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Biological Strategies for Phosphorus Mobilization: A Comparative Assessment of Crop and Fungal Phytase

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ABSTRACT

Phosphorus (P) is one of the major essential macronutrients required for plant growth, but its availability in soil is often limited due to fixation and presence in organic forms such as phytate. Phytase enzymes play a pivotal role in the hydrolysis of phytic acid, releasing inorganic phosphate that can be assimilated by plants. This study compares the phytase efficiency of selected legumes and cereals (cluster bean, mung bean, moth bean, pearl millet, and sorghum) under phosphorus-sufficient and phosphorus-deficient conditions. Simultaneously, it evaluates phytase activity from selected fungal isolates including Chaetomium globosum, Aspergillus ustus, Curvularia lunata, and Phoma spp., focusing on both intra- and extracellular activity. Results showed that legumes exhibited a higher phytase response under P-deficiency compared to cereals. Among fungi, Chaetomium globosum showed significantly higher extracellular phytase activity, correlating with superior inorganic P release from phytin and other organic P compounds. The study emphasizes the potential application of fungal phytase, particularly extracellular forms, in enhancing phosphorus bioavailability in arid and semi-arid soils, suggesting a complementary role for phytase-enriched biofertilizers.

***Keywords**: Phytase Efficiency, Phosphorus Mobilization, Legumes and Cereals, Fungal Phytase, Biofertilizer Potential, Sustainable Agriculture.*

Introduction

Phosphorus (P) is the second most limiting nutrient for crop productivity after nitrogen, particularly in arid and semi-arid regions. A significant portion of soil phosphorus exists in the organic form, predominantly as phytic acid or phytate, which is not directly available to plants. The mobilization of this bound phosphorus requires the activity of phytases—enzymes capable of hydrolyzing phytate into inorganic phosphate (Greiner & Konietzny, 2006).

Phytases are found in plants, microorganisms (bacteria and fungi), and animals, each possessing varying degrees of efficiency, pH optima, and substrate specificity. In soils, microbial phytases—especially those secreted by fungi—play a critical role in phosphorus cycling (Khavazi et al., 2022; Çalışkan-Özdemir et al., 2021). Simultaneously, plant roots also produce phytases, particularly under phosphorus-deficient conditions, to facilitate phosphorus uptake from organic sources in the rhizosphere (Tang et al., 2020).

Earlier studies have highlighted that while oilseed crops tend to secrete higher quantities of phytase, leguminous crops display higher specific activity for organic phosphorus hydrolysis (Sharma et al., 2024). Among fungi, genera such as *Aspergillus*, *Penicillium*, and *Chaetomium* are reported to be prolific phytase producers, capable of substantial extracellular enzyme secretion (Calizkan–Ozdemir et al., 2023).

Despite these insights, systematic comparative studies examining the phytase efficiency of arid-land crops (like cluster bean, mung bean, moth bean, pearl millet, and sorghum) versus fungal sources remain scarce. This study aims to bridge this gap by conducting a controlled investigation into the phytase activity of these crop species under differential phosphorus regimes, and by characterizing the

enzymatic behavior of isolated fungal strains across time intervals. The ultimate objective is to identify potent phytase sources for biofertilizer development and enhanced soil phosphorus management in low-input agricultural systems (Yadav & Tarafdar, 2001).

Materials and Methods

- **Plant Material and Growth Conditions:** Seeds of cluster bean (*Cyamopsis tetragonoloba*), mung bean (*Vigna radiata*), moth bean (*Vigna aconitifolia*), pearl millet (*Pennisetum glaucum*), and sorghum (*Sorghum bicolor*) were surface sterilized in a 1:1 mixture of ethanol and hydrogen peroxide for 2 minutes followed by treatment with 0.05% mercuric chloride for another 2 minutes. After washing thoroughly with sterile deionized water, seeds were allowed to germinate on moist filter paper for 5 days.

Germinated seedlings were transferred to growth chambers in a nutrient solution containing either 250 mg P L^{-1} (sufficient) or 5 µg P L^{-1} (deficient) phosphorus supplied as KH_2PO_4. Plants were grown in sterile conditions for 21 days with a 14-hour photoperiod (2,500–3,000 lux) at 30°C (day) and 20°C (night), and 65±5% relative humidity. Root exudates were collected weekly, and phytase activity in the solution was measured.

- **Isolation and Cultivation of Fungal Strains:** Soil samples were collected from 20 arid zone fields and serially diluted for fungal isolation on Martin's Rose Bengal agar supplemented with streptomycin. After purification, five dominant phytase-producing fungi were identified: *Chaetomium globosum*, *Aspergillus ustus*, *Curvularia lunata*, *Phoma* species, and *Aspergillus flavus*. Fungal cultures were maintained on potato dextrose agar (PDA) and later transferred to liquid Zapek-Dox broth for enzyme assays.
- **Enzyme Extraction and Activity Assays:** For extracellular activity, culture broth was filtered through Whatman No. 1 paper and diluted to a fixed volume. For intracellular activity, fungal biomass was homogenized with acid-washed quartz sand, and cell-free extract was obtained after centrifugation at 12,000 rpm for 20 minutes.
- **Hydrolysis of Organic P Compounds:** Enzyme units were standardized. Equal volumes of plant or fungal phytase (1 EU) were incubated with 300 µg of organic P (as ADP, ATP, glycerophosphate, or phytin) in 50 mL citrate buffer (pH 4.5) for up to 24 hours. Pi release was measured spectrophotometrically using the molybdenum blue method.
- **Soil Incubation Studies:** Soil samples were amended with 50 mL of phytase solution (1 EU) and incubated at 30°C for 2, 4, 6, 10, and 24 hours. The release of inorganic phosphate from soil phytin was recorded.
- **Data Analysis:** All experiments were performed in triplicates. Results were analyzed using one-way and two-way ANOVA followed by LSD (least significant difference) test at $p<0.05$.

Results

- **Phytase Activity in Plants under Phosphorus Stress:** Phytase activity in all five crop species increased under phosphorus-deficient conditions compared to sufficient phosphorus supply. The legumes (mung bean, moth bean, and cluster bean) demonstrated notably higher phytase release rates than cereals. At 21 days after sowing (DAS), cluster bean recorded 4.05 EU × 10^{-5} under –P, versus 3.12 EU × 10^{-5} under +P, representing a 29.8% increase. Similarly, mung bean and moth bean showed increases of 27.6% and 31.4% respectively. In contrast, pearl millet and sorghum showed lower increases of 12.1% and 15.7% respectively.

Table 1: Phytase Activity (EU × 10^{-5}) in Root Exudates at 21 DAS under P Conditions

Plant Species	+P	-P	% Increase
Cluster Bean	3.12 ± 0.10	4.05 ± 0.14	29.8%
Mung Bean	3.50 ± 0.12	4.47 ± 0.16	27.6%
Moth Bean	3.75 ± 0.13	4.93 ± 0.17	31.4%
Pearl Millet	2.80 ± 0.11	3.14 ± 0.12	12.1%
Sorghum	3.02 ± 0.10	3.50 ± 0.11	15.7%

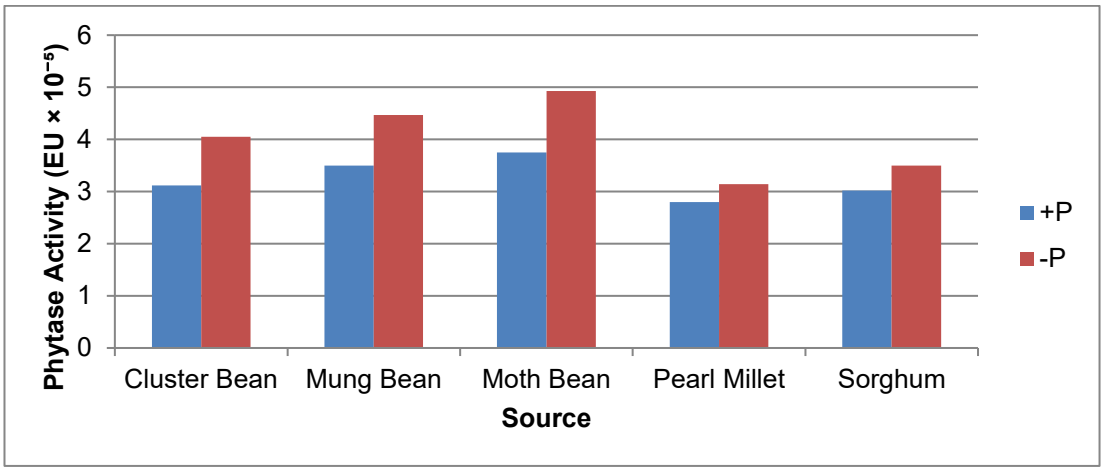

Figure 1: Increase in phytase activity in crop species under –P condition relative to +P

- **Fungal Phytase Activity: Intracellular vs Extracellular** Among fungal species, *Chaetomium globosum* showed the highest phytase production. Its extracellular activity at 21 days was 12.6 × 10^{-3} EU g^{-1} fungal mat, nearly 10 times higher than its intracellular counterpart. *Aspergillus ustus* and *Curvularia lunata* also showed substantial extracellular activity but lower than *C. globosum*.

Table 2: Fungal Phytase Activity at 21 Days Incubation

Fungal Species	Intracellular (EU × 10^{-3})	Extracellular (EU × 10^{-3})	Ratio (Extra/Intra)
Chaetomium globosum	1.25 ± 0.06	12.60 ± 0.34	10.1
Aspergillus ustus	0.85 ± 0.05	9.50 ± 0.31	11.2
Curvularia lunata	0.55 ± 0.03	4.15 ± 0.22	7.5
Phoma species	0.45 ± 0.02	3.88 ± 0.19	8.6

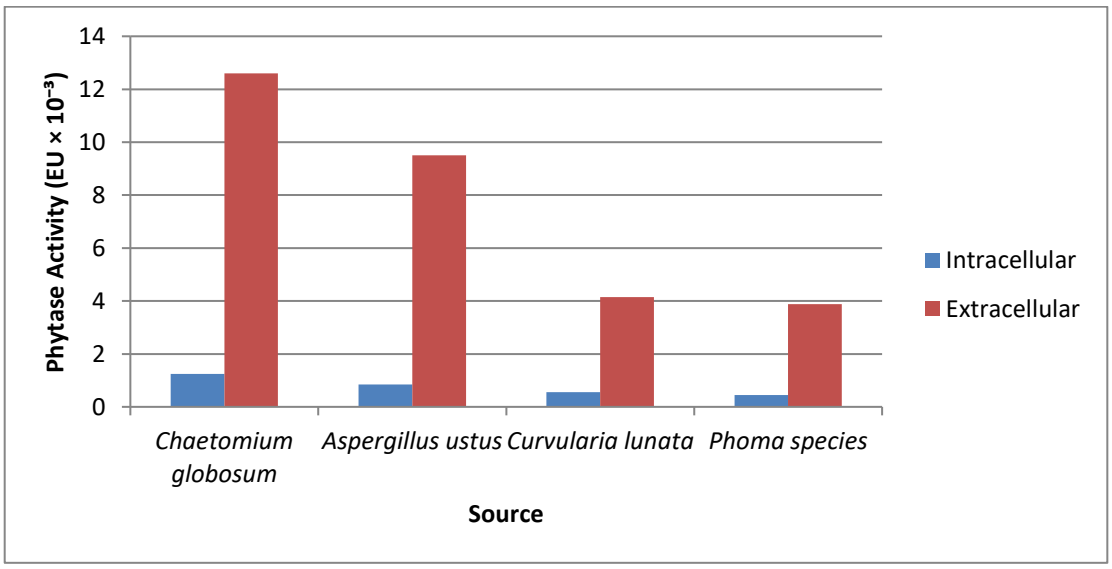

Figure 2: Comparison of intracellular and extracellular phytase activity

- **Hydrolysis of Organic P Compounds by Plant and Fungal Phytase** Phytase enzymes hydrolyzed all tested organic P substrates with varying efficiency. ADP showed the highest hydrolysis across all sources. Extracellular fungal phytase significantly outperformed plant phytase across all substrates.

Table 3: Percent Hydrolysis of Organic P Sources after 24 Hours (per 1 EU phytase)

Source Type	ADP (%)	ATP (%)	Glycerophosphate (%)	Phytin (%)
Plant Phytase	32.4	28.6	10.1	7.6
Fungal Intracell.	22.7	20.3	18.8	18.4
Fungal Extracell.	32.8	31.4	28.6	26.2

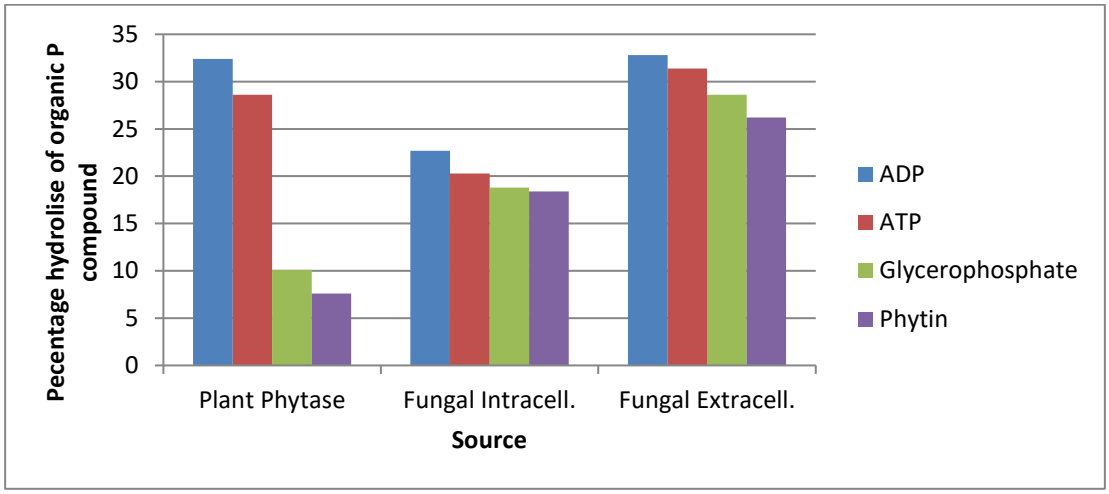

Figure 3: Substrate-specific hydrolysis efficiency of plant and fungal phytase

- **Soil Phytin P Hydrolysis Efficiency** Phytase from legumes hydrolyzed up to 57.8% of soil phytin P within 24 hours, followed by cereals. Among fungi, *Chaetomium globosum* demonstrated the highest capacity, achieving 47.1% hydrolysis.

Table 4: Phytin P hydrolysed at different time interval by plant and fungal phytase

Source	2h	6h	10h	24h	% Hydrolyzed
Cluster Bean	6.45	10.72	11.90	13.65	54.6%
Moth Bean	7.11	11.92	13.25	14.81	59.2%
Sorghum	5.12	8.02	9.35	10.15	40.6%
Chaetomium globosum	9.93	18.57	22.55	23.55	47.1%
Aspergillus ustus	8.29	15.40	15.00	16.58	33.2%

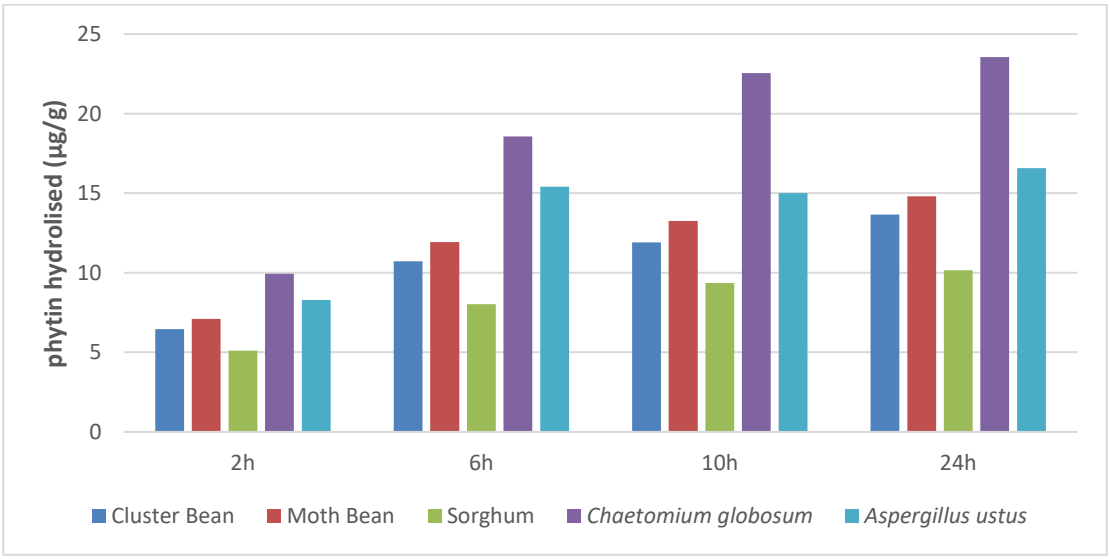

Figure 4: Phytin P hydrolysed at different time interval by plant and fungal phytase

Discussion

The comparative analysis of phytase efficiency between plant and fungal sources revealed several important insights relevant to phosphorus mobilization in agroecosystems. The findings suggest a significant variation in enzyme activity, not only among crop types but also between intra- and extracellular fractions of fungal phytase (Ma et al., 2023).

- **Plant Responses Under Phosphorus Deficiency** Leguminous crops, particularly moth bean, mung bean, and cluster bean, demonstrated a robust capacity to secrete phytase under P-deficient conditions. The increase in phytase activity under –P stress was more than 27% in legumes, compared to less than 16% in cereals. This trend supports previous studies indicating that legumes are better adapted for phosphorus solubilization due to their ability to modify the rhizosphere chemically and biologically.
- **Superior Efficiency of Extracellular Fungal Phytase** Fungal phytase, particularly from *Chaetomium globosum*, exhibited markedly higher extracellular enzyme activity compared to its intracellular counterpart and to phytase from plants. The extracellular fraction of *C. globosum* showed up to 12.6×10^{-3} EU g^{-1}, a tenfold increase over intracellular activity. This elevated secretion is crucial, as extracellular enzymes directly interact with organic phosphorus compounds in the soil matrix. The high extracellular-to-intracellular activity ratio observed across fungi supports their role as key microbial agents in phosphorus mobilization (Yadav et al., 2010).
- **Enzyme Specificity Toward Organic Substrates** ADP and ATP were consistently the most hydrolyzed substrates, followed by glycerophosphate and phytin. Notably, extracellular fungal phytase released nearly 26.2% of phosphorus from phytin, compared to just 7.6% by plant phytase. These findings align with the mechanistic understanding that fungal enzymes possess broader substrate specificity and higher catalytic turnover under acidic conditions typical of many soil environments (Caffaro et al., 2020; Patel et al., 2021; Hong et al., 2004; Hayes et al., 2000).
- **Time-Dependent Phytin Hydrolysis in Soil** Over a 24-hour incubation, legume-sourced phytase hydrolyzed up to 59.2% of soil phytin P, outperforming cereal-derived enzymes. Among fungal sources, *C. globosum* hydrolyzed 47.1% of phytin P, followed by *Aspergillus ustus* and *Phoma*. The release curve showed sharp increases between 2h and 10h, suggesting that fungal phytase acts rapidly, which is advantageous under fluctuating soil moisture and temperature regimes. This rapid mineralization window could be harnessed in designing controlled-release biofertilizer formulations (Xue et al., 2022; Tarafdar & Yadav, 2011).
- **Implications for Soil P Management** The differential performance of phytase sources implies that plant breeding for high phytase-exuding cultivars, particularly among legumes, may enhance P acquisition in low-input systems (Souid et al., 2022). Additionally, fungal strains such as *Chaetomium globosum* show potential for development as inoculant-based biofertilizers targeting organic P pools (Singh et al., 2023). These findings suggest integrated use of phytase-active plants and microbes can create synergistic zones of enhanced phosphorus availability in the rhizosphere, especially in arid and phosphorus-fixing soils (Rawat & Singh, 2022).

In practical terms, integrating high-phytase legumes and fungal bio-inoculants into crop rotations or intercropping systems could enhance P cycling, reduce reliance on chemical P fertilizers, and improve crop yields sustainably.

Conclusion

This study provides a comprehensive comparison of phytase activity from selected legumes and cereals with phytase from fungal sources, emphasizing their role in mobilizing native soil phosphorus. The findings clearly show that legumes—particularly moth bean, mung bean, and cluster bean—exhibit significantly higher phytase activity under phosphorus-deficient conditions compared to cereals like pearl millet and sorghum. This highlights their adaptive advantage and potential utility in sustainable cropping systems under low phosphorus input.

Fungal isolates, notably *Chaetomium globosum*, emerged as highly potent producers of extracellular phytase. The extracellular form of fungal phytase consistently showed superior efficiency in hydrolyzing organic P compounds, including recalcitrant substrates like phytin, compared to both plant-derived phytase and the intracellular fungal enzyme fractions. These results reinforce the potential application of fungi in phosphorus-solubilizing biofertilizer formulations.

Substrate-specific hydrolysis experiments demonstrated that fungal phytase had a broader and more efficient catalytic range than plant phytase, especially under acidic conditions mimicking those of many natural soils. Soil incubation studies confirmed the rapid action of fungal phytase in releasing inorganic phosphorus from native phytin reserves, suggesting its suitability for enhancing phosphorus availability in situ.

In practical terms, this research suggests a dual approach to improving phosphorus use efficiency: (1) developing and deploying legume varieties with enhanced phytase secretion under phosphorus stress; and (2) introducing fungal phytase, particularly from *Chaetomium globosum*, into agricultural systems through biofertilizers or inoculants. These strategies could reduce dependence on chemical P fertilizers, improve phosphorus recycling, and contribute to sustainable agricultural productivity, especially in arid and phosphorus-deficient regions.

Future research should focus on field trials assessing the synergistic impact of plant–fungus phytase interactions, long-term soil phosphorus dynamics, and the formulation and delivery mechanisms of fungal phytase biofertilizers under variable agronomic conditions.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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