Phosphate Solubilization by Earthworm-Associated Fungi Enhancing Soil Phosphorus Availability

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Abstract: Phosphate solubilization by fungi associated with earthworms was investigated to explore their potential role in enhancing phosphorus availability in soil. The study revealed that many of these fungi efficiently solubilized dicalcium phosphate and tricalcium phosphate, though their solubilization efficiency varied across species. The degree of phosphate solubilization was influenced by environmental factors such as pH and the presence of microbial nutrients, including carbon and nitrogen sources in the medium.

Phosphorus, a critical plant nutrient, is abundant in soils but often remains unavailable to plants due to its insoluble forms and soil conditions. Microorganisms, including fungi, bacteria, and actinomycetes, are known to play a vital role in phosphorus solubilization. The organic matter-to-phosphorus ratio particularly influences the biological transformation of phosphorus.

Earthworms, known for enhancing soil fertility, may contribute to phosphorus transformation through associated microorganisms. However, limited research exists on phosphate solubilization by earthworm-associated fungi. This study highlights the potential of earthworm-borne fungi in phosphate solubilization, offering insights into their role in improving soil phosphorus availability.

Keywords: Phosphate solubilization, Earthworm-associated fungi, Soil phosphorus availability, Sustainable agriculture, Microbial nutrient transformation, Dicalcium phosphate, Tricalcium phosphate, Soil fertility, Fungal efficiency, Organic matter-to-phosphorus ratio.

1. INTRODUCTION TO THE STUDY

Microorganisms and Their Role in Phosphorus Solubilization: Phosphorus is a vital nutrient for plant growth, but its availability is often limited due to its insolubility in soil. Microorganisms, including fungi, bacteria, and actinomycetes, play a crucial role in enhancing phosphorus bioavailability in plants by solubilizing and mineralizing it.

Phosphate Solubilization: Microorganisms convert insoluble inorganic phosphorus into soluble forms that plants can readily absorb. Among these, fungi such as *Aspergillus niger* and *Penicillium* species are particularly effective at solubilizing phosphorus compared to bacteria. They achieve this by producing organic acids like citric acid and gluconic acid, which chelate cations and release phosphorus into the soil.

Phosphate Mineralization: Microorganisms also break down insoluble organic phosphorus compounds, a process known as mineralization. Mesophilic and thermophilic bacteria are especially active in this pathway, converting organic phosphorus into inorganic forms through enzymatic activities.

Increasing Bioavailability: By solubilizing and mineralizing phosphorus, microorganisms significantly enhance the pool of bioavailable phosphorus in the soil. This increased availability supports better nutrient uptake by plants.

Promoting Plant Growth: Phosphate-solubilizing microorganisms (PSMs) contribute to plant growth beyond phosphorus availability. They enhance root development, strengthen root systems, and promote ramification. These improvements help plants resist diseases and mature more rapidly, leading to better crop yields.

Reducing Chemical Fertilizer Use: Inoculating soil with PSMs can minimize the dependency on chemical fertilizers, which are expensive and can have negative environmental impacts. The natural process of phosphorus solubilization by microorganisms supports sustainable agricultural practices.

Phosphate Solubilizing Microorganisms (PSMs), abundant in soil and the rhizosphere, are integral to sustainable agriculture. Their ability to enhance phosphorus availability while reducing chemical inputs makes them a valuable asset for improving soil fertility and environmental health.

Phosphorus is a vital macronutrient essential for plant growth and development. It plays a critical role in

various physiological processes, including energy transfer, photosynthesis, and the synthesis of nucleic acids and phospholipids. Despite its abundance in soils, phosphorus is often unavailable to plants due to its tendency to form insoluble compounds with calcium, iron, or aluminum under different soil pH conditions (Holford, 1997). This limited availability of phosphorus is a significant constraint in agricultural productivity, necessitating external application of chemical fertilizers to meet crop demands.

Microorganisms in the soil, including fungi, bacteria, and actinomycetes, contribute substantially to the solubilization of insoluble phosphate compounds, making phosphorus accessible to plants. Fungi such as *Aspergillus*, *Penicillium*, and other genera have been reported to solubilize phosphorus through the production of organic acids and enzymes (Garg & Neelima, 1989; Kapoor *et al.*, 1989). Similarly, bacterial species, including *Pseudomonas* and *Bacillus*, and actinomycetes such as *Streptomyces* have been studied for their phosphate-solubilizing capabilities (Rana *et al.*, 1984; Banik & Dey, 1983). The efficiency of biological phosphorus transformation is influenced by several factors, including the organic matter-to-phosphorus ratio in the soil (Mishra, 1985).

Earthworms are well-known soil engineers who improve soil fertility through their activities, such as burrowing, organic matter decomposition, and nutrient cycling. It is hypothesized that microorganisms associated with earthworms, particularly fungi, may play a direct or indirect role in phosphorus transformation in soil. However, little information is available regarding the phosphate-solubilizing ability of earthworm-associated fungi. Krishna Reddy and Reddy (1987a) emphasized the need to investigate such microorganisms, as they could offer an eco-friendly and sustainable approach to enhancing phosphorus availability in soils. This study aims to address this gap by evaluating the ability of earthworm-associated fungi to solubilize phosphate compounds and examining the environmental factors influencing their efficiency.

2. OBJECTIVE

The purpose of this study is to investigate the ability of fungi associated with earthworms to solubilize insoluble phosphate compounds and enhance phosphorus availability in soil. By examining the solubilization efficiency of earthworm-associated fungi under varying environmental conditions, this research aims to understand their potential role in sustainable soil fertility management and phosphorus transformation processes.

3. METHODOLOGY OVERVIEW

In this study, the fungi associated with earthworms were assessed for their ability to solubilize two types of phosphate compounds: dicalcium phosphate (DCP) and tricalcium phosphate (TCP). The fungi were cultured in a defined medium with either DCP or TCP as the sole phosphorus source, and solubilization efficiency was monitored by measuring the soluble phosphorus concentration in the medium at multiple time points (4, 9, and 12 days).

The study also focused on understanding the influence of key environmental and nutritional factors, such as pH and microbial nutrients, on solubilization efficiency:

1. pH: Changes in pH were monitored to determine its effect on the phosphate solubilization process, as pH is known to significantly influence the activity of phosphate-solubilizing microorganisms.
2. Microbial Nutrients: Carbon (e.g., glucose) and nitrogen (e.g., ammonium nitrate) sources were added to the culture medium to investigate their role in enhancing or inhibiting phosphate solubilization.

Methodology

1. Collection of Fungi

Fungi associated with earthworms were isolated from soil samples collected from earthworm habitats using standard microbiological isolation techniques.
The isolated fungal strains were identified and stored for further use in the experiment.

2. Culturing of Fungi

The fungi were cultured on a defined medium with either dicalcium phosphate or tricalcium phosphate as the sole phosphorus source.
The cultures were incubated at 28°C ± 2°C for 12 days to ensure optimal growth and solubilization.

3. Phosphate Solubilization Assay

Phosphate solubilization was assessed by measuring the soluble phosphorus concentration in the medium at 4, 9, and 12 days of incubation.

A spectrophotometric method, based on the molybdenum blue method, was employed to quantify phosphorus release.

4. pH Measurement

The pH of the medium was measured periodically throughout the incubation period to assess any changes in pH that might correlate with the solubilization of phosphorus.

5. Controls

Uninoculated media, served as the control group, to account for abiotic phosphorus release and ensure the observed phosphorus solubilization was microbial-driven. Through these methods, the study aimed to evaluate the solubilization efficiency of different fungi and explore the effect of environmental and nutritional factors on the phosphorus release process.

4. RESULTS

The efficiency of phosphate solubilization varies with fungal species and phosphate type.

Verticillium chlamydospores was highly effective with dicalcium phosphate but delayed its activity until the 9th day. *Neurospora crassa* and *Cucurbitaria cucurbitarum* excelled in solubilizing tricalcium phosphate. Changes in pH did not consistently correlate with solubilization, contrary to findings by Taha *et al.* (1969) and Bardiya and Gaur (1972). Peaks in solubilization were attributed to differential rates of phosphorus solubilization and fungal assimilation. (Table 1).

Table 1 Phosphate Solubilization Efficiency of Earthworm-Associated Fungi

Fungal Species	Phosphate Source	Phosphorus Release Efficiency	Solubilization Observations
Verticillium chalmydosporum	Dicalcium phosphate	Maximum	No solubilization until 9th day; highest release on the 12th day.
Hansfordea sp.	Dicalcium phosphate	Moderate	No liberation of phosphorus until the 4th day.
Gonatobotryum roseum	Dicalcium phosphate	Efficient	Continuous solubilization observed.
Gonytrichum state of M. inaequalis	Dicalcium phosphate	Efficient	Consistent solubilization activity.
Zygorrhynchus sachidanandii	Dicalcium phosphate	Poor	Minimal solubilization observed throughout.
Neurospora crassa	Tricalcium phosphate	Highly efficient	Significant liberation of phosphorus.
Cucurbitaria cucurbitarum	Tricalcium phosphate	Highly efficient	High phosphorus release with peaks during incubation.
Verticillium albo-atrum	Tricalcium phosphate	Moderate	Steady solubilization with detectable peaks.
Acremonium sp.	Tricalcium phosphate	Moderate	Phosphorus solubilization showed two peaks due to differences in solubilization and assimilation rates.
Aspergillus phaeospermum	Both	Moderate	Significant solubilization but dependent on pH and medium composition.

Influence of pH on Phosphate Solubilization

Table 2 highlights the significant influence of pH on the ability of earthworm-associated fungi to solubilize both dicalcium phosphate (DCP) and tricalcium phosphate (TCP). The results demonstrate that the solubilization of phosphate compounds is highly pH-dependent, with different fungi exhibiting optimal solubilization activity at varying pH levels.

Dicalcium Phosphate Solubilization

For DCP, the degree of solubilization increased as the acidity of the medium rose, with the maximum solubilization occurring at pH 5.5. This finding aligns with previous studies suggesting that certain fungi prefer slightly acidic conditions for optimal solubilization of phosphorus (Bardiya & Gaur, 1972). The solubilization activity declined both in highly acidic (lower pH) and alkaline (higher pH) conditions.

Interestingly, *C. cucurbitarum* exhibited a substantial increase in phosphate solubilization at pH 9.5,

indicating its unique ability to adapt to more alkaline conditions compared to other fungi in the study. Similar results have been reported by Taha *et al.* (1969), who observed an inverse relationship between pH and phosphate solubilization activity in some microorganisms.

For *G. murorum* and *N. crassa*, the maximum phosphate solubilization was observed at pH 6.5, a neutral to slightly acidic range, with a decrease in solubilization activity at higher pH levels. This is consistent with findings by Krishna Reddy and Reddy (1988), who reported that species of Aspergillus, including *Aspergillus niger*, showed optimal phosphate solubilization at slightly acidic pH values.

V. chlamydosporum exhibited the highest solubilization at pH 7.5, a neutral pH, and solubilization declined as the pH increased towards alkalinity. This result suggests that some fungi may be adapted to neutral conditions, where phosphate solubilization processes are maximized.

Z. sachidanandii failed to solubilize phosphate at pH 4.5, but showed the highest efficiency at pH 6.5, suggesting that this fungus requires a more neutral environment to perform optimal solubilization. This result is supported by earlier studies, such as Garg *et al.* (1992), which indicated that certain phosphate-solubilizing fungi have pH-specific activity ranges, highlighting the importance of environmental factors in microbial phosphorus cycling.

Tricalcium Phosphate Solubilization

For TCP, the maximum solubilization was achieved at pH 7.5, similar to the results observed for DCP. This aligns with findings by Banik and Dey (1983), who showed that neutral pH environments are often optimal for phosphorus solubilization. The fungi *N. crassa, V. chlamydosporum,* and *Z. sachidanandii* displayed similar efficiency in solubilizing TCP, demonstrating their high adaptability to neutral pH environments. These fungi are more efficient in solubilizing TCP compared to others in the study, confirming their potential as effective phosphate solubilizers.

At pH 4.5, *N. crassa* showed the highest phosphate solubilization, followed by *Z. sachidanandii, G. murorum, C. cucurbitarum,* and *V. chlamydosporum*, in descending order. This observation indicates that *N. crassa* is particularly efficient in acidic environments, similar to findings from studies on other fungi that are well-adapted to slightly acidic conditions (Krishna Reddy & Reddy, 1988).

pH Drift Towards Neutral

The pH of the medium in most cases tended to drift towards a more alkaline state as the fungi grew, which is a common phenomenon in phosphate solubilization studies. As microorganisms metabolize organic compounds and release acidic by-products, such as organic acids (e.g., citric acid and gluconic acid), the pH of the medium may initially decrease before stabilizing at a near-neutral value (Krishna Reddy & Reddy, 1987a). The final pH in all media, regardless of the initial conditions, remained near neutral, which aligns with previous studies indicating that pH stabilization often occurs during microbial growth (Bardiya & Gaur, 1972).

Overall, the results demonstrate that pH plays a crucial role in the phosphate solubilization process by fungi, with different fungi showing optimal activity at distinct pH ranges. These findings underscore the importance of understanding pH preferences in the selection of phosphate-solubilizing microorganisms for agricultural and environmental applications. The diverse pH optima observed in this study also highlight the need for more specific research to tailor the use of these fungi in different soil types and conditions.

Microbial nutrients play a significant role in influencing the solubilization of phosphorus by fungi. The rate of phosphate solubilization is affected by the type and concentration of microbial nutrients available in the medium. The results revealed in Table 3 show that certain microbial nutrients like malt extract, beef extract, yeast extract, and peptone had varying effects on the phosphate solubilization abilities of the fungi.

- Malt Extract and Beef Extract: These substances hurt phosphate solubilization, especially at higher concentrations. However, fungi such as *V. chlamydosporum*, *N. crassa*, and *Z. sachidanandii* were observed to be stimulated for solubilizing dicalcium phosphate (DCP) in the presence of malt extract and beef extract, suggesting that some fungi may thrive better under specific nutrient conditions.
- Yeast Extract: Yeast extract was found to stimulate the solubilization of dicalcium phosphate by all fungi under investigation, with solubilization

increasing as the concentration of yeast extract increased. This indicates that yeast extract may provide the necessary nutrients that promote the solubilization activity of the fungi.

Table 2 Solubilization Efficiency of Fungal Species on Dicalcium Phosphate (DCP) and Tricalcium Phosphate (TCP) at Various pH Levels

Fungai Species	Dicalcium Phosphate (DCP) Solubilization	pH for Maximum Solubilization (DCP)	Tricalcium Phosphate (TCP) Solubilization	pH for Maximum Solubilization (TCP)
C. cucurbitarum	High (at pH 9.5)	pH 9.5	Moderate	pH 7.5
G. murorum	Moderate	pH 6.5	Moderate	pH 7.5
N. crassa	High (at pH 6.5)	pH 6.5	High (at pH 4.5)	pH 4.5
V. chlamydosporum	High (at pH 7.5)	pH 7.5	Moderate	pH 7.5
Z. sachidanandii	Low (no solubilization at pH 4.5)	pH 6.5	Low (no solubilization at pH 4.5)	pH 6.5
Acremonium sp.	Moderate	pH 7.5	Moderate	pH 7.5
G. roseum	Moderate	pH 7.5	Moderate	pH 7.5
Hansfordea sp.	Low to Moderate	pH 6.5	Low	pH 7.5

- Peptone: Similar to yeast extract, peptone was effective in promoting the solubilization of tricalcium phosphate (TCP), with solubilization increasing as the concentration of peptone was raised. However, *Z. sachidanandii* showed only a marginal increase in solubilization when the peptone concentration was increased. *V. chlamydosporum* solubilized the maximum amount of phosphate at 0.1% peptone concentration, which may reflect the optimal nutrient concentration for this species.

These results underscore the importance of nutrient availability in enhancing the activity of phosphate-solubilizing fungi, which can be crucial for improving phosphorus availability in agricultural soils.

Table-3 Influence of Microbial Nutrients on Phosphate Solubilization by Fungi

Microbial Nutrients	Effect on Dicalcium Phosphate (DCP) Solubilization	Effect on Tricalcium Phosphate (TCP) Solubilization
Malt Extract	Adverse effect on DCP solubilization at higher concentrations. Stimulation of *V. chlamydosporum*, *N. crassa*, and *Z. sachidanandii*.	No significant effect observed.
Beef Extract	Adverse effect on DCP solubilization at higher concentrations. Stimulation of *V. chlamydosporum*, *N. crassa*, and *Z. sachidanandii*.	No significant effect observed.
Yeast Extract	Stimulation of DCP solubilization for all fungi, increased solubilization with concentration.	No significant effect observed.
Peptone	No significant effect on DCP solubilization.	Stimulation of TCP solubilization, increased solubilization with concentration, marginal effect on *Z. sachidanandii*.
0.1% Peptone	Moderate solubilization of DCP.	Maximum solubilization of TCP by *V. chlamydosporum* at 0.1% concentration.

The data in Table 4 show that the type of carbon source in the medium significantly influences phosphate solubilization by fungi. The degree of stimulation varies depending on both the fungus and the type of phosphate substrate (dicalcium phosphate or tricalcium phosphate).

- Sucrose: Sucrose was found to be the most effective carbon source for solubilizing dicalcium phosphate (DCP), particularly for *C. cucurbitarum*. However, succinic acid failed to support solubilization of either DCP or tricalcium phosphate (TCP) by *C. cucurbitarum*

and *V. chlamydosporum*, indicating that succinic acid is not an ideal substrate for these fungi. On the other hand, dextrin and mannitol were poor substrates for DCP solubilization by many fungi.

- Lactose and Glucose: *G. murorum* preferred lactose for maximum phosphate solubilization. Glucose was found to be the best carbon source for *N. crassa*, where it promoted maximum DCP solubilization, followed by lactose. *V. chlamydosporum* solubilized DCP most effectively in a sucrose-containing medium, while glucose was the preferred carbon source for *Z. sachidanandii*, leading to maximum phosphate solubilization.
- Succinic Acid and Dextrin: These carbon sources were less effective in stimulating phosphate solubilization in most fungi, with dextrin especially failing to induce phosphate solubilization by several species like *V. chlamydosporum*.

Interestingly, almost all fungi solubilized phosphorus to a considerable extent even in the absence of an added carbon source. This might be attributed to the yeast extract component in the medium, which contains naturally available carbon sources.

The same trends were observed with tricalcium phosphate (TCP) solubilization. Glucose was the best carbon source for inducing phosphate solubilization in *C. cucurbitarum*, *G. murorum*, and *N. crassa*, while galactose and sucrose were preferred by *V. chlamydosporum*. However, dextrin did not effectively promote TCP solubilization, and only marginal increases were observed for *V. chlamydosporum*.

It is also noteworthy that TCP was more readily solubilized than DCP in many cases, except when sucrose was the carbon source for fungi like *C. cucurbitarum* and *N. crassa*, where DCP solubilization was higher than TCP. Finally, while the pH generally shifted towards the alkaline side, no positive correlation between pH changes and phosphate solubilization was observed. The final pH was close to neutral, suggesting that the phosphate solubilization process might not be directly influenced by pH in this study.

The data from Table-5 indicate that the nitrogen source in the medium significantly influences the rate of phosphate solubilization by the fungi. The type of nitrogen source not only affected the phosphate solubilization ability of the fungi but also influenced the final pH of the medium.

1. *C. cucurbitarum:*

This fungus solubilized maximum phosphate in the presence of ammonium nitrate and sodium nitrite, while L-glycine had the least effect.

Interestingly, the solubilization of dicalcium phosphate (DCP) was higher in the absence of nitrogen source than in the presence of certain nitrogen sources like ammonium sulfate, L-glycine, L-tyrosine, and urea.

2. *G. murorum:*

L-asparagine was the most effective nitrogen source for phosphate solubilization, followed by sodium nitrite and sodium nitrate. On the other hand, ammonium sulfate, glycine, and urea were poor substrates for DCP solubilization.

3. *N. crassa:*

This fungus failed to liberate phosphorus in L-glycine medium and also in the medium without nitrogen. Nitrogen sources like tyrosine, urea, and ammonium sulfate were also poor for phosphate solubilization.

4. *V. chlamydosporum:*

This fungus showed maximum phosphate solubilization with ammonium nitrate, followed by L-asparagine, sodium nitrite, and sodium nitrate. The least solubilization was observed in media containing L-glycine, urea, and ammonium sulfate.

5. Z. sachidanandii: The best nitrogen sources for phosphate solubilization were sodium nitrate, L-asparagine, and sodium nitrite. Conversely, ammonium nitrate and L-glycine led to the least phosphate solubilization. This fungus also failed to solubilize phosphorus in the urea-containing medium.

Table-4 Influence of Carbon Sources on Phosphate Solubilization by Fungi

Fungus	Best Carbon Source for Dicalcium Phosphate (DCP)	Best Carbon Source for Tricalcium Phosphate (TCP)	Poor Carbon Source	Effect of Carbon Source on Solubilization
C. cucurbitarum	Sucrose	Glucose	Succinic Acid, Dextrin	Maximum solubilization in sucrose for DCP.

G. murorum	Lactose	Glucose	Succinic Acid	Lactose preferred for DCP, glucose for TCP.
N. crassa	Glucose, Lactose	Glucose	Dextrin	Maximum DCP solubilization in glucose, TCP in glucose.
V. chlamydosporum	Sucrose	Galactose, Sucrose	Dextrin	Maximum DCP solubilization in sucrose, marginal increase in TCP with dextrin.
Z. sachidanandii	Sucrose, Glucose	Glucose	Dextrin, Succinic Acid	Maximum DCP solubilization in sucrose and glucose, no solubilization in dextrin.
General Trends	Sucrose, Glucose	Glucose	Dextrin, Succinic Acid	TCP solubilization was generally more efficient than DCP, except with sucrose.

General Trends:

- Ammonium nitrate and sodium nitrite were among the best nitrogen sources for phosphate solubilization by several fungi.
- L-glycine and urea were generally poor nitrogen sources for solubilization across most of the fungi.
- The pH of the medium generally drifted towards the alkaline side, except in L-glycine-containing media, which was mildly acidic. The final pH of the medium was near neutral in most cases, suggesting that the nitrogen sources did not dramatically alter the pH in a way that would inhibit phosphate solubilization.

Table - 5: Influence of Nitrogen Sources on Phosphate Solubilization by Fungi

Fungus	Best Nitrogen Source for Dicalcium Phosphate (DCP)	Best Nitrogen Source for Tricalcium Phosphate (TCP)	Poor Nitrogen Sources	Effect of Nitrogen Source on Solubilization
C. cucurbitarum	Ammonium nitrate, Sodium nitrite	Sodium nitrate	L-Glycine, Urea	Maximum DCP solubilization in ammonium nitrate, least in L-glycine.
G. murorum	L-Asparagine	Ammonium nitrate	Urea, L-Glycine	Maximum solubilization in L-asparagine, least in urea and L-glycine.
N. crassa	Sodium nitrite	Sodium nitrite	L-Glycine, Urea	Maximum solubilization in sodium nitrite, least in L-glycine and urea.
V. chlamydosporum	Ammonium nitrate	L-Asparagine	L-Glycine, Urea	Maximum solubilization in ammonium nitrate, least in L-glycine.
Z. sachidanandii	Sodium nitrate, L-Asparagine, Sodium nitrite	Sodium nitrate, L-Asparagine	Ammonium nitrate, Urea	Maximum solubilization in sodium nitrate and L-asparagine, least in urea.
General Trends	Ammonium nitrate, Sodium nitrite	Sodium nitrate	L-Glycine, Urea	Nitrogen sources like ammonium nitrate and sodium nitrite promote solubilization, while L-glycine and urea are poor substrates.

5. DISCUSSION

The results presented in the tables highlight significant findings regarding the influence of various factors such as microbial nutrients, carbon sources, and nitrogen sources on the phosphate solubilization capacity of different fungi. The ability of these fungi to solubilize phosphorus from dicalcium and tricalcium phosphate has important implications for their potential use in soil fertility enhancement and sustainable agricultural practices.

Microbial Nutrients (Table-3)

The results from Table-3 demonstrate that different microbial nutrients significantly affect the solubilization of phosphorus, with the nature and concentration of the nutrient playing a crucial role. Among the nutrients tested, yeast extract was the most effective in stimulating phosphate solubilization by all the fungi studied, suggesting that it may provide essential growth factors that enhance the activity of phosphate-solubilizing microorganisms. On the contrary, malt extract and beef extract hurt solubilization, particularly at higher concentrations, although certain fungi like *V. chlamydosporum, N. crassa, and Z. sachidanandii* still exhibited some solubilization. This indicates that some fungi might possess adaptive mechanisms that allow them to utilize or tolerate these nutrient sources.

In contrast to traditional inorganic nitrogen sources, yeast extract seems to provide an ideal balance of organic nutrients that promote phosphorus solubilization. The ability of these fungi to solubilize phosphorus even in the absence of organic nutrients further highlights their adaptability, potentially suggesting a role for these fungi in environments where organic nutrients are limited.

Carbon Sources (Table-4)

The influence of different carbon sources on phosphate solubilization was also significant, as shown in Table-4. The solubilization rate varied with both the fungal species and the type of phosphate substrate. Sucrose was the most effective carbon source for promoting dicalcium phosphate solubilization by *C. cucurbitarum*, which is consistent with previous studies showing that sucrose is a preferred carbon source for many phosphate-solubilizing fungi (Khan *et al.*, 2007). Glucose was another excellent source for *N. crassa* and *Z. sachidanandii*, both of which showed high solubilization rates in its presence. This aligns with the general knowledge that simple sugars like glucose and sucrose are easily metabolized by fungi and serve as efficient energy sources for cellular activities, including phosphate solubilization (Zaidi *et al.*, 2009).

Interestingly, succinic acid failed to support solubilization by *C. cucurbitarum* and *V. chlamydosporum,* indicating that certain organic acids may not be as effective as simple sugars in promoting the solubilization of phosphate compounds. Additionally, the observation that fungi were able to solubilize phosphorus to a certain extent even in the absence of added carbon source suggests the possibility of utilizing carbon derived from yeast extract, as previously mentioned in the nutrient discussion. The ability of fungi to solubilize phosphate without an external carbon source demonstrates their inherent potential to perform phosphorus mobilization in nutrient-limited environments.

Nitrogen Sources (Table-5)

The nitrogen source had a profound impact on phosphate solubilization, as indicated by the results in Table-5. Ammonium nitrate and sodium nitrite were the most effective nitrogen sources for promoting phosphate solubilization in a majority of the fungi, particularly for *C. cucurbitarum* and *V. chlamydosporum.* These findings are in line with previous studies that have shown the importance of ammonium-based nitrogen sources in enhancing microbial phosphate solubilization (Rodriguez *et al.*, 2004). The preference for these nitrogen sources may be due to their role in providing readily available nitrogen for fungal growth and metabolic processes involved in phosphorus mobilization.

Interestingly, L-glycine and urea were among the least effective nitrogen sources for phosphate solubilization in most fungi. This may be due to the complex nature of these nitrogen compounds, which may require additional enzymatic processes for breakdown, thereby limiting their effectiveness in promoting phosphate solubilization under the tested conditions.

The pH trend observed across the different nitrogen sources indicated a general drift toward neutrality or mild alkalinity, with the medium becoming mildly acidic in the presence of L-glycine. This suggests that phosphate solubilization by these fungi may be linked to the production of organic acids such as citric acid, which lower the pH of the medium, enhancing the dissolution of phosphate compounds (Khan *et al.*, 2014).

Comparative Analysis and Significance

The comparison of the solubilization potential of dicalcium phosphate and tricalcium phosphate revealed that tricalcium phosphate was more readily solubilized by the fungi in most cases. This is consistent with the known higher solubility of

tricalcium phosphate in microbial systems (Rathore *et al.*, 2016). However, there were exceptions, such as *C. cucurbitarum,* which solubilized more dicalcium phosphate than tricalcium phosphate under sucrose-induced conditions. This suggests that the ability of fungi to solubilize different phosphate forms may depend on both the specific fungal species and the presence of certain carbon or nitrogen sources that enhance solubilization activity.

Moreover, it is important to note the positive effect of carbon and nitrogen sources on the overall solubilization process. The fungi investigated in this study showed considerable phosphate solubilization even under nutrient-limited conditions, highlighting their potential applications in low-input agricultural systems where organic and inorganic fertilizers are scarce or expensive.

6. CONCLUSION

The study emphasizes the complex interplay of microbial nutrients, carbon, and nitrogen sources in regulating the phosphate solubilization capacity of fungi. Yeast extract, sucrose, and ammonium nitrate were found to be the most effective in stimulating phosphate solubilization, though the specific preferences varied among fungal species. The findings contribute to a deeper understanding of microbial phosphate solubilization mechanisms and provide insights into optimizing nutrient conditions for sustainable agricultural practices, particularly in soil phosphorus management. Further studies focusing on field trials and the use of these fungi in soil will be essential to confirm their practical applications for enhancing soil fertility and crop production.

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