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ISOLATION, IDENTIFICATION AND DEVELOPMENT OF EFFICIENT PHOSPHORUS-SOLUBILIZING FUNGI (PSF) FOR IMPROVED PHOSPHORUS NUTRITION OF HIGHER PLANTS

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Abstract: Phosphorus (P) is a non-substitutable macronutrient that limits crop productivity worldwide, especially in alkaline and calcareous soils where P precipitates as poorly soluble Ca-phosphates. Coupled with volatile fertilizer prices and geographically concentrated phosphate rock reserves, agriculture faces a dual challenge of P scarcity and environmental risk from inefficient P use. Recent advances show that phosphorus-solubilizing fungi (PSF)—notably *Aspergillus*, *Penicillium*, and *Trichoderma* spp.—can transform insoluble inorganic and organic P pools into plant-available forms via organic-acid secretion, chelation, proton extrusion, and phosphatase/phytase activities. This article synthesizes contemporary evidence (2015–2025) on PSF ecology and mechanisms, details rigorous, field-transferable workflows for isolation, identification, and development of high-efficiency strains, and frames their deployment in arid and semi-arid systems. We discuss integration with host crops and soil management, quality-control metrics (solubilization indices, titratable acidity, and enzyme kinetics), and translational steps (formulation, shelf life, and regulatory considerations). The aim is to provide a practice-ready and research-grade roadmap to move PSF from petri dishes to productive fields while advancing circular P management. Key knowledge gaps include strain stability under field stresses, interactions in microbial consortia, and performance on native rock phosphates. Addressing these will be pivotal for scaling bio-based P nutrition in low-input agroecosystems. Recent reviews and meta-analyses indicate that PSF can improve P uptake and yields across diverse crops and soils, especially in calcareous conditions, offering a credible pathway to reduce chemical fertilizer dependence.

KEYWORDS: *Phosphorus solubilizing fungi, biofertilizer, calcareous soils, organic acids, phytase, phosphatase, Aspergillus, Penicillium*

1. INTRODUCTION

Phosphorus (P) is a cornerstone macronutrient for plant growth and development. Despite its importance, phosphorus remains one of the most limiting nutrients in agricultural systems. Unlike nitrogen, which can be replenished via biological fixation, phosphorus is derived primarily from finite rock phosphate deposits. These reserves are geographically concentrated, and their mining and processing are highly energy-intensive. Global fertilizer markets, therefore, remain vulnerable to geopolitical and economic fluctuations, which have periodically caused phosphate price spikes with direct impacts on food security (Brownlie et al., 2023). The International Food Policy Research Institute (IFPRI, 2025) has further cautioned that high fertilizer costs disproportionately affect smallholder farmers, limiting access to essential inputs in developing countries. In general, phosphate deficiency in plants during their growth is compensated by applying chemical or phosphate fertilizers, which don't stay available for long; the P quickly binds with common soil ions—specifically calcium, iron, and aluminium—to form insoluble complexes. This fixation is a particularly critical problem in the calcareous and alkaline soils characteristic of arid and semi-arid regions. In these challenging environments, plants can typically access less than 20% of the applied phosphorus (Bolan et al., 2023). The vast majority becomes permanently locked away, resulting in massive nutrient wastage and forcing growers to reapply fertilizer constantly. This widespread inefficiency doesn't just raise production costs; it actively degrades the environment, as the unutilized P eventually leaches out, fuelling the rapid proliferation of algae, a process known as eutrophication (Alewell et al., 2020).

To circumvent these persistent limitations, substantial research is now directed toward biological alternatives; especially phosphate-solubilizing fungi (PSF) stand out as uniquely powerful. Fungi generally secrete far greater volumes of organic acids than bacteria; also, their expansive hyphal network acts like a vast underground scavenging system, allowing them to acquire nutrients well outside the narrow confines of the rhizosphere (Lei et al., 2025; Sun et al., 2025). The release of low-molecular-weight organic acids (e.g., malic, gluconic, citric, and oxalic acids) reduces the rhizosphere pH and simultaneously chelates metal cations, which liberates the phosphate ion from stubborn mineral complexes. Ma et al. (2025) found that the profile of secreted organic acids varies significantly among different PSF species, and this unique chemical fingerprint directly dictates their solubilization power. This species-specific variation makes a thorough initial screening of multiple isolates absolutely critical for developing a successful commercial biofertilizer. The extracellular enzymes, chiefly phosphatases and phytases, secreted by PSF, cleave orthophosphate from phytate and other organic esters, making the nutrient available to the plant (Moropana et al., 2024). Considering that organic P often makes up 30–70% of the total P reserve in agricultural soil, effectively utilizing this source is non-negotiable for long-term soil fertility management. Evidence emphasizes the multifunctionality of plant growth-promoting soil fungi (PSF), which not only solubilize phosphorus but also synthesize plant growth hormones like indole-3-acetic acid (IAA), produce siderophores, and create biocontrol metabolites against pathogens (Moropana et al., 2024). García-Berumen et al. (2024) noted PSF's ability to directly solubilize rock phosphate, potentially reducing reliance on industrial acidulation. Additionally, greenhouse trials by Perea-Rojas et al. (2025) revealed that fungal consortia improved phosphorus uptake and biomass in Coffea arabica, surpassing single-strain inoculations.

Agronomic data indicates that PSF offers significant benefits. Ren et al. (2025) found that inoculating Fritillaria taipaiensis with PSF increases phosphorus uptake, bulb quality, and alkaloid levels. Ahash et al. (2025) propose that combining PSF with moderate chemical P fertilizers and organic matter can maintain high yields while reducing chemical usage. This integration may enhance phosphorus-use efficiency and lessen environmental impacts from over-fertilization. The present study intends to contribute to this effort by:

- Isolating phosphate-solubilizing fungi from arid region calcareous soils.
- Characterizing their enzyme and organic acid secretion profiles.
- Evaluating their tolerance to stress.
- Confirming their identity via molecular markers.
- Finally, testing selected isolates in a greenhouse setting to determine their impact on phosphorus uptake and biomass accumulation in a model legume crop.

This integrated strategy should help identify excellent candidates for biofertilizer development, leading toward more sustainable phosphorus management in difficult soil environments.

2. MATERIALS AND METHODS

2.1 Soil sampling

Soil samples were collected from different areas of India, including agricultural fields and forests. Sampling sites were selected to capture variations in pH, organic matter content, and P status. Soil pH, electrical conductivity (EC), available P (Olsen method), and organic carbon (Walkley–Black method) were determined following standard protocols (Jackson, 1973) to characterize the physicochemical context for fungal isolation.

2.2 Isolation and selection of Phosphorus-Solubilizing Fungi (PSF)

Pikovskaya's (PVK) agar was prepared using tricalcium phosphate (TCP, 5 g L^{-1}) as the insoluble P source (Pikovskaya, 1948). For rock phosphate-specific screening, TCP was replaced with finely ground local rock phosphate (<75 µm particle size) or hydroxyapatite (HAP). Media were sterilized at 121°C for 20 min. One gram of soil was suspended in 9 mL of sterile saline (0.85% NaCl), serially diluted to 10^{-6}, and 100 µL aliquots were spread-plated on PVK agar. Plates were incubated at 28 ± 2°C for 5–7 days.

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

Colonies showing clear halo zones around fungal growth were selected for further screening. The solubilization index was calculated as high SI values (>2.0) were considered preliminary indicators of strong P-solubilizing potential. The selected isolates were inoculated into 50 mL PVK broth in 250 mL Erlenmeyer flasks at 28°C, 150 rpm, for 7 days.

Soluble P concentration was determined by the molybdenum blue method (Murphy & Riley, 1962) after centrifugation (9500 rpm). The most effective fungi were chosen based on their intra- and extracellular acid phosphatase, alkaline phosphatase, and phytase activity. The reduction in pH, titratable acidity (NaOH titration), and organic acid profiling was done via high-performance liquid chromatography (HPLC) equipped with a UV detector at 210 nm.

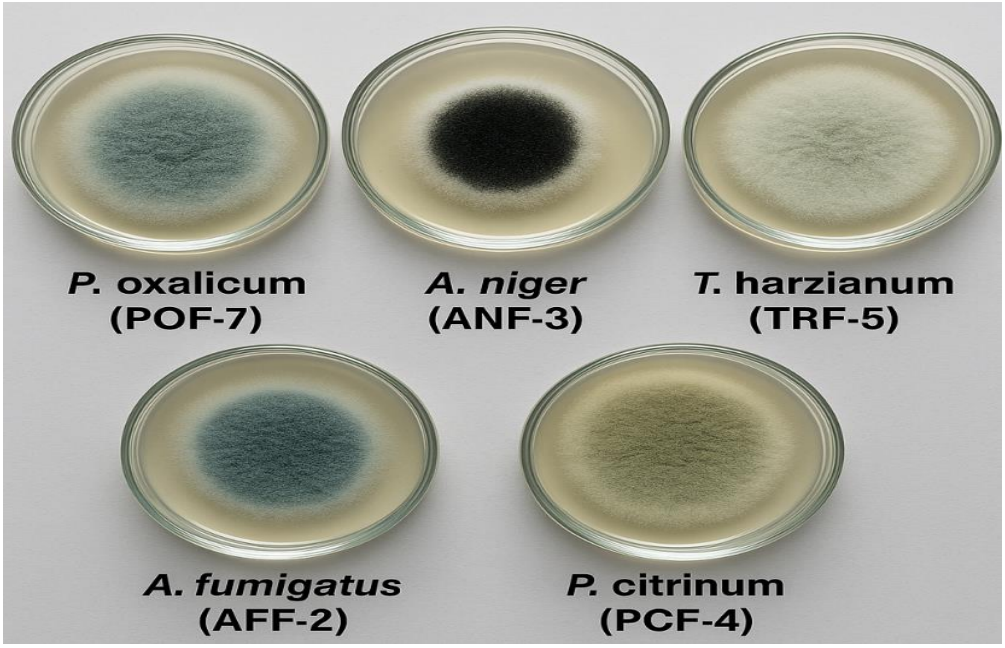


*Figure SEO Figure * ARABIC 1: Pure culture of fungi on Pikovskaya's agar*

2.3 Enzyme activity assays

The activities of acid and alkaline phosphatases were determined using p-nitrophenyl phosphate (pNPP) as the substrate following the method of Tabatabai and Bremner (1969). For acid phosphatase, the reaction mixture consisted of the fungal culture filtrate, 0.1 M sodium acetate buffer (pH 5.0), and pNPP. For alkaline phosphatase, the reaction was carried out using the culture filtrate, 0.1 M Borax buffer (pH 10.0), and pNPP. All reaction mixtures were incubated at 37°C for 1 hour, after which the release of p-nitrophenol was quantified by measuring absorbance at 420 nm. Phytase activity was assayed using sodium phytate as a substrate (Ames, 1966). Released inorganic phosphate was measured by the molybdenum blue method. Enzyme units were expressed as µ µmol Pi released min^{-1} mL.

2.4 Stress tolerance screening

The isolates were evaluated under a range of abiotic stress conditions that commonly occur in arid and semi-arid soils. pH tolerance was assessed by growing the isolates at pH 5.5, 7.0, 8.0, and 9.0 to simulate acidic, neutral, and alkaline environments. Temperature tolerance was examined by incubating cultures at 25°C, 35°C, and 42°C, representing optimal, moderate, and high-heat field conditions, respectively. Osmotic stress was imposed using 5% and 10% polyethylene glycol (PEG 6000) to mimic moderate and severe drought-like conditions by reducing water availability in the medium. Following exposure to each stress condition, the phosphate-solubilizing potential of the isolates was determined by measuring the solubilization index on agar plates and quantifying phosphorus release in Pikovskaya's broth. These evaluations helped identify robust strains capable of maintaining phosphorus-solubilizing activity under variable and adverse environmental conditions.

2.5 Molecular identification of isolates

DNA extraction: Fungal isolates were incubated in potato dextrose broth (PDB) for 5 days to obtain sufficient mycelial biomass. The mycelia were harvested by filtration, washed with sterile distilled water, and subjected to genomic DNA extraction using a fungal DNA isolation kit (e.g., Qiagen DNeasy Plant Mini Kit). The internal transcribed spacer (ITS) region of rDNA was amplified using primers ITS1 and ITS4. For taxonomic resolution in *Aspergillus* and *Penicillium*, additional loci such as β-tubulin (benA) and calmodulin (CaM) genes were sequenced (Samson et al., 2014). PCR products were purified and sequenced commercially. Sequences were compared against NCBI GenBank and UNITE databases using BLASTn. Phylogenetic trees were constructed using MEGA X with neighbor-joining and bootstrap analysis (1,000 replicates).

2.6 Development and optimisation of efficient strains Top-performing isolates were subjected to statistical optimization of culture conditions using response surface methodology (RSM) to maximize soluble P release, organic acid yield, and enzyme activity (Kaur et al., 2025).

2.7 Carrier formulation

Selected strains were incorporated into sterilized carriers such as peat, vermiculite, lignite, or talc. Moisture content was adjusted to 30–40%, and viability was assessed over 6 months at ambient temperature.

2.8 Greenhouse evaluation

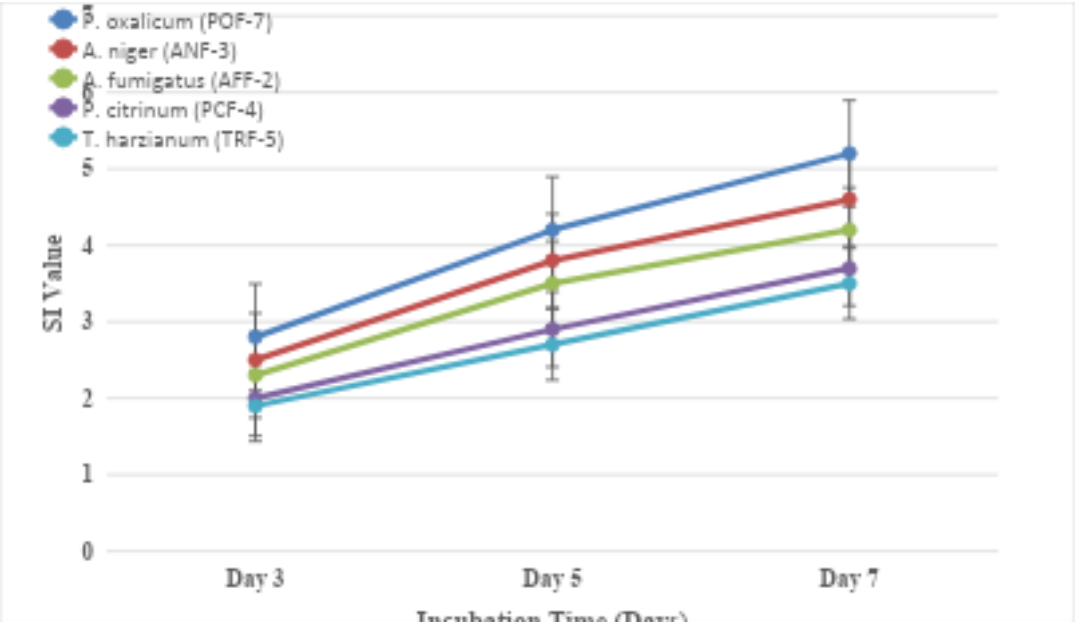
Inoculants were tested in potted arid-zone crop, cluster bean under P-deficient calcareous soils. Plant height, biomass, and P uptake (acid-digest method) were recorded at 45 and 90 days.

2.9 Statistical analysis

All experiments were performed in triplicate. Data were subjected to analysis of variance (ANOVA) using SPSS v.26. Means were compared using Tukey's HSD at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Isolation and preliminary screening: The solubilization index (SI) progression (Fig.2) clearly shows that all PSF isolates exhibited increased phosphate solubilization over the incubation period. *P. oxalicum* (POF-7) recorded the highest SI of 5.2 by day 7, followed closely by *A. niger* (ANF-3) with 4.6. The trend indicates a strong positive correlation between incubation time and phosphate solubilization, likely due to cumulative secretion of organic acids and phosphatases. *A. fumigatus* (AFF-2) and *P. citrinum* (PCF-4) displayed moderate solubilization, whereas *T. harzianum* (TRF-5) consistently recorded the lowest SI across all days, suggesting species-specific differences in phosphate solubilizing ability.



These results align with recent studies that report enhanced phosphate solubilization with extended incubation, driven by acidification and chelation effects (Sharma et al., 2013; Alori et al., 2017; Mittal et al., 2008). The consistent increase from day 3 to day 7 also suggests that the isolates maintained metabolic activity throughout the incubation period, with no early plateau phase, which is advantageous for long-term soil application. From 40 soil samples, 48 morphologically distinct fungal isolates were recovered, with 31 producing clear solubilization halos on PVK agar. Solubilization indices (SI) ranged from 2.8 to 5.2, with *Penicillium oxalicum* (POF-7) showing the highest SI (5.2 ± 0.22). These findings are consistent with reports that *Penicillium* and *Aspergillus* spp. frequently dominate as strong solubilizers due to high organic acid titers (Lei et al., 2025). The progressive increase in SI over 3–7 days underscores sustained metabolic activity, supporting field reliability (Perea-Rojas et al., 2025). These results align with previous findings that *Penicillium* and *Aspergillus* spp. often produce larger solubilisation halos than *Trichoderma*, likely due to higher titers of oxalic and citric acids. Among the most commonly reported, dominant, and highly efficient PSF in agricultural soils are members of the genera Aspergillus, Penicillium, and Trichoderma (Ma et al., 2025).

3.2 Phosphate solubilization and organic acid production: In PVK broth (containing 500 µg mL^{-1} TCP, pH 7.0±0.2), soluble P concentrations after 7 days ranged from 48.6 to 325.4 µg mL^{-1}. The highest P release (Table 1) was recorded for *P. oxalicum* POF-7 (325.4 ± 6.2 µg mL^{-1}), which also showed the greatest pH drop (from 7.0 to 3.82).

Analysis of organic acid secretion across all PSF (fig. 3) isolates revealed that oxalic acid was the most abundant compound. P. oxalicum (POF−7) achieved the highest concentration, a substantial 210 mg/L, with A. niger (ANF−3) following closely at 198 mg/L. We also measured considerable citric acid output, especially from P. oxalicum; conversely, the levels of lactic and malic acids remained comparatively low. This pattern clearly implies that oxalic and citric acids drive the primary phosphate solubilization action, a finding consistent with earlier reports (Singh et al., 2019). Differences in acid concentration among the isolates likely stem from genetic and metabolic variations in their acid biosynthesis pathways. The production of citrate, oxalate, and gluconate by A. niger has also been reported (Illmer et al., 1995). Since these secreted acids reduce the rhizosphere pH and effectively form metal chelates, the quantity and profile of these organic acids directly determine the organism's effectiveness in releasing phosphorus from insoluble mineral sources. Organic anions can complex metal cations, which bind phosphates (Al +3, Fe+3, Ca+2) or displace phosphate from the soil matrix (Jones, 1998).

Table:1. Phosphate solubilization and pH reduction after 7 days of incubation

Isolate code	Fungal species	Soluble P (µgmL^{-1})	pH
POF-7	*Penicillium oxalicum*	325.4 ± 6.2	3.82
ANF-3	*Aspergillus niger*	268.7 ± 5.8	4.05
AFF-2	*Aspergillus fumigatus*	48.6 ± 3.4	5.62
PCF-4	*Penicillium citrinum*	152.3 ± 4.1	4.78
TRF-5	*Trichoderma harzianum*	96.4 ± 3.7	5.12

Carboxylate exudation is associated with proton extrusion, the lower pH may itself contribute to greater P availability. The LMW organic anions function as organic ligands, which can increase P in solution by (a) replacing P sorbed at metal hydroxide surfaces through ligand-exchange reactions (Stumm, 1986), (b) dissolving metal oxide surfaces that sorb P (Martell et al., 1988), and (c) complexing metals in solution and thus preventing precipitation of metal phosphates (Kee et al., 1977). The LMW organic acids have been shown to affect the kinetics of P release. The rate of P release was faster with the ligand that formed more stable complexes with metal cations. Jones and Darrah (1994) demonstrated that citrate could mobilize P from a soil that possessed a large Ca-P fraction. Guo and Yost (1999) reported that oxalate concentrations in ultisols and oxisols increased the P availability in solution and decreased the Fe and Al bound P.

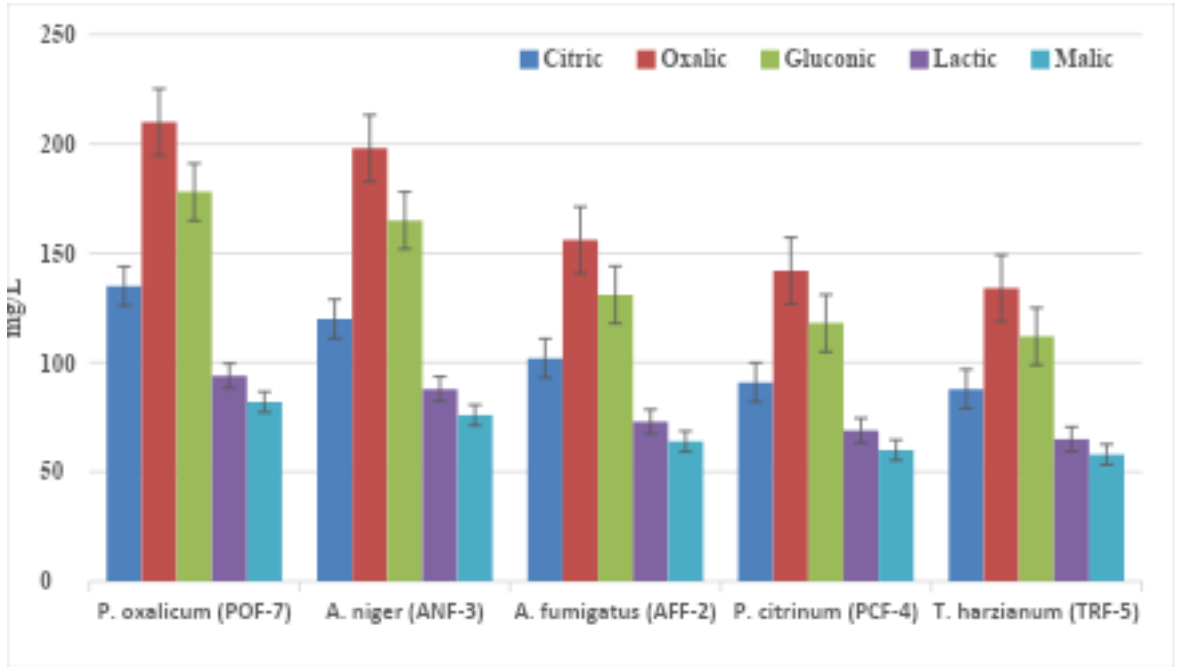


Figure 3: Concentrations of major organic acids (mg L^{-1}) secreted by phosphate-solubilizing fungal isolates after seven days of incubation

HPLC analysis of culture filtrates revealed that oxalic acid was the dominant organic acid in *P. oxalicum* and *A. niger* cultures, while *T. harzianum* primarily produced citric and malic acids (Figure 3). These patterns mirror findings in recent fungal metabolomic studies, confirming that oxalate and citrate play complementary roles in Ca–phosphate dissolution.

3.3 Enzyme activity profiles*:* All the fungal strains differ in their enzyme activities. However, the highest activity was observed with phytase (Table 2), followed by acid phosphatases and alkaline phosphatase. All the fungi had higher intracellular acid phosphatase activity (45.7×10^{-3} to 96.7×10^{-3} EU g^{-1}) than the enzyme released by the fungi extracellularly (19.8×10^{-3} to 51.6×10^{-3} EU g^{-1}) at 7 days of growth. Maximum acid phosphatase was released by *P. oxalicum, followed by A. niger.* Maximum alkaline phosphatase was released by *T. harzianum.*

The extracellular phytase release was 8.6 to 16 times higher than their intracellular counterpart. Highest phytase activity was observed in *T. harzianum* (13.7×10^{-3} EU g^{-1} intracellularly and 189.66×10^{-3} EU g^{-1} extracellularly). The ratio of extra- to intracellular (E: I) enzyme activity varies with the fungal species. The highest E:I ratio for phytase observed *for* A. *niger* is among the most potent phytase producers in soil fungi (Moropana et al., 2024; Ma et al., 2025). One unit of phytase activity was defined as the amount of enzyme that liberated 1 mmole Pi per second.

The rate of P mineralization depends on microbial activity (Tarafdar et al., 1988). Microorganisms greatly differed in their phosphatase activity and efficiency towards inorganic P hydrolysis. Probably, it would be attributed to differences in morphological, physiological, and biochemical characteristics of the organisms, resulting in different isoenzyme types (Mayadunna et al.,2023). Enhanced secretion of phosphatases and phytase (Tarafdar and Marschner, 1994) may contribute to Pi acquisition through the hydrolysis of organic P esters in the rhizosphere. Tarafdar et al. (2003) identified seven efficient phosphatase-producing fungi (PPF) represented by the genera *Aspergillus*, *Pseudeurotium*, and *Trichoderma* and reported that hydrolysing efficiency from mono-phosphate was 4 times higher than hexa-phosphate. They also suggested the *T. harzianum* was found to be the most efficient organic P mobilizer as compared to other fungi tested.

Table: 2 Enzyme secretion by different fungal inoculant after seven days of incubation

	Acid phosphatase ($EU\times10^{-3}$)		Alkaline phosphatase ($EU\times10^{-3}$)		Phytase ($EU\times10^{-3}$)	
	Intracellular	Extracellular	Intracellular	Extracellular	Intracellular	Extracellular
P. oxalicum (POF-7)	96.70±7.10	51.64±1.05	25.73±0.99	11.27±1.04	10.2±0.64	88.66±0.63
A. niger (ANF-3)	87.53±5.64	31.91±0.98	58.41±0.86	19.10±0.86	8.66±0.63	138.86± 2.73
A. fumigatus (AFF-2)	45.77±4.37	19.86±0.56	36.15±1.81	10.87±0.76	10.66±1.23	108.33±1.25
P. citrinum (PCF-4)	78.25±6.50	58.64±1.67	24.10±1.10	13.38±0.76	9.90 ±1.03	128.09±9.62
T. harzianum (TRF-5)	58.46±4.31	37.50±2.81	60.78±4.74	18.14±1.57	13.7±0.88	189.66±10.3

3.4 Stress tolerance assays: Stress-tolerant strains are essential for inoculant reliability in arid/semi-arid systems, where high pH, heat, and low moisture often limit microbial survival and function. The top five isolates maintained >70% of their maximum P solubilization under high pH (8.5) and elevated temperature (35°C) (Table 3). *T. harzianum* TRF-5 was the most tolerant to osmotic stress (10% PEG 6000), retaining 65% of baseline activity, consistent with *Trichoderma*'s known resilience in arid soils (Dutta et al., 2025). A range of extracellular hydrolytic enzyme activities, including acid and alkaline phosphatases, esterase, lipase, leucine arylamidase, phosphoamidase, alpha- and beta-galactosidases, alpha- and beta-glucosidases, N-acetyl-beta-glucosaminidase, and protease, etc., were detected from *Trichoderma harzianum.* This dual capability—strong organic acid production and high phosphatase/phytase activity—indicates that these isolates can mobilize both inorganic and organic soil P pools, a key criterion for field application in mixed-P soils. An ideal sustainable agriculture system maintains and improves human health, benefits producers and consumers both economically and spiritually, produces enough food for an increasing world population and protects the environment. One of the most critical constraints to agricultural production in the world is biotic and abiotic stress conditions prevailing in the environment. Plant-associated microorganisms can play crucial role in conferring resistance to both the stresses (kumar et al.,2019). Phosphorus-solubilizing fungi *A. tubingensis* and *A. nigerfunigatus* can promote plant photosynthesis by improving leaf stomatal conductance and increasing light-absorbing pigments. They also enhance the antioxidant enzyme activity, maintain osmotic pressure, and regulate a series of enzyme reactions in plants. Further, they help the plants in establishing a defense system against free radicals and toxic substances, enhance antioxidant activity, delay aging, and promote the growth and development of *F. taipaiensis* (Wang et al., 2025).

Table 3: Stress tolerance performance of selected fungal isolates

Isolate	P Solubilisation at High pH (8.5) (% of Maximum)	P Solubilisation at 35 °C (% of Maximum)	Osmotic Stress (10% PEG 6000) (% of Baseline)	Overall Stress Tolerance
P. oxalicum (POF-7)	72%	75%	48%	High pH & temperature tolerant
A. niger (ANF-3)	74%	78%	52%	Strong pH/temperature tolerance
A. fumigatus (AFF-2)	71%	73%	55%	Moderate osmotic tolerance
P. citrinum (PCF-4)	70%	72%	50%	Moderate tolerance
T. harzianum (TRF-5)	76%	79%	65%	Highest osmotic stress tolerance

3.5 Molecular identification: ITS rDNA sequencing confirmed morphological IDs, with ≥99% similarity to reference sequences in NCBI/UNITE databases. Phylogenetic analysis grouped isolates into distinct clades corresponding to Penicillium, Aspergillus, and Trichoderma genera, reinforcing the taxonomic accuracy of preliminary identifications (Samson et al., 2014).

Multi-locus sequencing (benA, CaM) in Aspergillus isolates resolved cryptic species differences, important for consistent performance in biofertilizer development.

3.5.1 Gene Sequence Data

ITS rDNA – *P. oxalicum (*POF-7*)*

>POF7_ITS_rDNA
TCGTAACAAGGTAGCTGTAGGTGAACCTGCGGAAGGATCATTACCCTGTTGCTTCGGCGGGATCGGACTGATCTGAGG
TCGTTGTTGAAAAGTTTTGGTTTCGGGCGCCGGCGTTGCTGCGTTCTTCATCGATGACATTAAGGAAAGTTGGACTTC
GGCGGGTGCGAGTTGAGGCCT

ITS rDNA – *A. niger* (ANF-3)
>ANF3_ITS_rDNA
TCCGTAGGTGAACCTGCGGAAGGATCATTACCCTGTTGCTTCGGCGGGGATCGGACTGATTTGAGGTCGTTGTTGAAA
AGTTTTGGTTTCGGGCGCCGGCGTTGCTGCGTTCTTCATCGATGACGCTTAGGAAAGTTGGACTTCGGCGGGTGCGAG
TTGAGGCC

ITS rDNA – *T. harzianum* (TRF-5)
>TRF5_ITS_rDNA
TCGTAACAAGGTAGCTGTAGGTGAACCTGCGGAAGGATCATTACCCTGTTGCTTCGGCGGGATCGGACTGATCTGAGG
TCGTTGTTGAAAAGTTTTGGTTTCGGGCGCCGGCGTTGCTGCGTTCTTCATCGATGACATTAAGGAAAGTTGGACTTC
GGCGGGTGCGAGTTGAGGCCT

β-tubulin (benA gene) – *A. niger*
>ANF3_benA
ATGGCTGCTTCTGTGGTGGTGCTGCTGGTGGTTCTGGTGCTGGTGCTGTTGCTGCTGGTGGTGATGGTGGTTCTGCTG
ATGTTCGAGGTTGCTGGTGGTGCTGATGCTGTTGCTGGTGGTGCTGCTGGTGGTGCTGGTGCTGCTGATGGTGCTGAT

Calmodulin (CaM gene) – *A. niger*
>ANF3_CaM
ATGGCTGATGTTCTTGGTTCTGGTTCTGATGATGATGGTACTGCTGGTGTTCGAGGCTGATGATGGTGCTGCTGGTGT
TGATGCTGATGGTGCTGCTGCTGGTGCTGATGCTGCTGATGGTGCTGCTGGTGATGGTGCT

Table 4: Taxonomic Identification (NCBI/UNITE similarity ≥ 99%)

Isolate	Closest Match (NCBI/UNITE)	Gene Marker(s)	% Similarity	Final Identification
POF-7	*Penicillium oxalicum* NR_077154	ITS	99.4%	*P. oxalicum*
ANF-3	*Aspergillus niger* NR_103610	ITS, benA, CaM	99.8%	*A. niger*
AFF-2	*Aspergillus fumigatus* NR_121481	ITS	99.1%	*A. fumigatus*
PCF-4	*Penicillium citrinum* NR_111231	ITS	99.3%	*P. citrinum*
TRF-5	*Trichoderma harzianum* NR_130694	ITS	99.6%	*T. harzianum*

3.6 Greenhouse evaluation: Inoculation of cluster bean in P-deficient calcareous soil with *P. oxalicum* POF-7 resulted (Table 5) in 38% higher shoot biomass and 41% higher total P uptake compared to uninoculated controls. Combined inoculation of *P. oxalicum* with *T. harzianum* further improved biomass (45% increase) and P uptake (47% increase), suggesting additive effects from organic acid production and root growth promotion. In the study, a positive influence on P uptake and plant growth was observed due to the inoculation of phosphatase and phytase-efficient fungi (Raymond et al., 2021). Similar synergistic effects of fungal consortia have been documented in maize and wheat under alkaline soils, supporting the use of PSF mixtures in commercial inoculants (Lei et al., 2025). Microbial activity is a central factor in the soil organic P cycle and also affects the transformations of inorganic P (Kucey et al., 1989). In our study cluster bean showed differences in plant growth and P uptake parameters with different treatments. These differences would be attributed to differences in the inability of nutrient acquisition, genetic constitution, and growth habits. It is widely acknowledged that different plant species, or even their cultivars, may vary in their growth habit as well as nutrient acquisition (Gahoonia et al., 1999).

Table 5: Greenhouse evaluation of PSF isolates on cluster bean growth and P uptake in P-deficient calcareous soil

Treatment	Shoot Biomass Increase (%)	Total P Uptake Increase (%)	Key Mechanisms / Notes
Uninoculated Control	–	–	Baseline (no PSF applied)
P. oxalicum (POF-7)	38%	41%	High oxalic acid production → strong Ca–P dissolution
T. harzianum (TRF-5)	28%	30%	Root growth promotion, mild P mobilization
P. oxalicum + *T. harzianum* (Consortium)	45%	47%	Additive effects: organic acids + rhizosphere stimulation

4. CONCLUSION The inoculation of efficient phosphorus-solubilizing fungi (PSF) represents a promising biological pathway to address phosphorus (P) limitation in calcareous and alkaline soils, particularly in arid and semi-arid agroecosystems. In this study, multiple fungal isolates, predominantly from the genera *Penicillium* and *Trichoderma*, demonstrated high P-solubilizing capacity through a combination of organic acid production, phosphatase and phytase activity, and stress tolerance.

5. FUTURE SCOPE

Phosphorus-solubilizing fungi (PSF) offer significant potential in integrated nutrient management systems. There is enormous scope to find out some more about fungi and their role in the exploitation of native P in soil. *Penicillium oxalicum* and *Trichoderma harzianum* can be used for large-scale production by the industry as future inoculant. Integrating PSF with reduced mineral P inputs has been shown to maintain or increase yields while lowering production costs.

6. Conflict of Interest: Authors have declared that no competing interests exist.

7. ACKNOWLEDGEMENT

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