pure cultured fungi were placed on a slide, stained with lactophenol cotton blue to visualize the fungal structures, covered with a coverslip, examined using a compound microscope and identified based on colony morphology, conidia, conidiophores, spore arrangement and surface texture. In addition, microbiological experts were consulted for the identification of fungal species and the following text books were used: Gilman, 1966; Barnett and Hunter, 1972; Nagamani *et al*, 2013.

### 2.5 Screening for Phosphate-Solubilization

After identifition, each pure fungal culture was streaked onto the center of PVK agar plates. The medium contained the following (g $L^{-1}$): ammonium sulfate ($(NH_4)_2SO_4$) 0.5, magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 0.5, ferrous sulfate ($FeSO_4$) 0.03, sodium chloride (NaCl) 0.3, potassium chloride (KCl) 0.3, manganese sulfate ($MnSO_4 \cdot 7H_2O$) 0.02, tricalcium phosphate ($Ca_3(PO_4)_2$) 10.0, glucose 10.0, and agar 15.0. The plates were incubated at 28–30°C for 7 days. After incubation, clear halo zones were observed around the colonies on the plates. The Phosphate Solubilization Index (PSI) was calculated using the following equation:

$$PSI = \{\text{Colony diameter} + \text{Halo zone diameter}\} / \text{Colony diameter}$$

Isolates with higher PSI values were considered efficient phosphate solubilizers. This metric provided a comparative assessment of solubilization capacity among the isolates.

### 2.6 Quantitative Phosphate Solubilization Assay

Fungal isolates were inoculated into Pikovskaya's broth containing 0.5% tricalcium phosphate and incubated at 28 ± 2 °C under shaking conditions for 7 days. The culture was centrifuged and soluble phosphate in the supernatant was estimated spectrophotometrically at a wavelength of 660 nm using the molybdenum blue method. Phosphate concentration was calculated from a standard curve and expressed as $\mu g ml^{-1}$.

## III. RESULTS AND DISCUSSION

### 3.1 Isolation and Identification of Fungal Isolates

Fourteen fungal isolates were obtained through isolation and purification procedures and were tentatively identified based on morphological characteristics. These isolates include five species of *Aspergillus*, two of the *Fusarium*, three of *Penicillium*, and one isolate each of *Curvularia*, *Alternaria*, and *Rhizopus*, as well as a non-sporulating fungus (*Mycelia sterilia*) was involved. The predominance of *Aspergillus* and *Penicillium* reflects their common occurrence in soil and their known adaptability to diverse environmental conditions.

### 3.2 Screening of Phosphate Solubilizing Fungi

For the phosphate solubility of all fourteen fungal isolates screening was done using the plate assay method on Pikovskaya's agar medium. Phosphate solubilization was indicated by the formation of a clear halo zone around the fungal colonies after incubation at 28 °C. The formation of the Halo zone was first observed on the third day of incubation and increased progressively up to seven days.

Only two isolates formed distinct clearance zones, indicating effective phosphate solubilizing activity (Fig. 1). The solubilization efficiency, assessed by the ratio of the diameter of the halo zone and the diameter of the colony confirmed these two isolates as the most efficient phosphate solubilizing fungi. The maximum halo zone diameters recorded were 12 mm and 9 mm, respectively (Table 1). The remaining isolates did not exhibit detectable phosphate solubility under the experimental conditions.


**Fig 1. Clear halo formation by representative fungal isolate in Pikovskaya's agar plates (A) *Aspergillus flavus* (B) *Aspergillus niger*.**

**Table 1. Determination of the phosphate solubilization index of fungal isolates**

| Fungal islolates | Halo zone (mm) | Colony diameter (mm) | Solubilizing index (SI) |
|---|---|---|---|
| *Aspergillus flavus* | 12 | 20 | 1.60 |
| *Aspergillus niger* | 9 | 22 | 1.40 |

As shown in Table 1, two fungal isolates demonstrated phosphate solubilizing activity on Pikovskaya's agar. *Aspergillus flavus* produced a halo zone of 12 mm with a colony diameter of 20 mm, yielding a solubilization index of 1.60. *Aspergillus niger* formed a 9 mm halo zone with a colony diameter of 22 mm, resulting in a solubilization index of 1.40.

These findings indicate that phosphate solubilizing ability was limited to a small fraction of the isolated fungal population, highlighting the need for targeted screening to identify efficient strains for further evaluation and application.

### 3.3 Quantitative Estimation of Soluble Phosphate

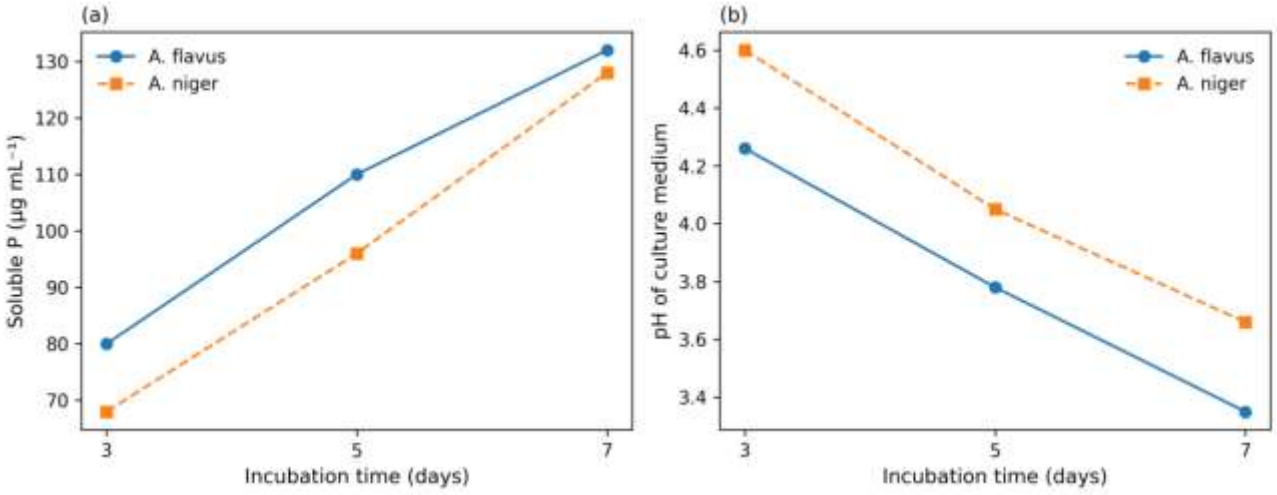
The quantitative assay revealed significant phosphate solubilization by the two selected fungal isolates in liquid medium supplemented with 0.5% tricalcium phosphate (Table 2). Both *Aspergillus flavus* and *Aspergillus niger* exhibited a gradual increase in soluble phosphate concentration over the incubation period, indicating sustained solubilization activity under liquid culture conditions.

**Table – 2 Estimation of soluble phosphate in liquid medium supplemented with 0.5% tricalcium phosphate**

| Fungal isolates | Day 3 | | Day 5 | | Day 7 | |
|---|---|---|---|---|---|---|
| | Sol. P conc. ($\mu g\ mL^{-1}$) | pH | Sol. P conc. ($\mu g\ mL^{-1}$) | pH | Sol. P conc. ($\mu g\ mL^{-1}$) | pH |
| ***Aspergillus* flavus** | 80 | 3.35 | 110 | 3.50 | 132 | 4.26 |
| ***Aspergillus niger*** | 68 | 3.66 | 96 | 3.80 | 128 | 4.60 |

Observation of this experiment revealed that on the third day of incubation, *A. flavus* released 80 µg $mL^{-1}$ of soluble phosphate, while *A. niger* released 68 µg $mL^{-1}$. By the fifth day, phosphate release had increased significantly, reaching 110 µg $mL^{-1}$ for *A. flavus* and 96 µg $mL^{-1}$ for *A. niger*. The highest solubility was observed on the seventh day, with concentrations of 132 µg $mL^{-1}$ and 128 µg $mL^{-1}$ for *A. flavus* and *A. niger*, respectively **(Graph-1).**

The increase in soluble phosphate was accompanied by a decrease in pH, indicating acidification of the medium. The pH values remained acidic throughout the experiment, ranging from 3.35 to 4.26 for *A. flavus* and from 3.66 to 4.60 for *A. niger*. This acidification is likely due to the release of organic acids, which aid the dissolution of tricalcium phosphate. Overall, *A. flavus* exhibited a higher capacity for phosphate solubilization efficiency than *A. niger* at all sampling intervals **(Graph-2).**




**Graph – 1 and Graph – 2**: Demonstrating Time-dependent phosphate solubilization and associated pH changes by *Aspergillus flavus* and *Aspergillus niger* in Pikovskaya's broth.

## IV. Conclusion

This study identified a diverse group of soil fungi; however, effective phosphate solubilization was observed in only two isolates, emphasizing the strain-specific nature of this characteristic. *Aspergillus flavus* and *Aspergillus niger* exhibited significant phosphate solubilizing activity in both solid and liquid media, with *A. flavus* demonstrating superior performance. The increase in soluble phosphate concentration over time, coupled with a decrease in pH, suggests that the production organic acids is a key mechanism underlying phosphate solubilization. These findings indicate that the selected *Aspergillus* strains, particularly *A. flavus*, have strong potential to be developed as a bioinoculant, which would improve phosphorus availability and reduce reliance on chemical fertilizers in sustainable agricultural systems.

## V. References


[1] Alam, S., Khalil, S., Ayub, N. Rashid, M. (2002): In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. *Int J Agric Biol* 4: 454-458.

[2] Barnett, H. L. and Hunter, B. B. (1972). Illustrated genera of imperfect fungi. 3$^{rd}$ edition *Burgess Publishing Company*. Printed in United states of America. 241pp.

[3] Gilman, J.C. (1966). A Manual of Soil Fungi. 2$^{nd}$ ed. The lowa Stare University Press, lowa and USA. 450pp.

[4] Gizaw, B., Tsegay, Z., Tefera, G., Aynalem, E., Wassie, M., & Abatneh, E. (2017). Phosphate solubilizing fungi isolated and characterized from Teff rhizosphere soil collected from North Showa zone, Ethiopia. *African Journal of Microbiology Research*, *11*(17), 687-696.

[5] Iman, M., & Azouni, E. (2008). Effect of phosphate solubilizing fungi on growth and nutrient uptake of soyabean (Glycine max L.) plants. *J. Appl. Sci. Res*, *4*(6), 592-598.

[6] Islam, M. K., Sano, A., HOSSAIN, M., & SAKAGAMI, J. I. (2019). Isolation and molecular characterization of phosphate solubilizing filamentous fungi from subtropical soils in Okinawa. *Applied Ecology & Environmental Research*, *17*(4).

[7] Khan, M. S., Zaidi, A., & Wani, P. A. (2009). Role of phosphate solubilizing microorganisms in sustainable agriculture-a review. *Sustainable agriculture*, 551-570.

[8] Mahadevmurthy, M., Channappa, T. M., Sidappa, M., Raghupathi, M. S. and Nagaraj, A. K. (2016). Isolation of phosphate solubilizing fungi from rhizosphere soil and its effect on seed growth parameters of different crop plants. *Journal of Applied Biology and Biotechnology* , Vol. *4*(6), 22-26.

[9] Majumder, M. S. I., Islam, M. K., AKAMINE, H., Sano, A., Onjo, M., & Hossain, M. A. (2019). Comparative study of phosphate solubilization potential of Talaromyces pinophilus strains. *Applied Ecology & Environmental Research*, *17*(6).

[10] Nagamani, A., Kunwar, I K. and Manoharachary, C. (2006). Hand book of soil Fungi, *I K. International Private Limited*, New Delhi.

[11] Raja, M., G. Praveena G. and John William, S. (2017). Isolation and Identification of Fungi from Soil in Loyola College Campus, Chennai, India. *International Journal of Current Microbiology and Applied Sciences*, *6*(2), 1789-1795.

[12] Ratna Kumar, P. K., Hemanth, G., Niharika, P. S. and Kolli, S. K. (2015). Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal Srikakulam District. *Int. J. Adv. Pharmacol*., *4*(2), 484-490.

[13] Saber, K.L., Nahla, A.D., Chedly, A. (2005) Effect of P on nodule formation and N fixation in bean. *Agron. Sustain. Dev*. 25: 389-393.

[14] Sundara, B., Natarajan, V., & Hari, K. (2002). Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. *Field crops research*, *77*(1), 43-49.

[15] Wang, Y.-Y., Li, P.-S., Zhang, B.-X., Wang, Y.-P., Meng, J., Gao, Y.-F., He, X.-M. and Hu, X. M. (2020). Identification of Phosphate-solubilizing Microorganisms and Determination of Their Phosphate-solubilizing Activity and Growth-promoting Capability. *BioResources*, *15*(2), 2560–2578.

[16] Toma, F.M. and Abdulla, N.Q.F. (2012). Isolation, Identification and Seasonal Distribution of Soilborne Fungi in Different Areas of Erbil Governorate. *Journal of advanced Laboratory Research in Biology*, *3*(4), 246-255.

[17] Waksman, S.A. (1922). A method for Counting the number of fungi in the soil. *J Bacteriol*., *7*, 339-341.