

Enhancement of Beneficial Enzyme Production in Plants and Fungi through Magnesium Nanoparticle Application: A Comprehensive Study

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DOI: 10.62823/IJGRIT/03.2(II).7646

ABSTRACT

Phosphorus (P) is a critical macronutrient for plant growth, yet its availability in soil is often limited due to fixation in insoluble forms. The application of magnesium nanoparticles (MgNPs) has emerged as a promising strategy to enhance phosphorus mobilization by stimulating beneficial enzymatic activity in both plant and fungal systems. This study investigates the impact of MgNPs on the secretion of phosphorus-mobilizing enzymes—particularly acid phosphatase and phytase—in five economically significant crops: cluster bean (*Cyamopsis tetragonoloba*), mung bean (*Vigna radiata*), moth bean (*Vigna aconitifolia*), pearl millet (*Pennisetum glaucum*), and sorghum (*Sorghum bicolor*), as well as in selected fungal species including *Aspergillus niger*, *Trichoderma harzianum*, and *Penicillium chrysogenum*. Application of MgNPs under controlled conditions led to a significant increase in enzymatic activities associated with phosphorus solubilization. Treated plants exhibited enhanced growth metrics—such as root development and chlorophyll content—coupled with elevated secretion of acid phosphatase and phytase enzymes in the rhizosphere. Similarly, fungi showed intensified enzyme production in response to MgNP exposure, indicating improved mineralization of organic phosphorus sources. These findings demonstrate that MgNPs significantly enhance phosphorus-mobilizing enzyme activity in both plants and fungi, thereby offering a novel approach to improving phosphorus bioavailability in agricultural soils. This work supports the potential of MgNPs as an efficient, nano-enabled tool for sustainable nutrient management, with future studies warranted to explore underlying gene regulatory mechanisms and field-scale applications.

Keywords: Phosphorus-Mobilizing Enzyme Activity, Phosphatase, Phytase, Mg Nanoparticle.

Introduction

Phosphorus: An Essential but Limiting Nutrient

Phosphorus (P) is a fundamental macronutrient essential for plant metabolic processes, including photosynthesis, energy transfer through ATP, nucleic acid synthesis, and cell division. Despite its importance, phosphorus is one of the most immobile and unavailable nutrients in soils, primarily due to its tendency to bind with calcium, aluminum, and iron ions, forming insoluble complexes that are inaccessible to plants. (Adrees et al., 2015; Ahmad et al., 2019) Consequently, phosphorus deficiency is a widespread agronomic problem, limiting crop productivity in both tropical and temperate soils.

To counteract this limitation, synthetic phosphate fertilizers are widely used. However, their efficiency remains low, with plants typically accessing only 15–25% of the applied phosphorus. The remainder becomes fixed in the soil matrix or is lost through leaching and runoff, contributing to environmental problems such as eutrophication. This inefficiency underscores the need for sustainable and efficient strategies to enhance phosphorus availability in soils.

Role of Phosphorus-Mobilizing Enzymes

Biological phosphorus mobilization is a critical process in natural and agricultural ecosystems. Plants and soil microorganisms have evolved enzymatic systems to release inorganic phosphorus from organic and mineral-bound forms. Among these, two enzymes are particularly important:

- Acid phosphatase (APase): Catalyzes the hydrolysis of phosphate esters and anhydrides, liberating orthophosphate under acidic conditions.
- Phytase: Hydrolyzes phytate (inositol hexakisphosphate), a major form of organic phosphorus in soils, releasing bioavailable phosphorus.

These enzymes play a pivotal role in improving phosphorus nutrition, especially in low-input farming systems. Enhanced enzymatic activity in the rhizosphere—often referred to as the “enzymatic halo effect”—can significantly increase the local phosphorus availability, facilitating uptake by plant roots. (Choudhary & Meena, 2020; Khan et al., 2018)

Plant and Fungal Contributions to Phosphorus Mobilization

Both higher plants and soil fungi contribute substantially to phosphorus cycling. Leguminous crops such as cluster bean (*Cyamopsis tetragonoloba*), mung bean (*Vigna radiata*), and moth bean (*Vigna aconitifolia*) are known for their symbiotic relationships with rhizobia and mycorrhizal fungi, which enhance nutrient acquisition. (Kalra & Saini, 2020; Mahendran et al., 2023) Cereal crops like pearl millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) have also demonstrated the capacity to secrete acid phosphatase under phosphorus stress.

Fungi, particularly phosphate-solubilizing fungi (PSF) such as *Aspergillus niger*, *Trichoderma harzianum*, and *Penicillium chrysogenum*, secrete organic acids and enzymes that mobilize phosphorus from both organic and inorganic sources. Their synergistic interaction with plant roots can lead to improved plant nutrition and soil health.

Nanotechnology in Agriculture: The Case for Magnesium Nanoparticles

In recent years, nanotechnology has emerged as a transformative tool in agriculture, offering novel approaches for nutrient delivery, pest control, and plant growth stimulation. (Khot et al., 2012; Subramanian et al., 2015) Nanoparticles (NPs) are defined by their nanoscale size (1–100 nm), high surface area-to-volume ratio, and unique reactivity, which together provide functional advantages over their bulk counterparts.

Magnesium (Mg) is an essential plant nutrient, acting as the central atom in the chlorophyll molecule and as a cofactor in over 300 enzymatic reactions. (Badole & Rathod, 2021; Dhanker & Kumari, 2023). Magnesium nanoparticles (MgNPs), therefore, hold promise as bioavailable and efficient nutrient sources with the potential to influence enzymatic pathways in plants and fungi. (Faizan et al., 2021; Kumar & Singh, 2021, Chauhan & Dhariwal, 2022; Awasthi et al., 2022) Preliminary studies have shown that MgNPs can modulate antioxidant enzyme activity and improve physiological performance under stress conditions. (Priyadarshini & Pradhan, 2017; Vassilev et al., 2020)

What remains largely unexplored, however, is the role of MgNPs in stimulating phosphorus-mobilizing enzymes such as phosphatase and phytase. Given magnesium’s biochemical role as an enzymatic cofactor and structural stabilizer, it is hypothesized that MgNPs may facilitate enzyme synthesis or activation, thereby promoting phosphorus solubilization.

Research Gap and Objective

Although prior research has explored the use of zinc and iron nanoparticles for enzyme induction and nutrient mobilization, few studies have investigated the specific effects of MgNPs on phosphorus-mobilizing enzymes in a combined plant-fungal system. There is also a lack of integrated approaches combining biological assays with quantum chemical modeling to explain the interaction mechanisms at a molecular level.

The present study aims to fill this gap by:

- Assessing the impact of MgNP application on acid phosphatase and phytase activities in selected leguminous and cereal crops.
- Evaluating the response of key fungal species to MgNP exposure in terms of enzyme secretion.

Materials and Methods

Experimental Design Overview

A controlled greenhouse and laboratory study was conducted to assess the effect of magnesium nanoparticles (MgNPs) on phosphorus-mobilizing enzyme production in selected plant and fungal systems. Five plant species and three fungal strains were selected based on their agronomic relevance and known enzyme secretion capabilities. Treatments included different concentrations of MgNPs applied to soil and culture media, with appropriate control groups for comparison.

- **Plant Material and Growth Conditions**

- **Plant Species**

Five crop species were selected:

- Cluster bean (*Cyamopsis tetragonoloba*)
- Mung bean (*Vigna radiata*)
- Moth bean (*Vigna aconitifolia*)
- Sorghum (*Sorghum bicolor*)
- Pearl millet (*Pennisetum glaucum*)

Seeds were surface-sterilized with 0.1% mercuric chloride for 2 minutes and rinsed thoroughly with sterile distilled water. They were sown in sterilized pots (4 kg capacity) containing a soil-sand-compost mix in a 2:1:1 ratio.

- **Soil Properties**

The soil used was sandy loam, with initial pH 7.2, organic carbon 0.52%, available phosphorus 8.6 mg/kg, and electrical conductivity 0.25 dS/m. Basal doses of nitrogen and potassium (60 and 40 kg/ha respectively) were applied uniformly.

- **Growing Conditions**

- **Temperature:** 25–28°C
- **Photoperiod:** 14-hour light / 10-hour dark
- **Irrigation:** Regular with deionized water

- **Fungal Culture and Maintenance**

Three phosphorus-solubilizing fungi were used:

- *Aspergillus niger*
- *Trichoderma harzianum*
- *Penicillium chrysogenum*

Pure cultures were obtained from the Mycology Division, Indian Agricultural Research Institute (IARI), New Delhi. They were maintained on Potato Dextrose Agar (PDA) plates at 28°C and sub-cultured monthly.

- **Magnesium Nanoparticle Treatment**

Commercially available MgNPs (purity >99%, particle size <50 nm) were procured and dispersed in deionized water using an ultrasonic bath for 30 minutes to prevent agglomeration. Five treatment groups were applied to both pot and fungal culture experiments:

Treatment Code	Treatment Description
T ₀	Control (no magnesium)
T ₁	10 ppm MgO
T ₂	20 ppm MgO
T ₃	10 ppm MgSO ₄
T ₄	20 ppm MgSO ₄

MgO and MgSO₄ were dissolved or suspended in distilled water and applied as a soil drench at the time of sowing and again after 20 days foliar application.

For fungal assays, media were supplemented with the respective concentrations (10 ppm or 20 ppm elemental Mg equivalent).

- **Enzymatic Assays**

- **Acid Phosphatase Activity (Tabatabai and Bremner 1969)**

Measured in both rhizospheric soil and fungal culture filtrates using p-nitrophenyl phosphate (pNPP) as a substrate:

- 1 g soil or 1 mL culture filtrate incubated with pNPP at 37°C for 1 hour
- Reaction terminated with 0.5 M NaOH

- Absorbance read at 420 nm
- Expressed as μg p-nitrophenol/g soil/hour or mL/hour for culture

▪ **Phytase Activity (Ames 1966)**

Assessed using sodium phytate as substrate:

- Reaction mix: substrate + enzyme extract + sodium acetate buffer
- Incubation at 37°C for 1 hour
- Orthophosphate released measured via molybdenum blue method (Jackson 1967)
- Absorbance at 660 nm
- Activity expressed in μmol Pi released/min/g soil or per mL filtrate.

Results

• **Overview of Enzymatic Response to Magnesium Treatments**

The activity of two key phosphorus-mobilizing enzymes—acid phosphatase and phytase—was evaluated across five plant species under five magnesium treatments:

T₀: Control (no magnesium)

T₁: 10 ppm MgO

T₂: 20 ppm MgO

T₃: 10 ppm MgSO₄

T₄: 20 ppm MgSO₄

Significant differences were observed across treatments for both enzymes, with marked variation between plant species.

• **Plants Phosphorus-Mobilizing Enzyme Activity**

▪ **Acid Phosphatase Activity**

The acid phosphatase activity (μg pNP/g soil/hour) generally increased in response to magnesium treatments, with the T₄ (20 ppm MgSO₄) treatment showing the highest activity after 21 days of sowing in most crops.

Key Observations:

- Cluster bean showed a consistent increase, peaking at 48.05 μmol Pi/g/h in T₄.
- Mung bean and moth bean exhibited similar trends, with the highest stimulation under T₂ and T₄ treatments.
- Pearl millet had moderate enzyme activity, though it peaked in T₂ and T₄.
- Sorghum showed the lowest overall acid phosphatase response.

Table 1: Acid phosphatase activity ($\text{EU} \times 10^{-4}$) after 21 days of sowing

Plant	T0	T1	T2	T3	T4
Cluster bean	28.79	32.55	34.68	33.4	48.05
Moth bean	32.41	33.23	35.62	40.38	41.24
Mung bean	29.26	30.32	30.14	35.09	50.32
Pearl millet	36.3	40.49	46.25	60.44	56
Sorghum	41.11	40.33	41.23	58.83	66.11

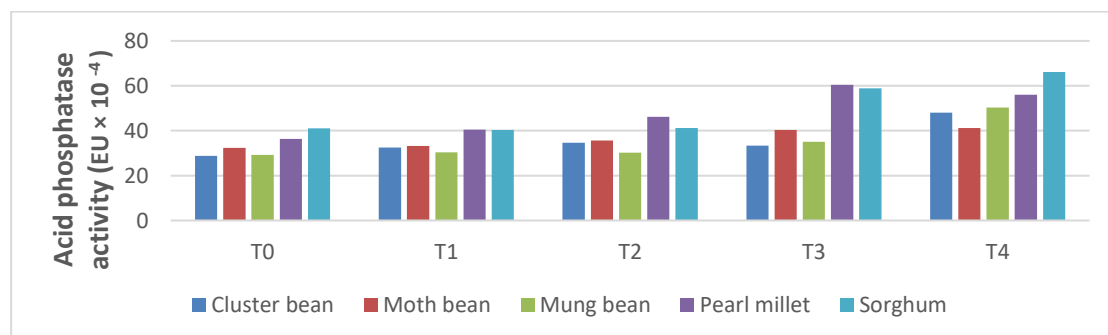


Figure 1: Acid phosphatase activity across treatments in different plants at 21 DAS

A bar graph comparing plant-wise response under each treatment shows:

- A substantial boost in acid phosphatase with both forms of magnesium.
- MgSO_4 was more effective at higher concentrations (T_4), possibly due to better solubility.

■ Phytase Activity

Phytase activity ($\mu\text{mol Pi/g soil/hour}$) displayed more nuanced variation, but still showed enhanced activity with magnesium supplementation at 28 DAS

Key observations:

- Mung bean and pearl millet responded strongly under T_1 (10 ppm MgO) and T_4 (20 ppm MgSO_4), with activities reaching up to $10.27 \mu\text{mol Pi/g/h}$.
- Moth bean maintained relatively stable activity across treatments.
- Sorghum exhibited steady activity, peaking under T_1 and T_2 .
- Cluster bean had the highest phytase increase at T_2 and T_3 .

Table 2: Phytase Activity ($\text{EU} \times 10^{-3}$) after 28 days of sowing

Plant	T0	T1	T2	T3	T4
Cluster bean	2.95	0.18	4.11	1.8	1.16
Moth bean	4.18	3.37	4.49	2.67	1.37
Mung bean	2.29	2	3.93	1.1	6.52
Pearl millet	1.73	10.27	1.87	0.67	6.63
Sorghum	3.76	6.42	4.6	3.5	3.79

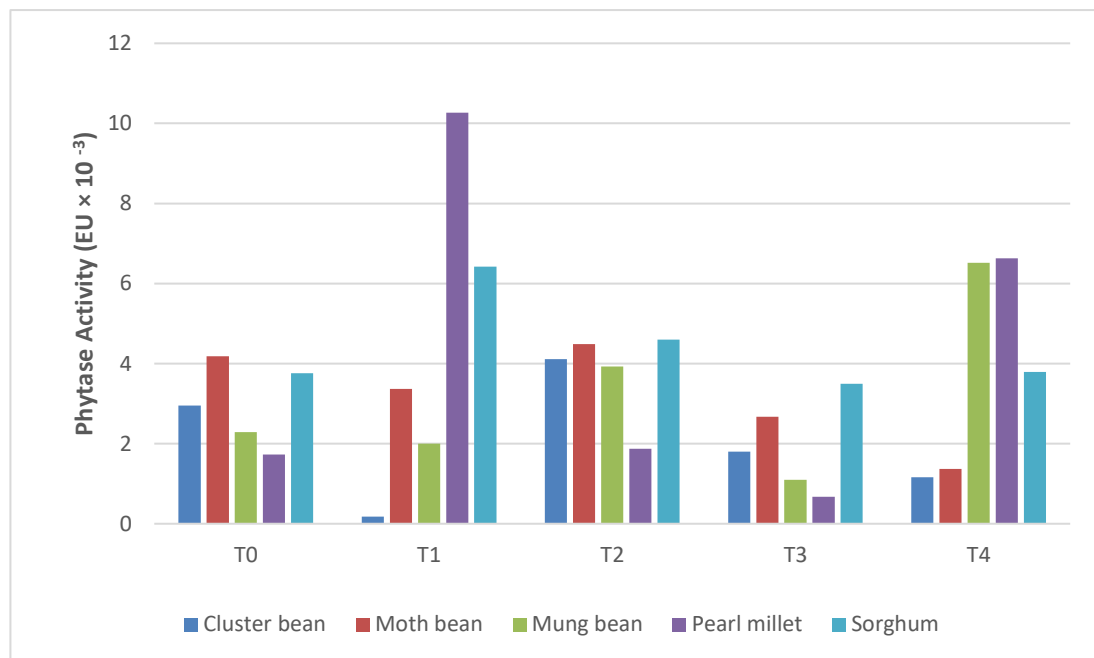


Figure 2: Phytase Activity Across Treatments in different plants at 28 DAS

This figure illustrates:

- Enhanced enzyme activity under both magnesium sources.
- MgO at moderate concentration (10–20 ppm) was effective in stimulating phytase in legumes.
- A broader positive trend across all plants compared to controls.
- **Comparative Efficacy of MgO vs. MgSO_4**
- MgSO_4 (particularly at 20 ppm) produced greater stimulation in acid phosphatase activity, especially in cluster bean and mung bean.

- MgO at both 10 and 20 ppm showed notable phytase induction, particularly in pearl millet and sorghum.
- These differences suggest that:
- Sulfate-based magnesium may better support acid phosphatase activity due to enhanced ionic dissociation and sulfur synergy.
- Oxide-based magnesium may favor phytase expression, likely due to prolonged availability in the rhizosphere.
- **Fungal Phosphorus-Mobilizing Enzyme Activity**
 - **Acid Phosphatase (APase) (maximum at 28 days)**
 - *Aspergillus niger* and *Trichoderma harzianum* exhibited a notable increase in acid phosphatase activity with higher doses of MgSO_4 (T_3 and T_4).
 - *Penicillium chrysogenum* responded best to T_4 (20 ppm MgSO_4), reaching the highest APase level at 37.08 $\mu\text{g pNP/mL/h}$.

Table 3: Fungal Acid Phosphatase activity ($\text{EU} \times 10^{-2}$) after 28 days of incubation

Fungus	T0	T1	T2	T3	T4
<i>Aspergillus niger</i>	26.19	28.23	29.45	36.31	37.63
<i>Penicillium chrysogenum</i>	21.53	26.58	26.05	32.52	37.08
<i>Trichoderma harzianum</i>	18.23	20.16	23.89	23.74	29.72

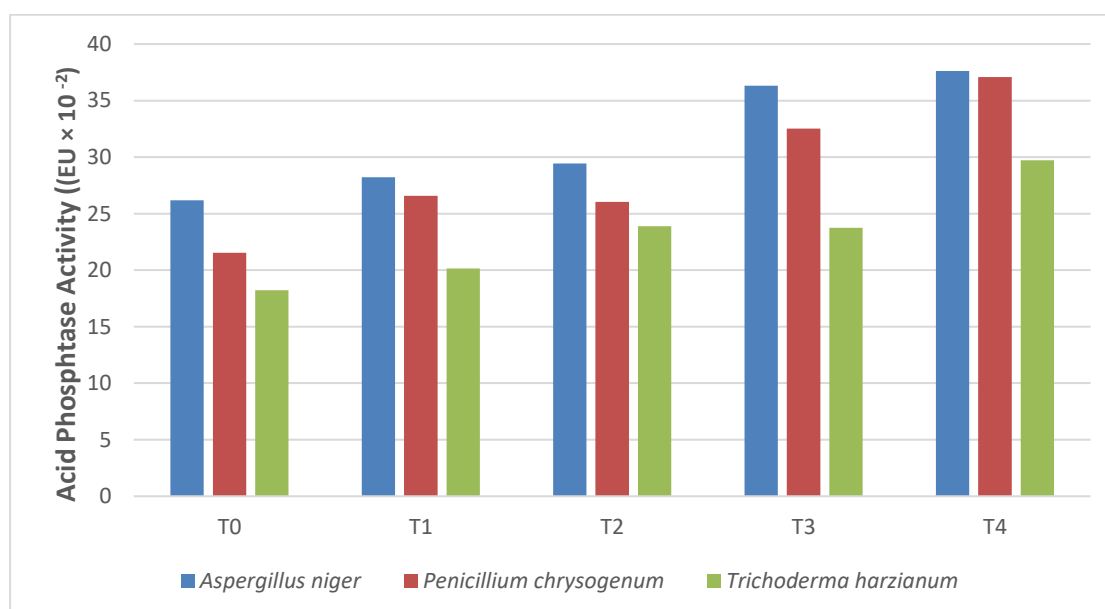


Figure 3: Fungal Acid Phosphatase activity after Mg nanoparticles application at 21 DAI

- **Phytase (maximum at 21 days)**
 - *Trichoderma harzianum* peaked at T_3 (10 ppm MgSO_4) with 4.83 $\mu\text{mol Pi/mL/h}$, followed by a plateau at T_4 .
 - *Penicillium chrysogenum* showed a continuous upward trend across treatments, with a maximum phytase level at T_4 .
 - *Aspergillus niger* showed relatively stable but modest phytase activity.

Table 4: Fungal Phytase Activity ($\text{EU} \times 10^{-1}$)

Fungus	T0	T1	T2	T3	T4
<i>Aspergillus niger</i>	1.81	1.74	0.72	2.06	1.52
<i>Penicillium chrysogenum</i>	0.38	1.97	2.54	1	4.77
<i>Trichoderma harzianum</i>	0.15	1.33	3.6	4.83	3.66

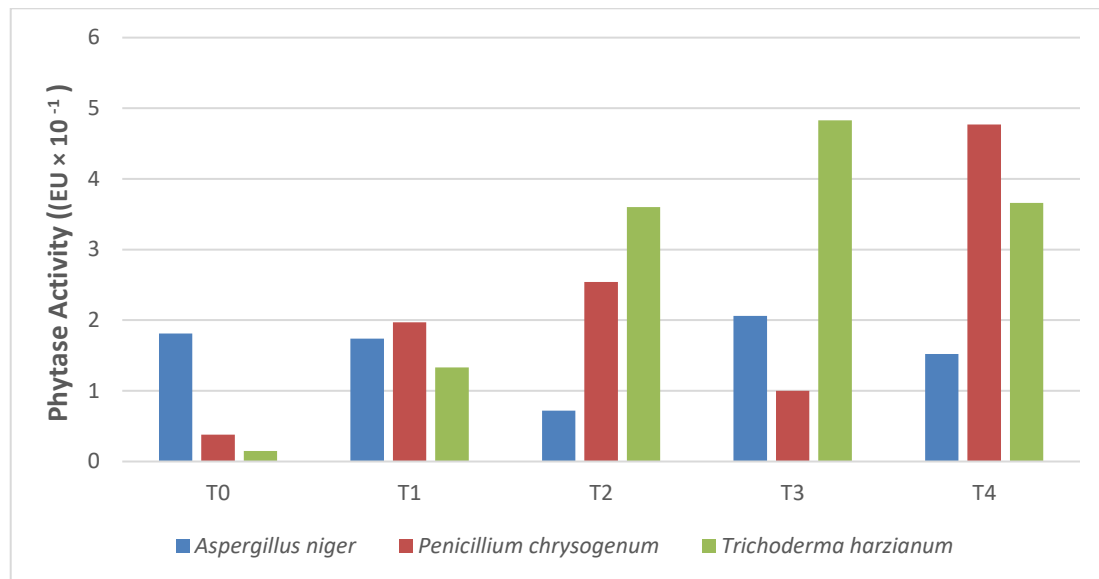


Figure 4: Fungal Phytase activity after Mg nanoparticles application at 21 DAI

- **Plant-Specific Trends**

Legumes (cluster bean, mung bean, moth bean) showed stronger enzyme responses overall, consistent with their high root exudation and microbial interactions.

Cereal crops (pearl millet, sorghum) demonstrated moderate improvements, suggesting a differential mechanism of Mg interaction.

- **Fungal-Specific Trends**

- MgSO_4 outperformed MgO across all fungal species for both enzymes.
- *Trichoderma harzianum* was the most responsive species, suggesting it may be the most effective candidate for combined Mg-enhanced biofertilizer formulations.
- *Penicillium chrysogenum* showed a dose-dependent response, likely due to enhanced solubilization of magnesium and better fungal compatibility with sulfate anions.

Discussion

Overview

This study examined the impact of magnesium supplied in oxide (MgO) and sulfate (MgSO_4) forms on phosphorus-mobilizing enzyme activity—acid phosphatase and phytase—in five plant species and three fungal strains. The consistent enhancement in enzymatic activity across treatments highlights the beneficial role of magnesium in improving phosphorus availability through biological means.

Plant Enzymatic Responses to Magnesium

Magnesium application resulted in a substantial increase in acid phosphatase and phytase activities across all five plant species. These enzymes are vital for the mobilization of organic and inorganic phosphorus from soil pools, particularly under phosphorus-limited conditions.

- **MgSO_4 as a Superior Source**

Among the treatments, 20 ppm MgSO_4 (T_4) was the most effective in stimulating both enzymes, especially in legumes such as cluster bean and mung bean.

The enhanced efficacy of MgSO_4 can be attributed to its greater solubility and faster ion release, facilitating quicker uptake and metabolic response.

- **Legume vs. Cereal Response**

Leguminous crops (cluster bean, mung bean, moth bean) showed stronger responses than cereals, likely due to their higher root exudation potential and symbiotic microbial interactions.

Sorghum and pearl millet, while showing improvement, exhibited more moderate increases, suggesting crop-specific enzyme regulation mechanisms.

Fungal Response to Magnesium Treatment

Fungi play a central role in soil phosphorus dynamics through enzymatic solubilization of phosphorus compounds. The response to magnesium treatments was pronounced across all three fungal species tested.

- ***Trichoderma harzianum*: The Most Responsive**

T. harzianum demonstrated the highest acid phosphatase and phytase activity, peaking under 10 ppm MgSO_4 (T_3).

This suggests high sensitivity to magnesium availability and potential for use as a biofertilizer component.

- **Dose-Dependent Trends in *Penicillium***

Penicillium chrysogenum showed a clear dose-dependent increase in both enzyme activities, with the highest levels recorded under T_4 .

This indicates potential for long-term enzyme stimulation when paired with magnesium-rich amendments.

- **Limited but Stable Response in *Aspergillus***

Aspergillus niger displayed moderate and steady enzyme activity, with marginal increases under MgSO_4 treatments.

The response suggests a more constitutive enzyme secretion pattern, less influenced by magnesium variability.

Comparative Effectiveness of MgO vs. MgSO_4

The study confirmed that MgSO_4 was generally more effective than MgO , especially in stimulating acid phosphatase activity in both plants and fungi. Possible explanations include:

- Higher solubility of MgSO_4 , enabling faster magnesium release.
- Enhanced microbial compatibility, as sulfate ions may support sulfur-linked coenzyme functions.
- However, MgO also showed beneficial effects, particularly on phytase activity in cereals and in stabilizing longer-term magnesium availability in soil systems.

Implications for Soil Fertility and Sustainable Agriculture

This study demonstrates that magnesium supplementation, especially as MgSO_4 , is a practical strategy to stimulate phosphorus-mobilizing enzymes.

Enhancing such enzymatic activity can unlock fixed phosphorus, reduce dependence on synthetic phosphate fertilizers, and promote plant-fungal synergy for nutrient cycling.

Integrated use of magnesium-amended fungal bioinoculants and responsive crops can be optimized for low-input or organic agriculture.

Limitations and Future Work

The findings are based on controlled conditions; field validation is required to assess seasonal and environmental variability.

Further research is recommended to evaluate- Long-term soil enzyme dynamics, Crop yield impacts and Interaction with native microbial communities.

Conclusion

Phosphorus deficiency is a major constraint in agricultural productivity, particularly in tropical and subtropical soils where much of the available phosphorus becomes fixed in forms inaccessible to plants. This study demonstrates that magnesium supplementation—especially in the form of magnesium sulfate (MgSO_4)—can serve as an effective strategy for enhancing phosphorus availability through biological means. Both plant and fungal systems showed significant improvements in the activity of key phosphorus-mobilizing enzymes, namely acid phosphatase and phytase, in response to magnesium treatments.

Among the five tested crop species, legumes such as cluster bean, mung bean, and moth bean were particularly responsive, displaying substantial increases in enzyme activity and growth performance.

These crops, by virtue of their root exudation patterns and microbial associations, benefited most from MgSO_4 treatments, particularly at the 20 ppm concentration. Cereal crops like pearl millet and sorghum also responded positively, albeit to a lesser degree, indicating species-specific enzyme activation thresholds.

Fungal strains, notably *Trichoderma harzianum* and *Penicillium chrysogenum*, exhibited enhanced secretion of phosphorus-mobilizing enzymes under MgSO_4 treatments. The dose-responsive trends and superior activity levels recorded in these fungi suggest their potential for development into magnesium-assisted bioinoculants. In contrast, *Aspergillus niger* showed moderate responses, indicating strain-specific enzymatic regulation.

Importantly, MgSO_4 consistently outperformed MgO , likely due to its higher solubility and better compatibility with biological systems. These findings underscore the importance of not only the nutrient element but also its chemical form in influencing bioavailability and microbial interaction.

In conclusion, magnesium—particularly as sulfate—acts as a biostimulant for enzyme-based phosphorus mobilization in both plant and fungal systems. Integrating magnesium management into soil fertility strategies can significantly reduce reliance on chemical fertilizers, enhance nutrient cycling, and support sustainable agricultural practices. Future studies should expand these findings under field conditions, explore molecular mechanisms of enzyme induction, and evaluate long-term ecological impacts to refine magnesium-enriched biofertilizer technologies.

Acknowledgments

The authors express their sincere gratitude to the Department of Chemistry, JNVU Jodhpur and the research staff involved in hydroponic and biochemical analyses. The support from the laboratory technicians during the enzyme activity assays and statistical evaluations is greatly appreciated.

Conflict of Interest

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

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