# Isolation and Molecular Characterization of Phosphate Solubilizing Fungi from Jhum Fields of Zunheboto District, Nagaland

**THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**


***Submitted By***

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**August, 2025**

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**Lumami 798 627, Nagaland, India**

**Department of Botany**

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This Ph. D. is being submitted to the Nagaland University for the degree of Doctor of Philosophy in Botany.

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**Supervisor**

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**(Ms. Imlimenla Jamir)**
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(**Imlimenla Jamir**)

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# Abbreviation

| | |
|---|---|
| **PSM** | **Phosphate solubilising microorganisms** |
| **PSF** | **Phosphate solubilizing fungi** |
| **P** | **Phosphate** |
| **-** | **Negative** |
| **+** | **Positive** |
| **%** | **percentage** |
| **µg** | **microgram** |
| **µl** | **microlitre** |
| ***p*H** | **Potential of Hydrogen** |
| **Min** | **Minute** |
| **mg** | **Milligram** |
| **ml** | **Millilitre** |
| **DNA** | **Deoxyribonucleic Acid** |
| **Nacl** | **Sodium Chloride** |
| **PCR** | **Polymerase Chain Reaction** |
| **$H_20$** | **Distilled water** |
| **$H_2S0_4$** | **Sulphuric acid** |
| **RBA** | **Rose bengal Agar** |
| **PDA** | **Potato Dextrose Agar** |
| **PVK agar** | **Pikovskaya's agar** |
| **NCBI** | **National Centre for Biotechnology Information** |
| **IAA** | **Indole Acidic Acid** |
| **RL** | **Root Length** |
| **SL** | **Shoot Length** |
| **RFW** | **Root Fresh Weight** |
| **RDW** | **Root Dry Weight** |
| **SFW** | **Shoot Fresh Weight** |
| **SDW** | **Shoot Dry Weight** |
| **ANOVA** | **Analysis of Variance** |

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# Abbreviation table

| | |
|---|---|
| **PGPM** | **Plant growth promoting microorganisms** |
| **PSM** | **Phosphate solubilising microorganisms** |
| **PSF** | **Phosphate solubilizing fungi** |
| **-** | **Negative** |
| + | **Positive** |
| **%** | **percentage** |
| | **microgram** |
| | **microlitre** |
| ***p*H** | **Potential of Hydrogen** |
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# CONTENTS

# Chapter-1

# Introduction and Review of literature

## INTRODUCTION

Fungi, encompassing a diverse array of eukaryotic organisms, including yeasts, molds, and mushrooms, wield considerable influence in soil ecosystems, exerting profound impacts on soil health and agricultural productivity. Renowned for their unique features such as chitin-rich cell walls and heterotrophic mode of nutrition, fungi occupy a distinct biological kingdom separate from plants, animals, and other microorganisms (Nougeira, 2003). Within the intricate web of soil life, fungi emerge as pivotal players, orchestrating essential ecological processes that underpin soil fertility, nutrient cycling, and ecosystem stability.

At the heart of fungi's ecological prowess lies their remarkable ability to decompose organic matter and recycle nutrients within the soil matrix. By secreting an arsenal of enzymes, fungi effectively break down complex organic compounds into simpler forms, including chitin and lignin, liberating vital nutrients such as nitrogen, phosphorus, and potassium (Himalini & Razia, 2019). This decomposition and nutrient mineralization process is the cornerstone of soil fertility, supplying plants with the essential elements necessary for growth and development. Moreover, fungi's adaptive capacity enables them to thrive across diverse environmental conditions, ensuring continuous nutrient cycling and soil enrichment over time (Yuvaraj & Ramasamy, 2020). Beyond their role as decomposers, fungi actively contribute in stabilizing soil organic matter, a key determinant of soil health and agricultural productivity. Through their extensive network of hyphae, fungi physically bind organic residues, preventing their rapid decomposition and loss from the soil system (Frac *et al*., 2023). This process enhances soil structure, promotes water retention, and mitigates erosion, fostering a conducive environment for plant growth and microbial activity. Furthermore, fungi play a crucial role in climate change mitigation by sequestering carbon as stable organic compounds in soil, particularly through higher ratios of fungal to bacterial activity, which is linked to reduced carbon dioxide ($CO_2$) emissions and enhancing soil resilience to environmental stressors (Civil, 2024).

Central to fungi's ecological significance is their ability to promote and maintain soil biodiversity, thereby promoting ecosystem resilience and function. As integral members of the soil microbiome, fungi engage in complex interactions with bacteria, archaea, and other microorganisms, creating a dynamic and diverse soil ecosystem (Frac *et al*., 2023). These interactions facilitate essential ecological functions such as organic matter decomposition, nitrogen fixation, and disease suppression, ultimately sustaining soil health and productivity (Himalini & Razia, 2019).

Moreover, fungi forge symbiotic relationships with plants, particularly through mycorrhizal associations, which confer numerous benefits to both partners. Mycorrhizal fungi, for instance, colonize and establish symbiotic associations with the root of most land plants, enhancing nutrient uptake, particularly phosphorus, and conferring resistance to environmental stresses (Nougeira, 2003). This mutualistic partnership improves plant growth and productivity and reduces dependence on chemical fertilizers, supporting more sustainable agricultural practices (Vassilev & Mendes, 2003). Thus, fungi emerge as indispensable actors in soil health and agricultural sustainability, wielding considerable influence over nutrient turnover, decompose organic matter, shape soil biodiversity and sustain plant-microbe interactions. By recognizing and applying these ecological functions of fungi opens pathways for improving soil fertility, conserving resources, and buffering agriculture against climate change pressures (Himalini & Razia, 2019). Thus, fungi stand as silent stewards of the soil, quietly orchestrating the intricate dance of life beneath our feet.

Among the many soil elements, Phosphorus (P) plays an important role in plant growth and development due to its involvement in various key plant functions. It is essential for energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant, and the transfer of genetic characteristics from one generation to the next. Phosphorus is vital for stimulating root development, boosting plant growth, enhancing fruit quality, and seed formation (Khan *et al*., 2023). Additionally, P is integral to DNA and RNA, crucial for genetic transfer and the development of new cells, making it essential for overall plant growth and reproduction. Adequate phosphorus levels are necessary for optimal growth rates, normal plant development, and the efficient functioning of various physiological processes in plants. When P is deficient, plants show stunted growth with shorter stems and smaller leaves, dark green or purplish leaves, delayed flowering, reduced

fruit and seed production, poor root development, and leaf necrosis in severe cases. Additionally, P deficiency can lead to reduced crop yield, increased susceptibility to diseases and pests, and long-term soil health issues. Symptoms may vary depending on the plant species, but common signs include dark green or reddish-purple leaves, tip dieback, leaf curling, distortion, smaller leaves, and premature leaf drop. In conifers, foliage may turn gray-green or dull blue-green, with fewer new needles produced and premature needle death in severe cases. Addressing P deficiency through proper soil testing, *p*H adjustment, organic matter incorporation, crop rotation, and avoiding overuse of P fertilizers is crucial for maintaining plant health and productivity (Weil & Brady, 2017). Thus, P limitation is a major nutritional constraint that affects plant growth, development, and yields. Efficient Phosphate solubilizing fungi (PSF) is a promising eco-friendly strategy to improve phosphate uptake and promote plant growth in modern agriculture.

Phosphate-solubilizing fungi (PSF) constitute a group of fungi renowned for their capacity to ameliorate soil P availability by converting insoluble P compounds into soluble forms through chelation and ion exchange. This enzymatic activity is primarily facilitated by the secretion of extracellular enzymes, notably phosphatase enzymes, which catalyze the conversion of organic P to inorganic P, thereby enhancing P avaliability for plants to absorb (Arias *et al*., 2022). A pivotal aspect of PSF functionality lies in their adeptness at enhancing phosphorus availability within soil matrices. This phenomenon is underscored by the secretion of organic acids and enzymes by PSF, which effectively mobilize P from calcium, iron, and aluminum phosphates (Chittora *et al*., 2019). Consequently, the solubilization of insoluble P compounds facilitates their uptake by plants, thereby bolstering nutrient availability and fostering enhanced crop productivity (Vassileva *et al*., 2022). Moreover, PSF exhibit notable adaptability to acidic soil environments, a trait exemplified by species such as *Talaromyces aurantiacus* and *Aspergillus niger*, which demonstrate robust growth across a wide *p*H range, from 6.5 to as low as 1.5 (Zhang *et al*., 2008). This attribute is particularly advantageous in agricultural contexts characterized by P-deficient and acidic soils, such as regions dedicated to moso bamboo production in China. Further augmenting the significance of PSF is their remarkable capability to solubilize diverse P sources, including recalcitrant compounds such as calcium, iron, and aluminum phosphates, as well as complex forms like phytate (Chittora *et al*., 2019). This broad spectrum of P-solubilization renders PSF efficacious across varied soil conditions and P-bearing substrates, thereby accentuating their utility in agricultural systems.

It is also equally critical to screen the different fungal populations about their ability to solubilize phosphate. Screening PSF is crucial due to their varying abilities to solubilize different forms of insoluble phosphorus (P) compounds (Adhikari *et al*., 2019). Further research shows significant variability in the P solubilization potential among different PSF species, with ranges spanning from 46.58 ± 0.79 to 83.42 ± 3.41 µg/ml, 35.92 ± 3.54 to 57.63 ± 0.79 µg/ml, and 42.50 ± 4.06 to 83.42 ± 3.41 µg/ml, depending on the P source (Adhikari *et al*., 2019). This variability highlights the importance of evaluating the specific phosphate-solubilizing capacity of individual fungal species or isolates.

The effectiveness of fungi in solubilizing P depends on multiple factors, including the chemical properties of the P source and the organic acids and enzymes produced by the fungi (Zhang *et al*., 2018). Additionally, interactions between the P source and the fungi, along with their ability to modulate the *p*H of their environment, can influence their P-solubilizing efficiency (Adhikari *et al*., 2019). Therefore, screening PSF becomes imperative to identify fungal strains with optimal phosphate-solubilizing capabilities, especially considering their diverse abilities to solubilize different forms of insoluble P in the soil (Chittora *et al*., 2019). This targeted screening approach ensures the selection of PSF strains that can effectively enhance soil phosphorus availability and contribute to sustainable agricultural practices.

Meanwhile, with the onset of the modern population increase, there is a serious concern about the influence of land use on the microbial population (Temjen *et al*., 2022). This is significant keeping in view that shifting cultivation practices, notably those employing the slash-and-burn method, have generated attention for their consequential impacts on soil fungal populations. Evidential findings suggest that shifting cultivation introduces dynamic alterations to soil fungi, manifesting across various facets:

Diverse Fungal Community Dynamics: The implementation of shifting cultivation practices induces perturbations in soil fungal diversity, primarily attributable to land clearing and burning activities. Such disturbances engender shifts in fungal species composition, thereby reconfiguring the fungal landscape within affected soils (Miah *et al*., 2010).

Fluctuations in Fungal Abundance: Jhum cultivation exerts discernible influences on the abundance of fungi inhabiting both surface and subsurface soil strata. Factors such as

moisture content fluctuations, organic matter fluctuations, and land disturbance regimes intricately modulate fungal population densities, shaping the ecological dynamics within soil fungal communities (Miah *et al*., 2014).

Soil Health Implications: Noteworthy repercussions on soil health parameters ensue from shifting cultivation practices, particularly in the absence of adequate soil conservation interventions. Diminished soil health metrics, including compromised soil aggregation, altered microbial populations, and perturbed nutrient profiles, are hallmark consequences. These alterations, in turn, reverberate through cascading impacts on soil fungal communities, perpetuating a cycle of ecological disruption (Ovung, 2021).

Another problem is also the effects of fertilizers and chemicals. The long-term application of inorganic fertilizers such as NPK has been linked with a decrease in fungal diversity in neutral soils, which results in a decline in fungal populations (Brauer *et al*., 2019). Pesticides, encompassing insecticides, fungicides, and herbicides, exert adverse effects on beneficial fungal populations within the soil and on plant foliage (Hooks, 2021). These chemicals diminish sensitive microbial communities, favouring pesticide-tolerant microbes that utilize organic compounds from deceased cells, potentially disrupting the equilibrium of fungal communities (Prashar & Shah, 2016). Furthermore, using fungicides aimed at combating fungal plant pathogens can detrimentally affect beneficial fungi, compromising the natural regulation of insect pests and potentially resulting in pest outbreaks (Brauer *et al*., 2019). Chemical inputs such as fertilizers and pesticides can disturb the soil microbiome, including fungal populations, thereby impacting soil health, nutrient cycling processes, and the equilibrium of beneficial microorganisms. While fungicide applications to control fungal plant diseases may enhance crop yield and quality, they can inadvertently affect non-target fungi, potentially disrupting the overall ecosystem balance.

In conclusion, the complex interplay between fungi, particularly PSF, and soil ecosystems highlights the importance of understanding their ecological roles and responses to anthropogenic activities. The implications of jhum cultivation practices on soil fungal populations further emphasize the need for comprehensive research in this field.

## REVIEW OF LITERATURE

Phosphorus (P) is a vital nutrient that supports plant growth and development, taking part in many important biological functions. Yet, its presence in soil is often limited, creating a major obstacle for agricultural productivity across the globe. Traditional methods of addressing P deficiency through chemical fertilizers have drawn concerns about environmental sustainability and long term economic viability. In the last few years, there has been growing interest in harnessing the potential of phosphate solubilizing microorganisms (PSMs) as eco-friendly alternatives to conventional P fertilizers. These microorganisms, particularly fungi, have demonstrated the ability to solubilize insoluble phosphates in soil, enhancing P availability to plants. This review chapter, aims to synthesize and critically evaluate the findings from these international, national, and regional studies, focusing on the mechanisms of P solubilization by PSF, their application in sustainable agriculture, and the prospects for harnessing their potential at the global level. Additionally, delve into broader implications of these findings for agricultural practices and policy-making at the national and regional levels, with a specific focus on Nagaland.

Khan *et al*., (2009) discuss the significance of phosphorus (P) deficiency in soil and its impact on crop productivity, highlighting the potential role of phosphate-solubilizing microorganisms, particularly fungi, in making P available to plants. While in vitro studies have shown promise in P solubilization by these microbes, their effectiveness in situ remains inconsistent. Fungi possess various beneficial traits, such as mineral solubilization and production of secondary metabolites, which can enhance plant growth when associated with roots. The review emphasizes understanding the mechanisms of phosphate solubilization, development, and application of fungal inoculants, and their role in promoting crop productivity across diverse agro-ecosystems. Strategies for managing P nutrition by applying phosphate-solubilizing fungi are also explored.

Vassilev *et al*., (2013) note that the recent surge in research on microbial solubilization of insoluble phosphates reflects a growing interest in biotechnological alternatives to chemically derived P-fertilizers. This interest is fuelled by rising P-fertilizer prices due to increased global demand and dwindling rock phosphate reserves. Microbial solubilization primarily produces organic acids such as citric, oxalic, gluconic, itaconic, and lactic acid, interacting with insoluble P sources. Cultivated through submerged and solid-state

fermentation systems, Fungi are the most commonly studied P-solubilizers. This review aims to consolidate data on how various abiotic factors influence fungal organic acid production. Factors such as nutrient medium composition, fermentation process parameters, interactions between insoluble P-particles and microbial systems, and fermentation modes are analyzed for their impact on organic acid production and P-solubilization. Additionally, suggestions for further research directions are provided.

De Oliveira Mendes *et al*., (2014) evaluates fungal isolates for their potential to solubilize P from various compounds, including AlPO4, FePO4, Ca3(PO4)2, and different types of rock phosphates (RP). Four isolates from Brazilian soils were tested, with *Aspergillus niger* FS1 showing the highest effectiveness in solubilizing all P sources, particularly through medium acidification and production of organic acids. Oxalic acid, produced exclusively by *A. niger* FS1, and citric acid played key roles in solubilizing AlPO4 and FePO4. While *Penicillium* isolates produced more gluconic acid, it didn't significantly contribute to solubilization. *A. niger* FS1 exhibited a superior capacity for medium acidification and production of organic acids with stronger metal-complexation activity, making it a strong candidate for P fertilization management.

Elias *et al*., (2016) aimed to isolate and characterize phosphate-solubilizing fungi from various rhizospheres using solid and liquid Pikovskaya (PVK) medium. 359 fungal isolates were obtained from rhizosphere soil samples of haricot bean, faba bean, cabbage, tomato, and sugarcane. Among these isolates, 167 (46.52%) demonstrated the ability to solubilize inorganic phosphate. The isolated phosphate-solubilizing fungi were primarily from the genera *Aspergillus* (55.69%), *Penicillium* spp. (23.35%), and *Fusarium* (9.58%). The solubilization index (SI) ranged from 1.10 to 3.05. Notably, isolates designated as JUHbF95 (*Aspergillu*s sp.) and JUFbF59 (*Penicillium* sp.) exhibited the highest phosphate solubilization, releasing 728.77 μg·mL−1 and 514.44 μg mL−1, respectively, from tricalcium phosphate (TCP) after 15 days of incubation. Moreover, inoculation of JUHbF95 resulted in the highest soluble-P release (363 μg mL−1) from rock phosphate (RP) in the PVK broth after 10 days of incubation. This study emphasizes the existence of diverse plant-associated PSF that hold potential as biofertilizers.

Nasr *et al*., (2021) aimed to assess the potential of isolated phosphate-solubilizing fungi (PSF) in inhibiting the growth of various pathogenic fungi. Out of 137 fungal isolates obtained from soil samples, nine solubilized inorganic phosphate, with eight isolated from different plant rhizospheres and one from free soil. These isolates were identified as belonging to the genera *Aspergillus* and *Penicillium*. *Aspergillus japonicus* 2 exhibited the highest solubilization index (1.5) and effectively inhibited the growth of *Alternaria alternata* by 70%. This isolate showed promise as a bio-control agent against *Fusarium solani*, *Geotrichum candidum*, and *Alternaria alternata*. The highest P solubilization by *Aspergillus japonicus* was observed with sucrose as the carbon source and ammonium chloride or tryptophan as the nitrogen source. Maximum solubilization percentage (78.2%) was achieved with 5% glucose concentration, and pH 3 provided optimal solubilization (90%). After eight days of incubation, the solubilization percentage peaked at 80.2%.
Tian *et al*., (2021) review addresses the vital role of P in biological systems, emphasizing its limitation on biomass production due to the small fraction of plant-available P in soil. With increasing global food demand and extensive use of P fertilizers in agriculture, there's a risk of excessive P inputs leading to environmental issues like eutrophication. While phosphate-solubilizing microorganisms (PSMs) are recognized as eco-friendly P fertilizers, their role in managing P deficiency and influencing P geochemical processes needs deeper exploration. The review outlines the various forms of P and their cycles in soil systems, elucidates how PSMs mediate soil P biogeochemical cycles, and discusses the metabolic and enzymatic mechanisms involved. Additionally, it underscores the significance of PSMs in the biogeochemical P cycle and suggests environmental considerations for future applications of PSMs.

Arias *et al*., (2022) aimed to explore the ability of PSF as an alternative to traditional fertilizers in coffee cultivation. It analyzed the underlying mechanisms involved in the P solubilization of fungal strains and evaluated the impact of a phosphate-solubilizing strain on coffee plants. Among 151 strains analyzed, *Sagenomella diversispora*, *Penicillium waksmanii*, and *Penicillium brevicompactum* exhibited the highest solubilization potential. *Aspergillus niger* and *P. waksmanii* showed the highest soluble phosphorus values, while *P. brevicompactum* displayed the highest phosphatase activity. Inoculation of *P. brevicompactum* on coffee plants increased soil soluble phosphorus content in two out of three plantations but did not significantly affect foliar phosphorus content. However,

coffee plants inoculated with the phosphate-solubilizing strain showed an increase in coffee bean weight across all plantations, with significant improvements observed in two of every three plantations. This suggests the potential of PSF as biofertilizers for enhancing coffee bean yield.

Vassileva, *et al*., (2022) further highlights the role of fungi in phosphorus (P) solubilization and plant nutrition, underscoring their importance as beneficial microorganisms in agriculture. While fungi like *Aspergillus, Penicillium*, and *Trichoderma* have shown efficacy in solubilizing P and aiding plant P uptake, their role has been less studied than bacteria. Despite recent doubts about the effectiveness of microbial P-solubilizers in soil, the review synthesizes established facts and emerging perspectives to advance our understanding of fungal-mediated P-solubilization. It advocates for leveraging fungal resources to enhance nutrient cycling and promote sustainable agricultural practices in line with the principles of the circular economy.

**National level**

In India, agriculture is the backbone of the economy, supporting millions of livelihoods and contributing significantly to the country's GDP. However, sustaining agricultural productivity amid growing population pressure, diminishing arable land, and environmental concerns poses a formidable challenge. Phosphorus, a crucial nutrient for plant growth, is often limited in Indian soils, impacting crop yield and quality. In light of this, the exploration and utilization of phosphate solubilizing microorganisms (PSMs) present a promising avenue to sustainably enhance soil fertility and agricultural productivity. This introduction lays the stage for understanding the importance of PSMs in Indian agriculture and underscores the continued need for research and innovation in this critical field.

Gupta *et al*., (2012) investigated the impact of four phosphate solubilizing bacteria (PSB) on the growth and aloin-A content of *Aloe barbadensis* in soil containing tricalcium phosphate (TCP). These PSB were identified as *Pseudomonas synxantha*, *Burkholderia gladioli*, *Enterobacter hormaechei*, and *Serratia marcescens* based on 16S rRNA gene sequencing. The PSB could solubilize 25-340 µg ml (-1) of TCP into the liquid phase. Treating plants with individual PSB or a mixture of these increases soil's available phosphorus, phosphorus uptake, and plant growth. The increase in aloin-A content,

attributed to higher plant biomass and unit biomass production, ranged from 108% to 673% across treatments, with the PSB consortium exhibiting the highest increase.

Tari *et al*., (2022) current research project focuses on isolating and characterizing phosphate solubilizing microorganisms (PSM) from the rhizospheric soil of several medicinal plants and assessing their impact on plant growth. Medicinal plants including *Aloe vera*, *Bauhinia variegata*, *Cannabis sativa*, *Lantana camara*, and *Menthaviridis* were chosen for PSM isolation, with the soil status of these plants also evaluated. Phosphate solubilizing bacteria (PSB) were examined for morphological characteristics and Gram staining under a stereo microscope, while PSF were identified through microscopic analysis. Various parameters, such as colony diameter, halo zone diameter, and solubilization index, were determined on PVK agar plates. TLC results indicate citric acid as the predominant acid produced by PSM strains. Pathogenicity tests confirmed that all strains were non-pathogenic. The study demonstrated a positive plant growth response to PSM inoculation across all experiments. In the first study, individual inoculation of PSM resulted in a significant increase in plant growth parameters, including fresh and dry weight, plant height, and root and shoot length, compared to the control. In the second study, composite inoculation of PSM with different P sources, particularly rock phosphate (RP), significantly enhanced plant growth. These findings suggest that PSM inoculation, particularly in combination with RP amendment, can be an effective biofertilizer for promoting plant growth in medicinal plant cultivation.

Doilom *et al*., (2020) extensively characterized thirteen fungal strains, one isolated from air and twelve from the soil, evaluating their capacity to solubilize tricalcium phosphate (TCP) on solid and liquid Pikovskaya (PVK) media. Remarkably, the airborne fungal strain KUMCC 18-0196 (identified as *Aspergillus hydei* sp. nov.) exhibited significant phosphate-solubilizing activity, marking the first report of a phosphate-solubilizing fungus isolated from air. Additionally, the study identified species within *Aspergillus*, *Penicillium*, and *Talaromyces* genera, commonly reported as PSF. Four new species, *A. hydei*, *P. soli*, and *Talaromyces yunnanensis*, were introduced to science, identified based on morphological characteristics and multigene phylogenetic analyses. This comprehensive approach underscores the importance of accurate species identification for understanding PSF diversity and identifying potential taxa for future plant growth-promoting applications.

Alam *et al*., (2022) aimed to assess changes in soil P distribution influenced by PSF, P fertilization and liming and their impact on soybean growth. Conducted in Negheriting tea estate, Assam, India, the study applied varying levels of P, lime, and PSF to soil samples, followed by post-harvest soil P fractionation. Results showed that different P fractions in the soil, led by residual P, varied significantly with treatments. Inorganic P fractions, except residual P, increased notably with P fertilization. Liming and PSF application increased soluble P fractions and reduced Al and Fe-bound P. Soybean growth responded positively to P, lime, and PSF application. Liming enhanced P uptake and dry matter yield, while PSF inoculation moderately increased P uptake and yield. The findings underscored the short-term efficacy of liming or PSF in solubilizing native soil P, suggesting potential synergies between P, lime, and PSF for future agricultural practices.

Krishna & Suka (2022) assess the phosphorus and nutritional status of soil samples collected from coastal districts in Odisha, India, and isolate potential fungal strains capable of phosphate solubilization. Twelve soil samples from three districts were analyzed for key soil parameters, showing significant correlations among *p*H, electrical conductivity, organic carbon, nitrogen, phosphorus, and potassium. Fungal colonies were isolated and screened from rhizospheric soil, identifying four potential fungal strains. These strains demonstrated P solubilization efficiency on Pikovskaya's agar selective media, forming halo zones around their colonies within 10 days of incubation. Among them, *A. niger* exhibited the highest P solubilization efficiency, with a solubilization index (SI) of 3.56 cm and significant *p*H reduction in PVK broth containing rock phosphate.

**Regional:**

The northeastern region of India, particularly Nagaland, is characterized by its rich biodiversity and diverse agricultural landscapes. However, despite its ecological importance, the literature on this subject is substantially limited on certain crucial aspects of agricultural microbiology, particularly regarding PSF. PSF have significant impact in soil fertility and plant nutrition, yet their diversity and potential applications remain relatively unexplored in this region. In recent years, researchers have recognized the importance of understanding the microbial communities present in agricultural soils,

especially in areas like Zunheboto and Mokokchung districts of Nagaland. These regions are known for their tea gardens and diverse agricultural practices, serve as excellent ideal locations for investigating the indigenous microbial populations and their contributions to soil health and plant productivity.

Imlimenla *et al*., (2021) aims to document PSF in Zunheboto district, Nagaland, due to the lack of information on these fungi in the region. PSF were collected from the rhizospheric region, with Aspergillus and Penicillium identified as the dominant genera. The findings hold significance for promoting sustainable farming practices in Nagaland by exploring PSF as an alternative to chemical fertilizers.

Jamir *et al.*, (2022) investigated indigenous dominant microfungi in tea garden soils of Mokokchung district, Nagaland, India, assessing their P solubilization activity. A total of 110 fungal isolates from 19 genera were identified, with *Aspergillus*, *Penicillium*, and *Trichoderma* dominating the soil microfungi. Significant differences were observed in culture plates and microscopic studies of the isolates. *Aspergillus* species exhibited the highest P solubilization activity, followed by *Penicillium* and *Trichoderma* species. The findings provide insights into indigenous microfungi in tea garden soils and their potential applications as biofertilizers for tea and other plants, contributing to expanding knowledge on indigenous fungi and their roles.

## ORIGIN AND SCOPE OF THE RESEARCH PROBLEM

Zunheboto's agricultural practices face a significant challenge: maintaining soil health in the face of traditional shifting cultivation. While this practice provides a livelihood for the community, it can lead to soil degradation and depletion of essential nutrients, particularly P. P is crucial for plant growth and development, and its insufficient levels can profoundly impact crop yields. Shifting cultivation practices often involve clearing land, burning vegetation, and planting crops for a few seasons before moving on to new areas. This cycle can lead to Soil erosion. Exposed soils are vulnerable to erosion by wind and rain, washing away valuable topsoil and nutrients like phosphorus. Further, heavy rainfall can leach soluble nutrients, including phosphorus, deeper into the soil profile, making them inaccessible for plant uptake. Despite these concerns, research on solutions specifically for Zunheboto agricultural system is limited.

One promising approach lies in exploring the potential of naturally occurring beneficial soil microbes. PSF is a diverse group of fungi with the remarkable ability to convert unavailable phosphorus forms in the soil into plant-usable phosphates. They accomplish this through several mechanisms. Organic acid production: PSF secretes organic acids that bind with soil particles containing phosphorus, making them more soluble and readily available for plants. These fungi can directly break down certain mineral forms of phosphorus, releasing it for plant absorption

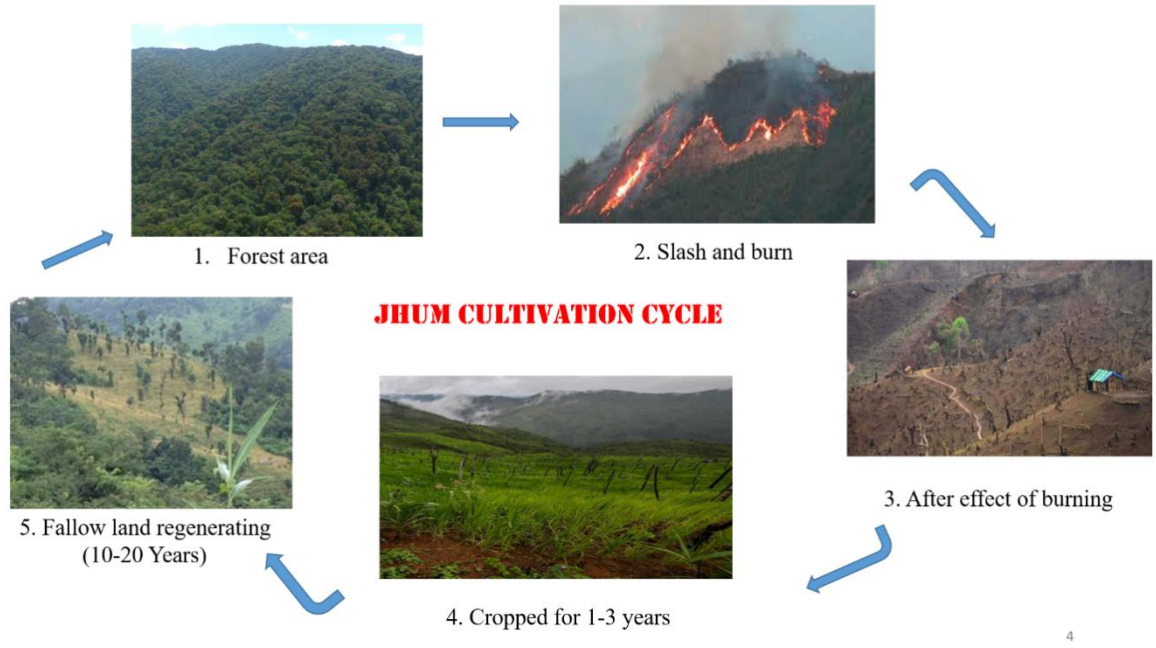


**Figure 1: Schematic representation of Jhum cultivation cycle.**

The importance of studying PSF in the soils of Zunheboto holds immense potential benefits for the region's agricultural sustainability. Further, strategies can be developed to promote their activity in cultivated soils by understanding the presence and effectiveness of indigenous PSF strains. This can enhance plant access to phosphorus, improving crop yields and food security for the community. PSF also offers a natural and eco-friendly approach to improve phosphorus availability in soils. This reduces dependence on expensive and potentially harmful chemical fertilizers, promoting sustainable and environmentally responsible agricultural practices. PSF not only improves phosphorus availability but also contributes to overall soil health by promoting beneficial microbial

communities and improving soil structure. By identifying and evaluating native PSF strains, strategies can be designed to enhance their activity in farmlands, thereby increasing phosphorus availability, boosting crop yields, and improving food security for local communities.

This study therefore investigates PSF in the jhum fields of Zunheboto, Nagaland. By characterizing these fungi, it aims to expand our understanding of soil microbial ecology while offering insights into sustainable approaches to improve soil fertility in shifting cultivation systems. Based on this background, the present study was undertaken for my Doctoral Research with the following objectives:

1. Isolation of phosphate solubilizing microorganisms from the selected crops under jhum field.
2. Screening of potential phosphate solubilizing fungi.
3. Morphological and molecular characterization of the isolated strains.
4. To compare the efficiency of isolated phosphate solubilizing strains in pot experiment.

# Chapter - 2

# Isolation of Rhizospheric Fungi from the Selected Crops Under the Jhum Fields

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# Chapter - 2

# Isolation of Rhizospheric Fungi from the Selected Crops Under the Jhum Fields

## Introduction

Microbial populations are instrumental to fundamental processes that drive the stability and productivity of agro-ecosystems. Chemical fertilizers like urea, calcium nitrate, ammonium sulfate and diammonium phosphate have transformed farming by giving crops an instant supply of nutrients. They work almost like fast food for plant easy to take in, quick to act, and powerful in effect. With this boost, crops grow faster, stronger, and more efficiently, which is a big reason global food production has risen so dramatically. However, the excessive and imbalanced use of these synthetic inputs has adverse effects, including subverting soil ecology, disrupting the environment, degrading soil fertility, and posing harmful risks to human health and groundwater contamination. Biofertilizers, which use microorganisms to enhance soil fertility, present a sustainable alternative. By relying on beneficial microbes, they naturally enrich the soil through processes like nitrogen fixation, P solubilization, and growth stimulation. Unlike synthetic inputs, they are environmentally safe and ensure a steady release of nutrients from natural sources. Numerous studies have pointed to bio fertilizers as promising alternatives to chemical fertilizers (Suhag, 2016).

PSF are vital for improving soil fertility and supporting plant growth because they convert insoluble forms of phosphate to soluble forms, thus making it available for plant uptake. Among these microorganisms, PSF are particularly effective due to their diverse mechanisms of solubilization, including the secretion of organic acids, enzymes, and siderophores (Rodriguez & Fraga, 1999; Richardson, 2001). The isolation and study of these fungi have significant implications for sustainable agriculture, especially in regions where phosphate availability limits crop productivity (Gyaneshwar *et al*., 2002). In traditional agricultural systems such as jhum cultivation, also known as shifting cultivation, the role of PSMs becomes even more critical. Jhum cultivation, predominantly practiced in northeastern India and other Southeast Asian regions, involves clearing forest patches, burning biomass, and cultivating crops on the resultant ash-enriched soil (Nath *et al*., 2022). While this method can temporarily increase nutrient availability, it often leads to soil degradation and nutrient depletion over time. Thus, identifying and utilizing PSMs from jhum fields could offer a sustainable solution to enhance soil fertility and crop yields

in these ecologically fragile areas (Sharma *et al.,* 2013**).** Research has shown that jhum fields, due to their unique soil conditions and diverse plant interactions, harbor a rich diversity of microorganisms, including PSF. These fungi can be isolated from various crop rhizospheres, where they play a pivotal role in nutrient cycling and plant growth promotion (Singh *et al*., 2011). By studying these indigenous PSF, we can not only understand their ecological roles but also explore their potential application in biofertilizer development, thus promoting sustainable agriculture practices in jhum region**s (**Vassilev *et al*., 2006**).** This chapter focuses on the isolation of PSF from selected jhum fields. The isolation process involves collecting soil samples from the rhizosphere of different crops, culturing the fungi on specific media, and identifying the isolates capable of solubilizing phosphate. The outcomes of this research could provide valuable insights into the diversity and functionality of PSF in jhum fields, paving the way for their application in enhancing soil fertility and crop productivity in these traditional agricultural systems.

## Material and methods

### 2. 1 Description of the study site

Nagaland is a mountainous region in the corner of North-East India, bordering Myanmar, Assam, Arunachal Pradesh, and Manipur, and it is of critical importance for global biodiversity conservation (Myers *et al*., 2000). The region provides a strong potential for biodiversity benefits while mitigating climate change with a high emission mitigation potential (Murthy *et al*., 2013). Regarding Jhumming, almost 72% of the total arable land employs shifting cultivation, and the ethnic population in the rural areas depends on farming in Nagaland (Solo and Kikhi, 2021). Zunheboto district, nestled within Nagaland, India, encompasses an area of approximately 1,255 sq km (26.0129° N, 94.5242° E). The dominant inhabitants are the Sumi Naga people, renowned for their distinct culture and traditions. The specific research sites were at Lumami and Sumi Settsu villages. Zünheboto lies north of the Satoi Range. Due to its elevation, Zünheboto features a more moderate version of a humid subtropical climate. Shifting cultivation is deeply ingrained in the socio-cultural fabric of the Sumi Naga community in Zunheboto. This practice is reflected in various agricultural festivals celebrated throughout the year. The "*Tuluni*" festival, typically held in the second week of July, is a prominent celebration that unites the community. Due to its rich cultural heritage and strong connection to the land, shifting

cultivation remains the cornerstone of agricultural practices in the surrounding hills. This village serves as a prime location for the study due to numerous fallow lands available. **Figure 2.1** depicts the location of the study sites. **Figure 2.2** depicts the photographic depiction of the jhum sites selected.

## 2.2 Soil physico-chemical parameter:

### Soil analysis

To assess the soil properties, we collected soil samples from each chosen site at two distinct times: spring (March-May) and summer (June-August). Samples were obtained in layers from a depth of 0-30 cm. Following collection, each sample was placed in a tightly sealed polyether bag and transported securely to the laboratory for further analysis. Soil temperature was measured in the field using a digital soil thermometer. Most soil tests were conducted on air-dried samples that were sieved through a 2 mm nylon sieve. Exceptions were bulk density and soil moisture, which were determined using fresh samples (**Table 2.1**). All analyses were performed in triplicate, and the results are presented as mean values with standard deviation. The following section provides a brief explanation of the protocols used for each test.

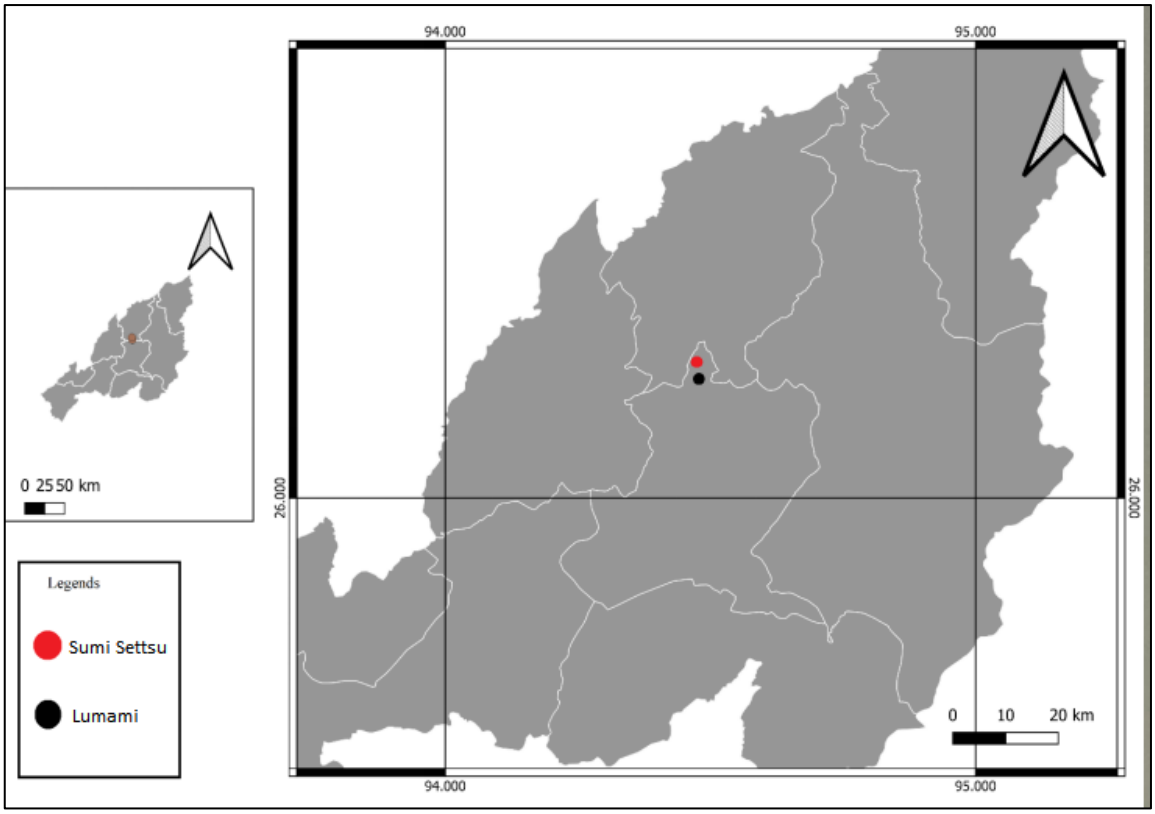



**Figure 2.1: Study map area with the two selected sites.**


**Figure 2.2. Photographic view of Jhum field from A: Lumami and B: Sumi Settsu**


**Figure 2.3. Photographic view of Jhum field of Lumami. (A) Maize plant (B) Chilli plant**


**Figure 2.4. Photographic view of Jhum field of Sumi Settsu. (A) Rice plant (B) Chilli plant**

### 2.2.1 *p*H and Electrical conductivity

To 50 ml of distilled water, 10 gm of soil samples was added into a conical flask and shaken for 30 minutes. Next, the supernatant is then transferred to a beaker and its pH and EC are recorded utilizing the HM Digital *p*H-200 and LMCM-20, respectively.

### 2.2.2 Soil Moisture

From the fresh collected soil samples, 50 gm were kept in an oven for 24 hours at $105^0$C. Soil moisture in percentage is then obtained by utilizing the formula

$$\text{Soil moisture (\%)} = \frac{\text{weight of the oven dried soil}}{\text{weight of the fresh soil}} x\ 100$$

### 2.2.3 Bulk Density (BD)

Fresh soil samples were collected in a core sampler (10x10cm) and oven dried at 24 hours at $105^0$C.

$$\text{Bulk density (gm/cm}^3\text{)} = \frac{\text{Mass of the dried sample}}{\text{Core sampler volume}}$$

### 2.2.4 Soil texture

20 gm of the soil samples were transferred to a 500 ml graduated cylinder. Next 50 ml of sodium hexametaphosphate and 10 ml of water is introduced into the cylinder. This mixture is then stirred for 5 minutes making the final volume to 500 ml. At the 48 second mark, utilizing a pipette, 25 ml of the aliquot is removed. This aliquot is then placed under the oven at $105^0$C with the help of an evaporating dish (labelled Silt+clay). The next aliquot is then collect at the 40 min from the upper 5 cm region of the suspension and then placed into the oven. The evaporating dish are then collected after 24 hours and weight. The soil texture is then calculated utilizing the formula

$$\text{Clay (\%)} = \left(20 \times \frac{\text{dried weight of the second aliquot}}{\text{weight the soil sample}}\right) \times 100$$

$$\text{Silt (\%)} = \left(20 \times \frac{[\text{dried weight of first aliquot} - \text{dried weight of the second aliquot}]}{\text{weight the soil sample}}\right) \text{x } 100$$

Sand (%) = 100 – (silt % + clay %)

### 2.2.5 Soil organic carbon (SOC)

1 gm of the soil sample is added to 10 ml of $K_2Cr_2O_7$ and 20 ml of conc. $H_2SO_4$ into a conical flask and allowed to react for 30 mins. To this mixture, 200ml of distilled water and 10ml of phosphoric acid is introduced. 1ml of indicator (diphenylamine indicator) is added and titrated against 1N ferrous ammonium sulphate (FAS). The end point of the titration process is when the mixture changes to green.

$$\text{Organic carbon (\%)} = \frac{A1 - A2}{W} X\ 0.003\ X\ 100$$

*Where, A1 = volume of ml of 1N K2Cr2O7 utilized, A2 = volume of FAS used in titration, W = gm of soil sample.

### 2.2.6. Available Phosphorus ($P_{av}$)

Reagents A and B are as follows.

$$\text{Reagent A} = \text{Ammonium molybdate A. R. (17.14 gm)} + \text{potassium antimonyl tartrate A. R. (0.392 gm)} + \text{Sulphuric acid (200 ml)} + \text{deionized water (850 ml).}$$

$$\text{Reagent B} = \text{L} - \text{Ascorbc acid A. R. (0.53 gm)} + \text{deionized water (5 ml)} + \text{reagent A (70 ml)}$$

In a centrifuge tube, 7 ml of Bray extracting solution (i.e. 2.22 g of Ammonium fluoride + 5 ml conc. HCl) and 1 gm of the soil sample is taken. The tube is then spun at 6000 rpm for 5 minutes. Dispense the supernatant (0.50 ml) and reagent B (2.0 ml) into a colorimeter stand. This is then allowed to stand for half an hour. Next, prepare a standard solution from the P solution (2.50 mg/l) of concentrations 0.05, 0.10, 0.20, 0.30, 36, 0.40 and 0.50 mg/l. Finally, set the instruments instrument absorbance at 882nm and record. The phosphorus (available) is obtained by plotting concentration against absorbance.

$$\text{Available phosphorus (kg/ha)} = \frac{\text{Phosphorus concentration} \times \text{Dilution factor} \times 2.24 \times \text{aliquot utilized}}{\text{sample utilized}}$$

### 2.2.7 Available potassium ($K_{ex}$)

50 gm of soil sample and 25ml of ammonium Acetate are transferred to an Erlenmeyer flask (150ml). After shaking it for 5 minutes in a mechanical shaker, it is then filtered

through a filter paper (Whatman No. 1). Utilizing a flame photometer, the sample is adjusted to zero with blank. Next, for preparation of a standard graph, from the working K solution 0, 5, 10, 15, 20 and 25 ppm was prepared. The concentration of K in the sample is obtained by plotting against the standard graph.

$$\text{Available Potassium (kg/ha)} = \frac{\text{RxExtractvoumex2.24}}{\text{weight of sample}}$$

*Where, R=ppm of K value from the standard graph.

### 2.2.8 Available Nitrogen ($N_{av}$)

Soil samples (5gm), distilled water (20ml) and 0.32% $KMNO_4$ (25ml) are introduced into digestion tubes and fitted into the Kelplus distillation unit. To this 2.5% NaOH (25ml) *via* the distillation unit. At the receiving end of the distillation unit 2.5 % boric acid (25ml) is mixed with indicator i.e., Bromocresol green (0.3 gm), methyl red (0.2 gm) and 95% ethanol (400 ml) to receive the liquid ammonia from the receiving end. This is then titrated against 0.02N $H_2SO_4$.

$$N_{av}(\text{Kg/ha}) = \frac{14 \times (\text{Normality of the acid}) \times (\text{titrant value reading}) \times 2.24 \times 106}{\text{sample weightx } 10000}$$

### 2.2.9 Cation Exchange Capacity

In a 40 ml centrifuge tube, add soil sample (45 gm) and Sodium acetate trihydrate (33 ml) and centrifuge at 33 rpm. Gradually pour the supernatant, and repeat with N Sodium acetate trihydrate (33 ml) at least four times and discard the supernatant liquid. Now add 95% ethanol (33ml) and centrifuge till a clear and decant supernatant is obtained. Repeat this step with 95% ethanol at least 3 more times. Now, replace the absorb sodium by utilizing 1N Ammonium acetate (33 ml) for three more times. Decant the three supernatant liquids into an 100ml volumetric flask and make the final volume with 1 N Ammonium acetate solution. Prepare a series of suitable Na standards by Diluting 2, 4, 6 and 8 ml of 250 ppm Na solution. Lastly add 1 N Ammonium acetate to each of the flask (100ml) to obtain 0, 5, 10, 15 and 20 ppm Na solution. Take the readings at Flame photometer.

CEC

$$(\text{meq}/100\text{g}) = \frac{\text{meq}}{\text{L}} \text{Na (from calibration curve)} \times \frac{\text{Total volume of the extract (ml)}}{\text{weight of soil sample}} \times \frac{100}{1000} \times 20$$

**2.2.10 Statistical analysis:**

All statistical analyses, including ANOVA and PCA, were carried out using SPSS software (version 26.0). A one-way ANOVA was applied to assess seasonal differences in soil characteristics and to evaluate variations in soil depth across different sites. Mean comparisons were conducted with Duncan's Multiple Range Test (DMRT) at a 5% significance level ($p < 0.05$).

**Table 2.1:** Methods utilized in analysis of soil parameters in the present study

| Soil parameters | Methods |
|---|---|
| *p*H | Digital *p*H meter |
| Electrical conductivity (EC) | EC meter |
| Soil moisture | Gravimetric method (Misra, 1968) |
| Clay content | Pipette method (Piper, 1942) |
| Bulk density (BD) | Core sampler method (Allen, 1989) |
| Soil organic carbon (SOC) | Walkley and Black method (1934) |
| Available nitrogen (N) | Kjeldahl method (1883) |
| Available phosphorus (P) | Bray's no. 1 extract method (Bray and Kurtz 1945) |
| Exchangeable potassium (K) | Photometric method (Trivedy and Goel 1986) |
| Cation exchange capacity (CEC) | Bower *et al*. (1952) |

## 2.3. Isolation of the fungal isolates.

### 2.3.1 Rhizospheric soil collection

The rhizospheric soil samples were collected from selected crops under jhum fields of Lumami and Sumi Settsu, Zunheboto. The soil was were taken from a depth of 5-20cm

with intact roots and were brought to the laboratory in sterile plastic bags. The collected soil samples were kept at 4 °C for futher study.

### 2.3.2 Media Preparation:

Rose-Bengal Agar (RBA) (Martin, 1950) media was used for mix culture of fungi. The required materials, agar, $KH_2$ PO4, Mg.$So_4$ $H_2O$, peptone, dextrose, and Rose Bengal were weighed and placed into a conical flask and dissolved in the 500ml distilled water. While Potato Dextrose Agar (PDA) with composition of dexrose, potato extract and agar was used for pure culture. The preparation of both media's first begins with sterilizing which is followed by autoclaving the required equipments.

The solution was continuously stirred as it boiled. After that, the flask`s mouth was stuffed with cotton and covered with aluminium foil before being autoclaved at 15lb pressure for 15-20 minutes. The medium was allowed to cool down before adding streptomycin before it solidified. The medium was poured into sterilized petriplates in the laminar air flow chamber under sterilized conditions. After the media has solidified, it is exposed to UV light for a few minutes before it is ready for culture. Plates were sealed with parafilm and incubated at 28 ± 2°C for 1 week.

### 2.3.3 Soil dilution plate method (Waksman, 1922)

Soil dilutions were made by suspending 1g of each soil sample in 10ml of sterile distilled water and thoroughly mixing the suspension for few minutes, followed by additional five test tubes with 9ml each as before. 1ml of the soil suspension is drawn with the micro pipettes and added to the first test tube to make a total volume of $10^{-1}$. Now, 1ml of the mixture from the $10^{-1}$ dilution is collected and placed into the next test tube, which now has a total dilution factor of $10^{-2}$. The same procedure is done for the remaining test tubes, yielding $10^{-3}$, $10^{-4}$, and $10^{-5}$, correspondingly. $10^{-2}$ and $10^{-3}$ and $10^{-4}$ were employed to isolate fungi in order to reduce fungal colony overcrowding. Under laminar air flow, 1ml of each concentration of suspension was uniformly added to the sterile petri plates containing the sterile RBA medium. To prevent air microbial contamination, the petri plates were covered and then bonded with a thin sheet of transparent polythene. The plates were inverted and incubated for 7 days at 28 ± 2°C. Following that, the number of fungal colonies was counted, and pure culture in PDA media was performed.

## 2.4 Fungal diversity

Throughout the study period, soil samples were collected seasonally from sites. To obtain a representative sample, soil was collected from five randomly chosen locations within each site and then combined into a single composite sample. Following collection, the soil was transported to the laboratory under sterile conditions for storage at 4°C until further analysis. RBA and PDA were chosen for fungal isolation. Streptomycin was added to both RBA and PDA media to suppress bacterial growth. The Waksman (1922) method for serial dilution was employed to achieve appropriate fungal concentration for isolation. Following Selman and Waksman (1921), 1 gram of soil was diluted in 10 ml of sterilized distilled water, creating a 1:10 dilution series (10^-1 to 10^-5). Dilutions from 10^-2 to 10^-5 were used for fungal isolation. One milliliter from each dilution was transferred, in triplicate, to separate plates containing PDA or RBA media supplemented with streptomycin sulfate (0.03g/L). Plates were incubated in the dark at 25°C ± 10°C for 5-7 days. Fungal colonies observed on the plates were then transferred to fresh PDA and RBA plates for further isolation and incubated under the same conditions (25°C ± 10°C, dark, 5-7 days). Colony morphology on the plates was documented. Lacto-phenol cotton blue temporary slides were prepared from the isolated fungi and examined under a compound microscope (Motic Model BA210LED) for identification purposes. The fungi were identified with the help of literature (Gillman, 1957; Watanabe, 2002; Ho *et al*., 2003; Hauser, 2006; Nagmani *et al*., 2006; Webster and Weber, 2007; Afzal *et al*., 2013).

Morphological Examination

Temporary mounts of fungal mycelium and spores were prepared using lacto-phenol cotton blue. Micro-morphological features were observed under a compound microscope (Motic Model BA210LED) following standardized mycological keys (Gillman, 1957; Watanabe, 2002; Webster and Weber, 2007; Afzal et al., 2013) for preliminary genus and species assignment.

## 2.5 Fungal diversity indices

Fungal data obtained were then analysed in PAST (Palaeontological Statistics) 4.03 for diversity indices. The diversity indices selected to access the impact of fallow on fungal population are briefly explained as follows

### 2.5.1 Simpson's index of diversity (1-D)

This diversity index represents not only the number of classes but also the proportion of classes (Simpson, 1949). The value ranges from 0 to 1, with 0 representing infinite diversity, and 1 representing no diversity. However, the Simpson's index of diversity is utilized instead of D, where 0 represents no diversity, and 1 represents infinite diversity.

$$D = 1 - (\frac{\varepsilon n(n-1)}{N(N-1)})$$

*Where, D= Simpsons diversity, n= total no of a particular species, N= total number of organism of all species.

### 2.5.2. Pielou's evenness (J) index

Pielou's evenness ranges from 0 (no evenness) to 1 (complete evenness), and evenness is high if all species have similar distribution (Pielou, 1969). It is represented by the formula:

$$E = \frac{H}{\ln S}$$

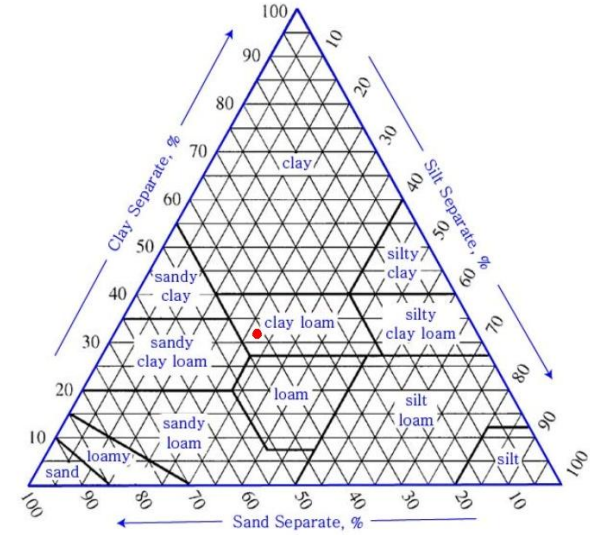
*Where, H is Shannon Weiner diversity, S is total number of species.

### 2.5.3 Berger–Parker index (D)

Berger-Parker dominance reports the proportional abundance of only the most abundant species in the population (Berger, 1970). Higher values of Berger-Parker indicate that the most common species dominate the community at the site.

$$D = N_{max} / N$$

*Where, Nmax represents the number of individuals in the most abundant species, N represents the total number of individuals in the sample.



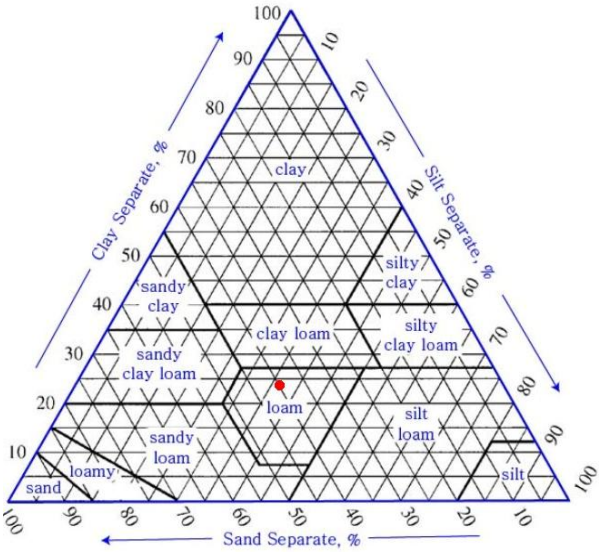




**Figure 2.1: Clay loamy at site I spring and loam at site I summer**

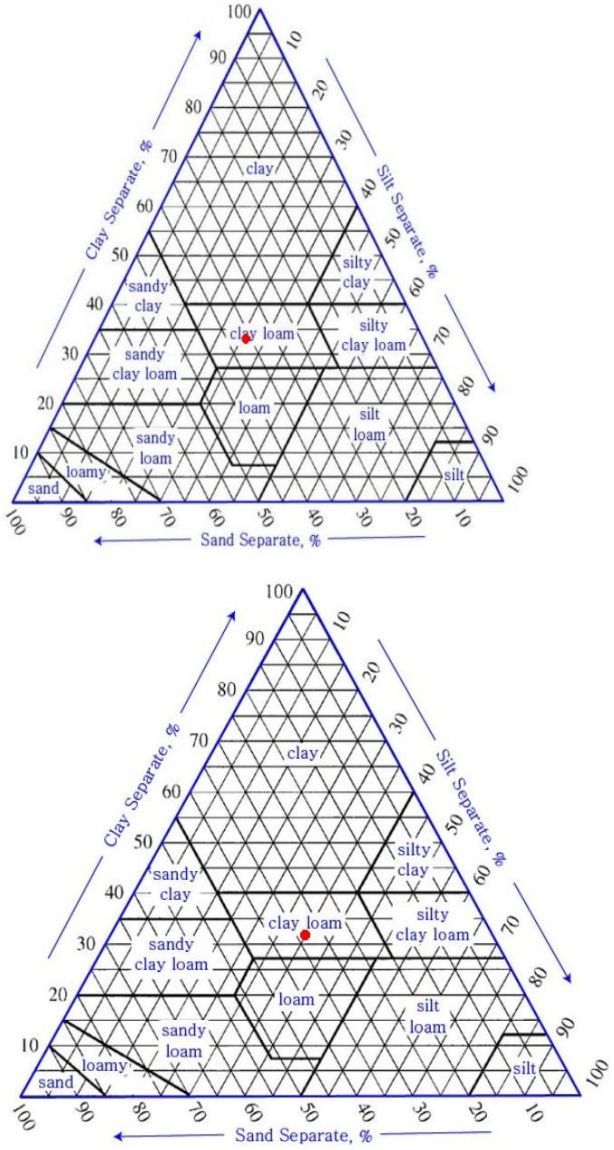



Figure b: clay loamy at site II during spring.

**Fig 2.2: Soil texture class of a: clay loamy at site I spring and b: loam at site I summer c: clay loamy at site II during spring and d: summer**

**Table 2.2: Descriptive statistics of soil**

| Physicochemical characters of soil | Sites | Season | Mean | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|---|
| *p*H | **Site I** Lumami | Spring | 5.38 | 0.20 | 5.26 | 5.63 |
| | | Summer | 5.18 | 0.10 | 5.07 | 5.24 |
| | **Site II** Sumi Settsu | Spring | 5.35 | 0.18 | 5.23 | 5.56 |
| | | Summer | 5.28 | 0.04 | 5.55 | 5.63 |
| EC( dS $m^{-1}$) | **Site I** Lumami | Spring | 0.51 | 0.01 | 0.50 | 0.52 |
| | | Summer | 0.48 | 0.01 | 0.47 | 0.49 |
| | **Site II** Sumi Settsu | Spring | 0.62 | 0.01 | 0.61 | 0.63 |
| | | Summer | 0.59 | 0.01 | 0.58 | 0.60 |
| SOC (%) | **Site I** Lumami | Spring | 2.48 | 0.28 | 2.20 | 2.75 |
| | | Summer | 2.82 | 0.14 | 2.72 | 2.98 |
| | **Site II** Sumi Settsu | Spring | 2.30 | 0.01 | 2.29 | 2.30 |
| | | Summer | 2.55 | 0.25 | 2.26 | 2.70 |
| N ($kg/ha^{-1}$) | **Site I** Lumami | Spring | 491.00 | 14.00 | 477.00 | 505.00 |
| | | Summer | 533.00 | 14.00 | 519.00 | 547.00 |
| | **Site II** Sumi Settsu | Spring | 480.00 | 20.00 | 460.00 | 500.00 |
| | | Summer | 540.00 | 20.00 | 520.00 | 560.00 |
| K ($kg/ha^{-1}$) | **Site I** Lumami | Spring | 341.03 | 35.56 | 300.40 | 366.50 |
| | | Summer | 363.99 | 20.15 | 345.10 | 385.20 |
| | **Site II** Sumi Settsu | Spring | 341.13 | 31.99 | 305.10 | 366.20 |
| | | Summer | 344.27 | 19.14 | 330.95 | 366.20 |
| P ($kg/ha^{-1}$) | **Site I** Lumami | Spring | 33.63 | 0.75 | 33.20 | 34.50 |
| | | Summer | 38.12 | 0.47 | 37.66 | 38.60 |
| | **Site II** | Spring | 33.93 | 0.31 | 33.60 | 34.20 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Sumi Settsu | Summer | 37.02 | 1.23 | 35.60 | 37.80 |
| | **Site I** Lumami | Spring | 31.30 | 2.30 | 29.00 | 33.60 |
| | | Summer | 38.20 | 2.30 | 35.90 | 40.50 |
| | **Site II** Sumi Settsu | Spring | 50.86 | 0.56 | 50.30 | 51.42 |
| Moisture (%) | **Site I** Lumami | Summer | 52.54 | 0.56 | 51.98 | 53.10 |
| | | Spring | 1.14 | 0.04 | 1.10 | 1.18 |
| | **Site II** Sumi Settsu | Summer | 1.26 | 0.04 | 1.22 | 1.29 |
| | | Spring | 1.26 | 0.06 | 1.20 | 1.32 |
| BD (gm/cm$^{-3}$) | | Summer | 1.08 | 0.06 | 1.02 | 1.14 |
| | **Site I** Lumami | Spring | 18.24 | 0.70 | 17.53 | 18.94 |
| | | Summer | 20.35 | 0.70 | 19.64 | 21.05 |
| | **Site II** Sumi Settsu | Spring | 19.50 | 1.14 | 18.37 | 20.64 |
| CEC (meq 100g$^{-1}$) | | Summer | 22.91 | 1.14 | 21.78 | 24.05 |
| | **Site I** Lumami | Spring | 42.00 | 0.50 | 41.50 | 42.50 |
| | | Summer | 40.50 | 0.50 | 40.00 | 41.00 |
| | **Site II** Sumi Settsu | Spring | 36.67 | 1.15 | 36.00 | 38.00 |
| Sand (%) | | Summer | 34.00 | 1.73 | 32.00 | 35.00 |
| | **Site I** Lumami | Spring | 26.00 | 0.50 | 25.50 | 26.50 |
| | | Summer | 24.50 | 0.50 | 24.00 | 25.00 |
| | **Site II** Sumi Settsu | Spring | 29.00 | 1.73 | 27.00 | 30.00 |
| Silt (%) | | Summer | 29.67 | 0.58 | 29.00 | 30.00 |
| | **Site I** Lumami | Spring | 32.30 | 2.70 | 29.60 | 35.00 |
| | | Summer | 24.20 | 2.70 | 21.50 | 26.90 |
| | **Site II** Sumi Settsu | Spring | 33.67 | 1.15 | 33.00 | 35.00 |
| Clay (%) | | Summer | 32.33 | 1.15 | 31.00 | 33.00 |

## RESULTS AND DISCUSSION:

### Soil properties from the selected sites.

This section analyzes the soil properties collected from two different sites, Lumami (Site I) and Sumi Settsu (Site II), during the spring and summer seasons, respectively. The data includes measurements of *p*H, electrical conductivity (EC), soil organic carbon (SOC), nitrogen (N), potassium (K), phosphorus (P), moisture, bulk density (BD), cation exchange capacity (CEC), and the percentages of sand, silt, and clay. At both sites, the pH levels were lower during the summer compared to the spring. Specifically, at Site I (Lumami), the *p*H decreased from 5.38 in spring to 5.18 in summer. Similarly, at Site II (Sumi Settsu), the *p*H dropped from 5.35 in spring to 5.28 in summer. This indicates slight soil acidification during the warmer months, which can influence nutrient availability and microbial activity (Temjen *et al*., 2023). Next, the electrical conductivity, which measures the soil's ability to conduct electrical current and indicates soil salinity, showed a slight decrease from spring to summer at both sites. At Site I (Lumami), EC decreased from 0.51 $dS/m^{-1}$ in spring to 0.48 $dS/m^{-1}$ in summer. Site II (Sumi Settsu) decreased from 0.62 $dS/m^{-1}$ in spring to 0.59 $dS/m^{-1}$ in summer. This slight reduction could be attributed to changes in soil moisture content and ion concentrations. The concentration of dissolved ions, such as sodium, calcium, and chloride, which contribute to the soil's electrical conductivity, may have decreased from spring to summer. This could be due to factors like leaching, plant uptake, or other chemical processes in the soil (Hust, 1976). Soil organic carbon content increased from spring to summer at both sites. Site I (Lumami) increased from 2.48% in the spring to 2.82% in the summer, while Site II (Sumi Settsu) saw an increase from 2.30% in the spring to 2.55% in the summer. Higher SOC in summer might be due to increased microbial activity and organic matter decomposition during the warmer season (Zhou *et al*., 2018; Temjen *et al*., 2023). The soil's nitrogen content also increased from spring to summer. At Site I (Lumami), nitrogen levels increased from 491 $kg/ha^{-1}$ in spring to 533 $kg/ha^{-1}$ in summer. Similarly, at Site II (Sumi Settsu), nitrogen levels rose from 480 $kg/ha^{-1}$ in spring to 540 kg/ha in summer. This increase can benefit plant growth, as nitrogen is a critical nutrient. Potassium levels remained relatively stable from spring to summer at both sites, with a slight increase observed at Site I (Lumami) from 341.03 $kg/ha^{-1}$ in spring to 363.99 $kg/ha^{-1}$ in summer and a minor increase at Site II (Sumi Settsu) from

341.13 kg/ha$^{-1}$ in spring to 344.27 kg/ha$^{-1}$ in summer. Potassium is essential for various plant physiological processes, and its stable presence indicates good soil fertility. Similarly, Phosphorus content increased slightly from spring to summer at both sites. At Site I (Lumami), phosphorus levels rose from 33.63 kg/ha$^{-1}$ in spring to 38.12 kg/ha$^{-1}$ in summer. At Site II (Sumi Settsu), the levels increased from 33.93 kg/ha$^{-1}$ in spring to 37.02 kg/ha in summer. Phosphorus is vital for plant energy transfer and root development.Soil moisture content increased from spring to summer at both sites, with Site I (Lumami) recording an increase from 31.30% in spring to 38.20% in summer. Site II (Sumi Settsu) showed a more significant increase from 50.86% in spring to 52.54% in summer. Higher soil moisture in summer can enhance plant nutrient uptake but might also influence soil aeration and microbial activity. Further, the increased soil temperature can improve nutritional availability due to faster rates of chemical processes that free nutrients (Pregitzer & King, 2005). Next, Bulk density, which affects soil porosity and root penetration, varies between the seasons. At Site I (Lumami), BD increased from 1.14 g/cm$^{-3}$ in spring to 1.26 g/cm$^{-3}$ in summer. Conversely, at Site II (Sumi Settsui), BD decreased from 1.26 g/cm$^{-3}$ in spring to 1.08 g/cm$^{-3}$ in summer. Lower BD in summer at Site II might indicate improved soil structure and porosity. BD is a crucial indicator of soil structure, with lower values due to increased soil moisture, temperature, and biological activity during this season, typically associated with improved soil structure and porosity. Lower bulk density indicates a higher volume of pore spaces, which benefits root growth, soil permeability, and overall soil health (Zhou *et al*., 2018). Cation exchange capacity, indicative of the soil's ability to hold positively charged ions, increased from spring to summer. At Site I (Lumami), CEC increased from 18.24 meq 100g$^{-1}$in spring to 20.35 meq 100g$^{-1}$in summer. Site II (Sumi Settsu) rose from 19.50 meq 100g$^{-1}$in spring to 22.91 meq 100g$^{-1}$in summer. Higher CEC values in summer suggest improved nutrient retention capacity. The soil texture components showed slight seasonal variations: Sand decreased slightly at both sites from spring to summer. At site I (Lumami) decreased from 42.00% to 40.50%, and Site II (Sumi Settsu) decreased from 36.67% to 34.00%. At Site I (Lumami), silt content decreased from 26.00% in spring to 24.50% in summer. However, at Site II (Sumi Settsu), it increased from 29.00% to 29.67%. The clay content decreased at Site I (Lumami) from 32.30% in spring to 24.20% in summer. At Site II (Sumi Setsu), the clay content was relatively stable, with a minor decrease from 33.67% to 32.33%. These observations highlight the seasonal changes in

soil properties, reflecting the dynamic nature of soil chemistry and structure in response to environmental factors. The season significantly influences soil prokaryote and fungal diversity and composition, with soil *p*H and organic carbon being key drivers for prokaryotes and soil *p*H and electrical conductivity for fungi (Han *et al*., 2021). Chen *et al*., (2021) also state that soil properties, microbial biomass, and extracellular enzyme activities vary significantly with sampling time in alpine ecosystems, affecting soil carbon dynamics. Monitoring soil properties is crucial for understanding and managing soil health and fertility, especially about seasonal changes. Such monitoring can help determine the availability and effectiveness of PSF, which are vital in enhancing soil fertility and promoting sustainable agricultural practices. The variation in soil properties across seasons and locations can significantly influence the presence and activity of PSF, making soil monitoring an essential practice for optimizing agricultural productivity and sustainability.

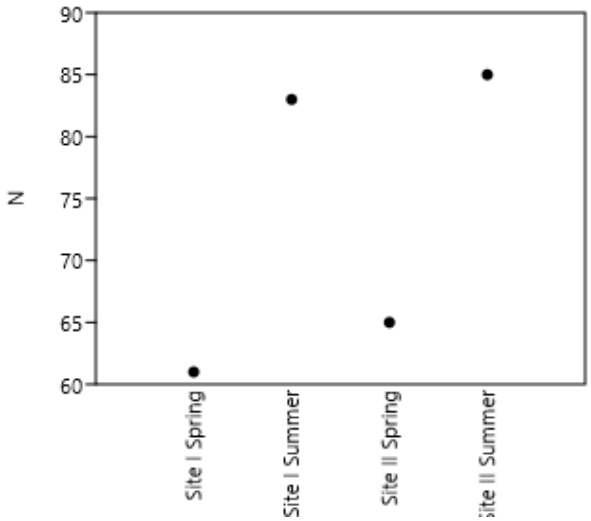



**Figure 2.3: No of Individuals**

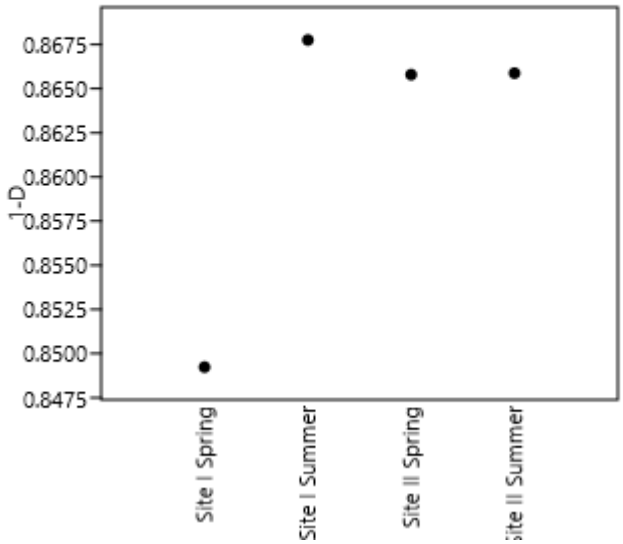



**Figure 2.4: Simpsons index of diversity (1-D) values of the study sites**

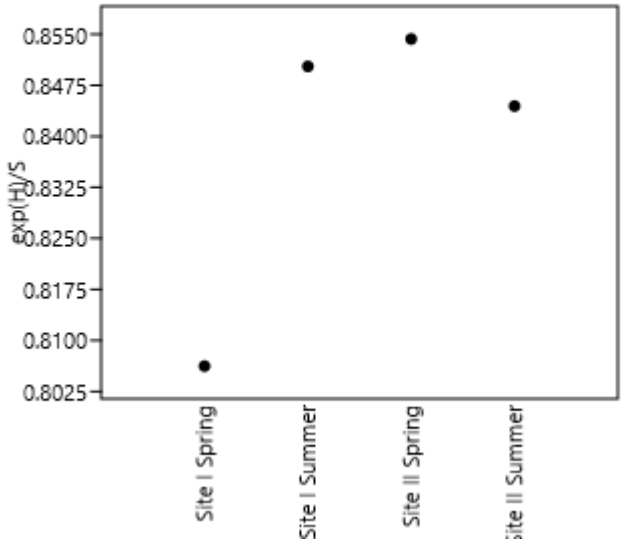



**Fig 2.5: Pielou's evenness (J) index values of the study sites**

**Isolation of the rhizospheric fungi:**

**Diversity indices**

The number of individuals increases from Spring to Summer in both sites. At Site I, the number of individuals goes from 61 in Spring to 83 in Summer. At Site II, it increases from 65 in Spring to 85 in Summer. This indicates a seasonal increase in the population size at both sites, likely due to favourable summer conditions for the species. Simpson's Diversity Index, which estimates the chances that two individuals randomly selected from a sample will belong to different species, shows relatively high diversity at both sites and in both seasons. The index is closer to 1, indicating high diversity. The values increase slightly from Spring to Summer at Site I (from 0.8492 to 0.8678) and remain almost constant at Site II (0.8658 to 0.8659). This suggests a slight increase in diversity in Site I during Summer and stable diversity at Site II. Evenness measures how evenly the individuals are distributed among the different taxa. Higher values indicate more even distribution. At Site I, evenness increases from Spring to Summer (0.8062 to 0.8503), indicating a more balanced distribution of individuals among species in Summer. At Site II, evenness is high in both seasons but slightly decreases from Spring to Summer (0.8543 to 0.8444), indicating a minor shift towards a less even distribution in Summer.

The observed increase in individual numbers from spring to summer at both sites underscores how seasonal conditions—particularly warmer temperatures, increased soil moisture, and improved fertility—can boost species abundance. Soil analyses from both sites confirmed marked improvements in fertility markers during summer, notably elevated nitrogen, phosphorus, and soil organic carbon levels, all conducive to greater growth and survival (Solanki *et al*., 2024; Wang *et al*., 2018). These nutrient gains are typical of summer due to enhanced biological activity and organic matter turnover.

Assessments using Simpson’s Diversity Index indicated persistently high diversity at both sites and seasons, with values near 1. At Site I, a modest rise in diversity during summer was aligned with increased species evenness, implying that more favorable summer conditions allowed species to be more equitably distributed. Conversely, at Site II, while diversity remained stable across seasons, evenness declined slightly in summer. This suggests a shift toward dominance by specific taxa, even as overall population sizes grew, highlighting that species-specific responses to environmental improvement can

alter community balance (Ji *et al*., 2022). Changes in soil properties help explain these community patterns. Both sites exhibited summer elevations in nitrogen, phosphorus, organic carbon, and moisture content, each fostering productivity. Increased cation exchange capacity (CEC) during summer further reflected the soil's enhanced capacity to supply essential nutrients, supporting higher biological activity and abundance (Sarkar *et al*., 2024). Notably, Site I experienced a summer shift in soil texture, with reduced clay and increased sand content—conditions that facilitate nutrient turnover and water infiltration, likely contributing to more balanced community structure. In contrast, the relatively stable texture at Site II appeared to encourage the dominance of certain taxa, leading to reduced evenness despite abundant resources. In summary, the findings suggest that seasonal changes in soil characteristics are primary drivers of community composition, affecting both abundance and species distribution. While both sites benefited from improved summer soil conditions, the distinct community evenness responses emphasize the moderating role of local soil features in shaping biodiversity outcomes.

**Table 2.3**: Lists of fungal species isolated from rhizospheric soil in lumami Site

| SL. NO | Fungal species | Spring | Summer | Autumn |
|---|---|---|---|---|
| 1 | *Aspergillus* sp. | - | + | + |
| 2 | *Aspergillus flavus* | + | + | + |
| 3 | *Aspergillus niger* | + | + | + |
| 4 | E*upenicillium* | + | + | + |
| 5 | *Penicillium* sp | + | + | + |
| 6 | *Penicillium spinulosum* | + | + | + |

| | | | | |
|---|---|---|---|---|
| 7 | *Paecilomyces* sp | + | + | + |
| 8 | *Mucor* sp | - | + | + |
| 9 | *Cladosporium* sp | - | + | + |
| 10 | *Trichoderma* sp | + | + | - |
| 11 | *Talaromyces purpureogens* | + | + | - |
| 12 | *Talaromyces amestolicae* | + | + | + |
| 13 | *Acremonium* sp. | + | + | + |
| 14 | Sterile hyphae (white) | + | + | + |

**Table 2.4:** Lists of fungal species isolated from rhizospheric soil in Sumi-settsu Site

| Sl no. | Fungal species | Spring | Summer | Autumn |
|---|---|---|---|---|
| 1 | *Aerophialophora levis* | - | + | - |
| 2 | *Aspergillus* sp. | + | + | + |
| 3 | *Aspergillus flavus* | - | - | + |
| 4 | *Eupenicillium* | + | + | + |
| 5 | *Penicillium* sp | + | + | + |
| 6 | *Penicillium breviladianum* | + | + | + |

| | | | | |
|---|---|---|---|---|
| 7 | *Penicillium sclerotiorum* | - | + | + |
| 8 | *Trichoderma* sp | - | + | + |
| 9 | *Trichoderma harzianum* | + | + | + |
| 10 | *Microsporium* sp | + | - | + |
| 11 | P*aecilomyces* sp | + | + | - |
| 12 | Sterile hyphae (white) | + | + | + |
| 13 | Sterile hyphae (brown) | + | + | + |

The tables above show the seasonal distribution of fungal species isolated from rhizospheric soil at two different sites. The Lumami site yielded 14 different fungal species, while the Sumi-settsu site yielded 13 species. The presence (+) or absence (-) of each species is indicated for spring, summer, and autumn seasons.

Influence on Fungal Abundance and Diversity: The summer improvements in nutrient availability and moisture coincided with a surge in fungal population size at both sites. The total number of fungal isolates (colonies) increased from spring to summer by a substantial margin which reflects how favorable summer conditions are warmer temperatures, ample moisture and higher soil fertility which boost fungal growth and reproduction. With more organic matter decomposing and roots exuding nutrients in summer, fungi had more resources to exploit, leading to greater abundance. Prior research supports this link: soil biota flourish with increased temperature and moisture up to optimal levels, and indeed the summer in Nagaland provides exactly that pulse of biological activity.

Species Presence and Seasonal Patterns: The composition of fungal species also shifted with seasons, though many taxa persisted throughout. In spring, fewer species were active – e.g. at Sumi Settsu only 9 species were detected in spring, compared to 11 by

summer and autumn. Certain fungi were season specific or showed marked seasonal preference. For instance, *Aerophialophora levis* was not found in spring or autumn at Sumi Settsu but appeared in summer, suggesting it might be a heat tolerant or moisture-requiring fungus that proliferates only under peak monsoon conditions. Conversely, *Aspergillus flavus* was absent in Sumi Settsu's spring and summer but present in autumn, implying perhaps it sporulates after the peak rainy season or could be in response to the harvesting period/disturbance. At the Lumami site, where maize and chili were grown, some genera like *Mucor* and *Cladosporium* were not seen in spring but emerged by summer and autumn. Meanwhile, *Trichoderma* and *Talaromyces purpureogenus* were found in spring and summer at Lumami but seemingly disappeared by autumn, perhaps outcompeted or having completed their life cycle earlier. These patterns highlight that the fungal community is dynamic across the growing season: some species sporulate or become detectable only under certain environmental conditions (e.g. high moisture or after crop growth), while others are perennial residents.

Site Differences: Despite both being jhum field soils, Lumami and Sumi Settsu showed some differences in fungal community structure. Lumami (Site I) overall yielded one more species than Sumi (14 vs 13), and a few genera were unique to each site. For example, *Cladosporium* and *Mucor* were isolated in Lumami but not in Sumi Settsu's samples, whereas *Aerophialophora* and a *Microsporium* sp. were found at Sumi Settsu but not at Lumami. These differences could be due to microclimatic and edaphic variations between the sites – Lumami's slightly sandier soil can create distinct niches. Lumami's better drainage might support fungi that prefer aerated conditions (e.g. *Mucor* thrives in fast-draining soils with high organic matter like decaying litter). Moreover, the slight differences in bulk density and texture changes discussed earlier may have led Lumami's summer community to be more evenly balanced, whereas Sumi Settsu's conditions allowed a few species to become more dominant by summer. In essence, seasonal improvements in soil fertility benefited both sites' fungal communities, but local soil features modulated the outcome. As noted, both sites saw increased fungal abundance in summer, yet community evenness diverged: Lumami became more even (no single fungus dominating) while Sumi Settsu became a bit less even as certain taxa took over. This underscores that site-specific factors (soil texture, crop differences, initial community makeup) can moderate how a fungal community responds to seasonal change. Overall, the results paint a picture of a resilient and diverse fungal community in

jhum soils, one that expands with resource availability but still reflects the imprint of its local environment. In summary, the findings suggest that seasonal changes in soil characteristics are primary drivers of community composition, strongly affecting both abundance and species distribution. While both sites benefited from improved summer soil conditions, the distinct community evenness responses emphasize the moderating role of local soil features in shaping biodiversity outcomes and sustaining productivity in jhum fields.

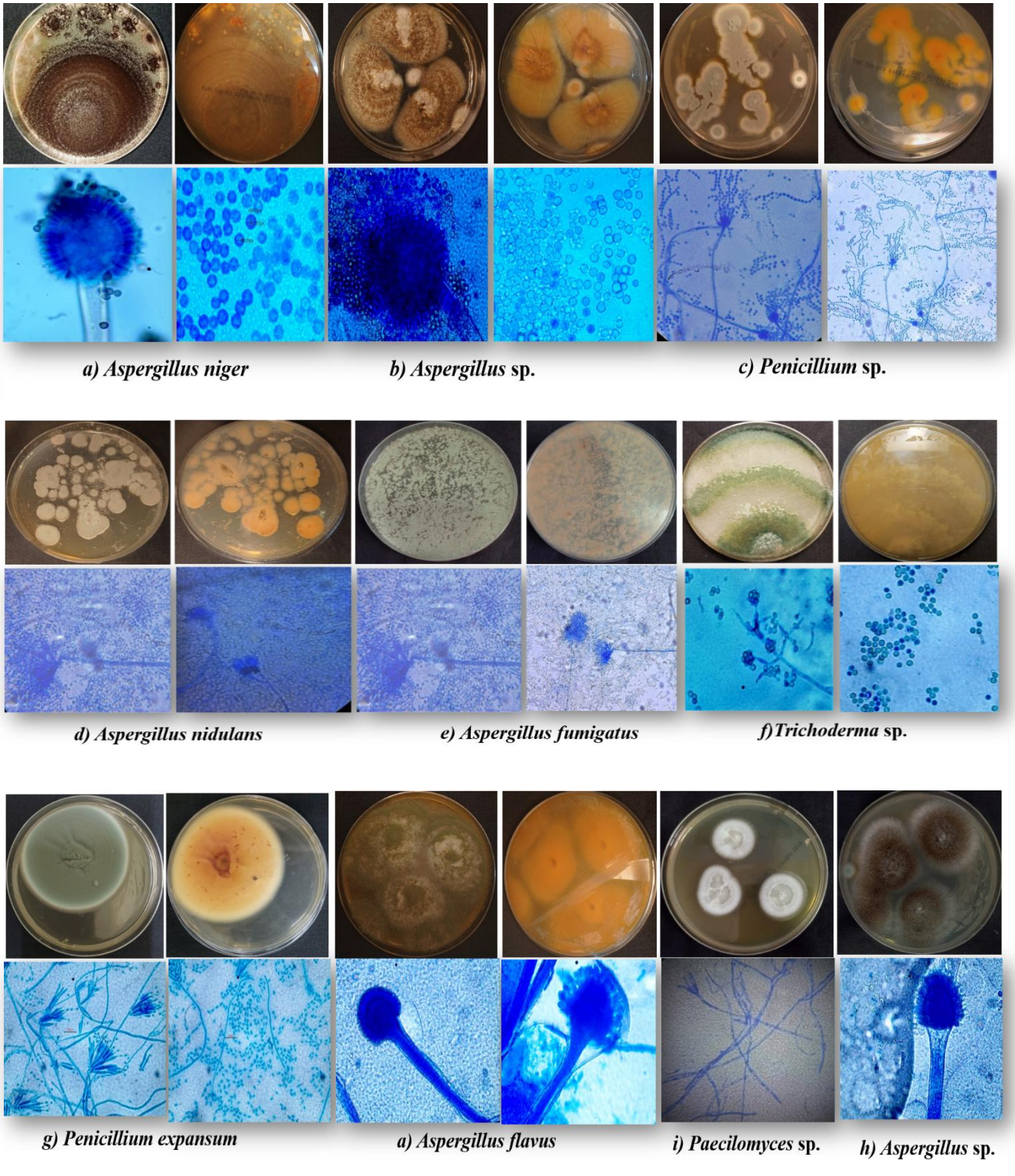



**Figure 2.7: Fungal culture plates showing pure culture on PDA stained with lactophenol cotton blue drop. Microscopic images under different magnifications (40X). Scale bar: 10 µm.**

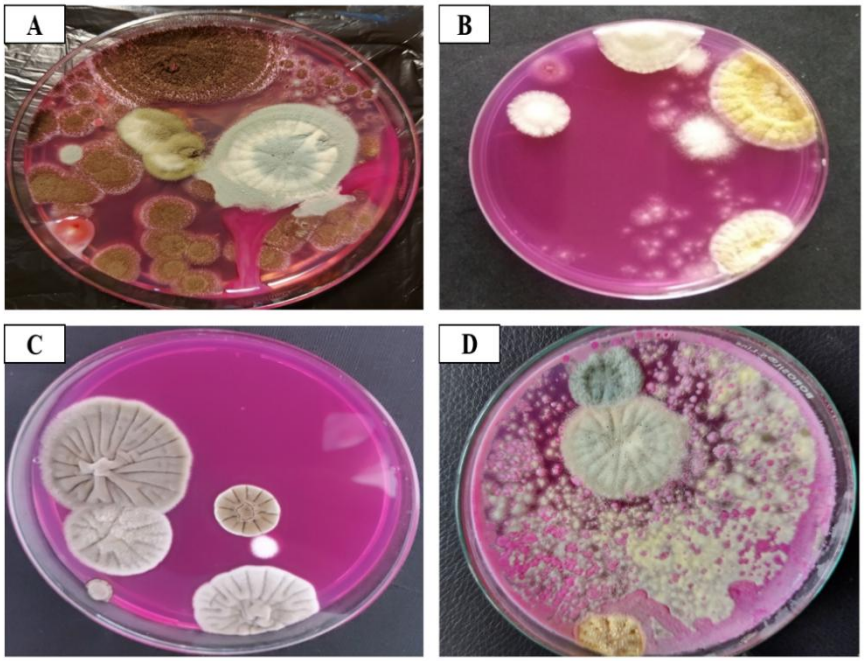



**Figure 2.5: Pure fungal isolates on rose bengal agar medium**

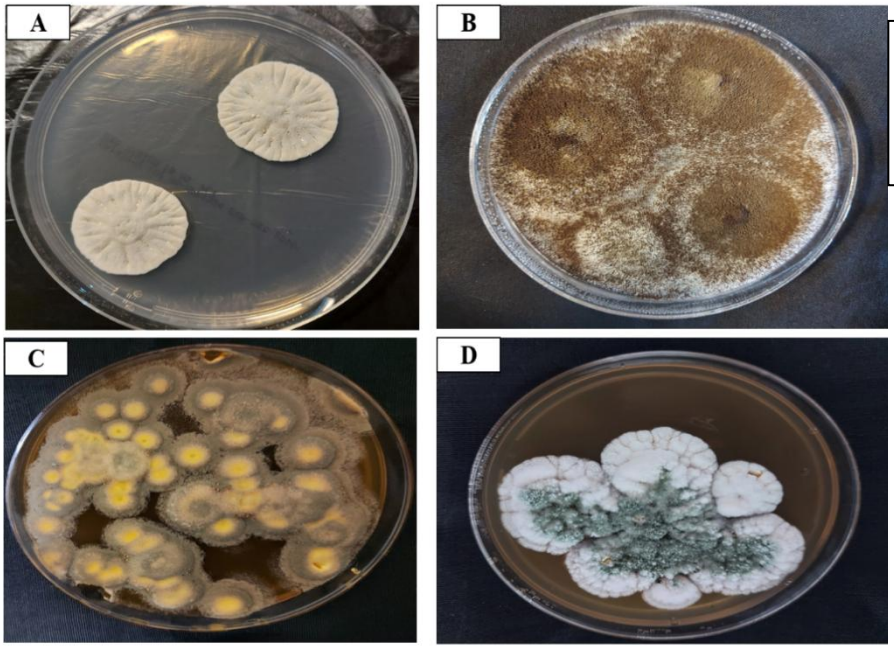



**Figure 2.6: Pure fungal isolates on potato dextrose agar medium**

# Chapter – 3

# Screening for PSF and Molecular Characterization of the Selected Strains.

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**Introduction**

PSF enhance P uptake efficiency by dissolving it via organic acid production and enzymatic action. P deficiency remains a major limiting factor in agricultural productivity worldwide, primarily due to the low solubility and non availability of soluble phosphate forms. PSF provide an eco-friendly approach to improving soil fertility by breaking down insoluble phosphate compounds. Through the release of organic acids and specific enzymes, they convert these compounds into forms that plants can readily absorb. These fungi thus play a pivotal role in enhancing nutrient availability and improving soil fertility. These fungi not only contribute to phosphorus solubilization but also produce phytohormones like indole-3-acetic acid (IAA), which promote root elongation and plant vigour. Fungal plant-growth-promoting traits such as auxin (indole-3-acetic acid, IAA) production and phosphate solubilization are critical for enhancing nutrient uptake and plant growth. Many endophytic and rhizosphere fungi synthesize IAA, which promote plant root development and growth. For example, Numponsak *et al*., (2018) reported that an endophytic *Colletotrichum* sp. produced over 1200 µg/mL IAA when cultured long-term with high tryptophan levels. Similarly, several *Penicillium* and *Aspergillus* species are known to produce IAA under tryptophan supplementation. In parallel, many soil-borne fungi can also solubilize insoluble phosphates, making phosphorus available to plants. PSF are typically screened on Pikovskaya's (PVK) medium compirisng of chemicals such as tri calcium phosphate. Clear zones or elevated soluble phosphate in this medium indicate solubilization (often quantified as a solubilization index, PSI).

The accurate identification and molecular characterization of PSF are crucial to understanding species diversity, functionality, and their application potential

as biofertilizers. Although morphological and biochemical methods provide preliminary identification, molecular tools, especially targeting the internal transcribed spacer (ITS) region of the ribosomal DNA, allow precise and reliable fungal species delineation. For comprehensive characterization of PSF, molecular tools complement classical morphological and biochemical screening techniques. The highly conserved internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) is employed for fungal identification due to its discriminatory power at the species level. Molecular characterization using ITS sequencing allows precise phylogenetic placement and understanding of fungal diversity, crucial for selecting promising bioinoculants.

This chapter documents the isolation, identification, and molecular characterization of PSF from rhizospheric soils, emphasizing their phosphate solubilization and IAA production capabilities. Morphological examinations under microscope were supplemented by ITS-rDNA amplification, sequencing, and phylogenetic analysis for reliable species identification. In this chapter, we screen rhizospheric fungi for their abilities to produce IAA (in potato dextrose broth with 0.5 mM tryptophan) and solubilize phosphate in PVK medium. Also focuses on the isolation, phenotypic screening, and molecular characterization of diverse PSF from rhizospheric soils, emphasizing their phosphate solubilization potential.

**Material and methods:**

**3.1 Quantitative estimation phosphate solubilization**

Pure fungal cultures were cultured and maintained on potato dextrose agar (PDA) at 28°C. For each assay, 5 mm mycelial plugs from 7-day-old PDA plates were transferred to liquid media. Three replicates of each fungus and an uninoculated control were used. Fungal strains were isolated and cultured on Pikovskaya's medium (10gm glucose, 0.5gm ammonium sulfate, 0.2gm sodium chloride, 0.1gm magnesium sulfate, 0.2 gm potassium chloride, 0.5gm of yeast extract, 0.002 gm manganese sulfate, 0.002 gm ferrous sulfate and 5.0 gm tri calcium phosphate). For SI measurement, each fungal strain was spot-inoculated onto PVK agar and incubated at 28°C. Halo (clearing) diameters were measured on days 3, 5, and 7. The phosphate solubilization index (SI) was calculated as (colony diameter + halo diameter) / colony diameter. Additionally, for broth assays, 5 mL of spore suspension ($10^7$ spores/mL) was inoculated into 100 mL PVK broth (0.5% TCP, pH 7.0). Cultures were shaken (130 rpm) at 28°C. On days 3, 5,

and 7, supernatant samples (centrifuged to remove solids) were analyzed for soluble phosphate using the molybdenum blue method. The uninoculated PVK medium served as a negative control. All treatments were in triplicate.

### 3.2 Qualitative estimation of phosphate solubilization

Fungal strains showing halo zone in PVK agar were isolated and cultured on Potato Dextrose Agar (PDA) for 7 days. Spore suspensions were prepared and adjusted using a hemocytometer. Phosphate solubilization was evaluated in PVK's broth containing tri calcium phosphate (TCP) as an insoluble P source. Solubilization index (PSI) was estimated based on the diameter of halo zones formed around fungal colonies on solid media. The phosphate solubilization index (PSI) was recorded by measuring the diameter of the clearing zone/halo diameter(Premono *et al*., 1996).

$$\textbf{PSI} = \frac{\textbf{Colony diameter + Halozone diameter}}{\textbf{Colony diameter}}$$

### 3.3 Quantitative estimation IAA production

For IAA production, the isolates were grown in PDA broth supplemented with 0.5 mM L-tryptophan, following the protocol of Numponsak *et al*., (2018). Quantification of IAA was done using Salkowski's reagent spectrophotometrically at 530 nm. Each plug was inoculated into 20 mL potato dextrose broth (PDB) supplemented with 0.5 mM L-tryptophan (Hi media). Cultures were incubated at 28°C, shaking at 120 rpm, in the dark. To assess IAA production, both fungi were cultured in PDA broth supplemented with 0.1% (w/v) L-tryptophan and incubated at 28 ± 2°C on a rotary shaker for 7 days. After incubation, the cultures were centrifuged at 10,000 rpm for 10 minutes to collect the supernatant.

One milliliter of the supernatant was mixed with 2 ml of freshly prepared Salkowski reagent (1 ml of 0.5 M $FeCl_3$ in 35% perchloric acid). The mixtures were incubated at room temperature for 25 minutes. A change in color to pink or reddish-pink indicated the presence of IAA. The optical density (OD) was measured at 530 nm using a spectrophotometer, and IAA concentrations were estimated by comparing the absorbance

values against a standard curve prepared using known concentrations of IAA. Values (mean ± SD) were calculated from triplicate flasks for each strain and control.

**Genomic DNA Extraction**

Pure fungal isolates were cultured in Potato Dextrose Broth at 28°C for 5 days with shaking (120 rpm). Mycelium was harvested and ground in liquid nitrogen. Genomic DNA was extracted using a CTAB-based method with subsequent chloroform:isoamyl alcohol extraction. DNA was precipitated by cold 70% ethanol, air-dried, and resuspended in TE buffer. Quality and quantity were verified by 1% agarose gel electrophoresis and spectrophotometry.

Morphological Characterization

Microscopic features of fungal isolates were observed by preparing temporary slides mounted with lacto-phenol cotton blue stain. Observations of spore shape, size, and hyphal structures were made with a compound microscope (Motic Model BA210LED). Identification was conducted based on taxonomic keys and standard literature (Gillman, 1957; Watanabe, 2002; Webster and Weber, 2007; Afzal *et al*., 2013).

**3.4 Molecular and phylogenetic analysis.**

For DNA extraction, the mycelia of actively growing fungi, grown in PDA broth for five days at 25± 2°C were harvested and ground under liquid nitrogen. Total genomic DNA was extracted via a CTAB method adapted from Cenis (1992) with the following key steps:

Cell lysis with extraction buffer containing 0.2M Tris-HCl, 0.5M HCl, 0.01M EDTA, 1% SDS, and 1M sodium acetate.

Phase separation using chloroform:isoamyl alcohol (24:1, v/v).

DNA precipitation with chilled 70% ethanol, washing, and air drying.

DNA dissolution in TE buffer with RNase treatment to remove RNA contamination.

DNA quality and quantity were assessed by agarose gel electrophoresis (1% agarose, ethidium bromide stained) and spectrophotometrically.

PCR Amplification of ITS Region

ITS DNA regions of fungal isolates were amplified using universal primers of ITS1 (5'-ICCGTAGGTGAACCTGCGG- 3) and ITS (5'-ICCTCCCTTATTGATATGC-3). A 96-well thermal cycler (Bio Rad T100Thermal Cycler) was used for PCR. The reaction

volume was 25 uL and contained 10x buffer (2.5ML), 25 mM MgC12 (2 L), 2 mM dNTP (2.2 uL), 10 uM primers (1.5 uL each), Tag polymerase DNA (0.375 L), and template DNA (2.5 L). The PCR process was performed with 35 cycles with denaturation temperature of 94 •C for 5 min, denaturation 94 •C for 30sec, annealing at 50 •C for 1 min, extension at 72 •C for 1 min, and final extension at 72 •C for 10 min. PCR amplified products were analyzed by electrophoresis on 1.5% agarose gel. A 4 uL of the PCR amplified sample was loaded on the gel by diluting it in 1 uL 6X DNA loading dye. A 1 kb ladder was also loaded on gel with samples to estimate the size of the PCR-amplified product. Electrophoresis of gel was performed for 30 min at 100 volts, and ethidium bromide-stained gel was viewed on a UV illuminator (Biostep UV Transilluminator UST-20M-8E).

Molecular Identification and Phylogenetic Analysis

The ITS-rDNA sequences obtained from PSF isolates were analyzed by BLAST (Basic local alignment search tool) and compared with similar sequences available in the nucleotide database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Next, the phylogenetic tree was constructed in Mega 11 using the neighbor-joining method with 1000 bootstrap replications.

**Results and discussion:**

The study successfully isolated and molecularly identified diverse phosphate-solubilizing fungi from rhizospheric soils, predominantly belonging to *Aspergillus* and *Penicillium*. The molecular characterization by ITS sequencing afforded precise species-level identification, overcoming the limitations inherent in morphological methods alone.

The superior phosphate solubilizing efficacy of *Aspergillus niger* aligns with global literature documenting its organic acid mediated phosphate mobilization. The sustained high solubilization index over extended incubation periods highlights its potential applicability in agricultural biofertilizer formulations aimed at enhancing phosphorus nutrition. Phylogenetic analysis provided evolutionary insights, confirming the taxonomic positions and relatedness of isolates within established fungal clades. This supports their ecological classification and helps predict functional traits relevant to plant growth promotion. The integration of molecular tools such as ITS sequencing with traditional microbiological techniques underscores a comprehensive approach essential for identifying potent PSF strains. These results lay the groundwork for future applied

studies, including greenhouse and field experiments, to evaluate the effectiveness and compatibility of these fungi within sustainable crop production systems. This research highlights the genetic diversity and phosphate-solubilizing capacity of rhizospheric fungi isolated from agricultural soils.

Molecular characterization via ITS-rDNA sequencing affirming the identity of key fungal strains predominantly in the genera *Aspergillus* and *Penicillium.* Among isolates, *Aspergillus niger* demonstrated the greatest phosphate solubilization potential, suggesting its candidacy as an effective biofertilizer agent. Comprehensive molecular identification and phylogenetic analyses provide a solid foundation for the selection of elite PSF strains, reinforcing their promising role in enhancing plant phosphorus nutrition sustainably.

**Discussion**

This study set out to investigate the growth-promoting potential of various rhizospheric fungi, emphasizing their capacity to solubilize phosphate and synthesize indole-3-acetic acid (IAA), an essential auxin that supports root development. The significance of this work lies in addressing the pressing demand for sustainable agricultural approaches that boost nutrient availability and strengthen plant health while reducing dependence on synthetic fertilizers. All tested fungal isolates demonstrated the capacity to solubilize inorganic phosphate, although the degree of effectiveness varied among species and strains. This was quantified using the Solubilization Index (SI), assessed on Pikovskaya's medium. Notably, *Aspergillus niger* exhibited the highest SI values throughout the incubation period.

The screening and molecular characterization of PSF from Jhum fields revealed a rich diversity of fungal genera, as summarized in Table 3.1. The isolates primarily belonged to *Aspergillus*, *Penicillium, Trichoderma*, and *Talaromyces*, all known to contribute to phosphorus solubilization and soil fertility enhancement.

Quantitative phosphate solubilization data (Table 3.2) indicate significant variability in solubilization efficiency among the isolates over time. Notably, *Aspergillus niger* demonstrated the highest phosphate solubilization index consistently on days 3, 5, and 7 (3.20±0.25 to 3.54±0.22), indicating robust potential for improving phosphorus availability in soils. Other species like *Penicillium sclerotiorum* and *Talaromyces*

spp. also exhibited moderate solubilizing activity, reinforcing their biofertilizer candidacy.

Table 3.3, highlights the dual functionality of some isolates in both phosphate solubilization and Indole Acetic Acid (IAA) production. For instance, *A. niger* and *P. spinulosum* displayed positive results for both traits, suggesting their potential as multifaceted plant growth-promoting fungi.

The molecular data reinforced the ecological and functional significance of the isolates. The phylogenetic clustering supported this by showing well-defined evolutionary relationships among the isolates, corresponding to their respective genera and species. Collectively, these findings underscore the considerable genetic diversity and multifunctional traits of PSF from Jhum soils, making them promising candidates for biofertilizer development aimed at sustainable agriculture. The integration of morphological, biochemical, and molecular approaches, as shown in the data tables, provides a comprehensive basis for selecting efficient strains for future application.

The co-occurrence of phosphate solubilization and phytohormone (IAA) synthesis by these fungal isolates points to strong synergistic effects on plant health and productivity. Incorporating such biofertilizers into soil management schemes offers a sustainable alternative to excessive chemical fertilizer use, with clear benefits for crop yield, soil fertility, and environmental conservation. In particular, *A. niger* emerges as a standout candidate due to its consistently high SI and IAA production, suggesting potential as a keystone biofertilizer in cropping systems suffering from poor phosphorus bioavailability. Moreover, the observed differences in performance among fungal taxa suggest that using a consortium of such beneficial strains may further enhance plant-microbe interactions and nutrient uptake synergistically. Tailoring biofertilizer formulations to leverage the unique strengths of diverse fungal species could broaden their field efficacy across varying agro-ecological condition

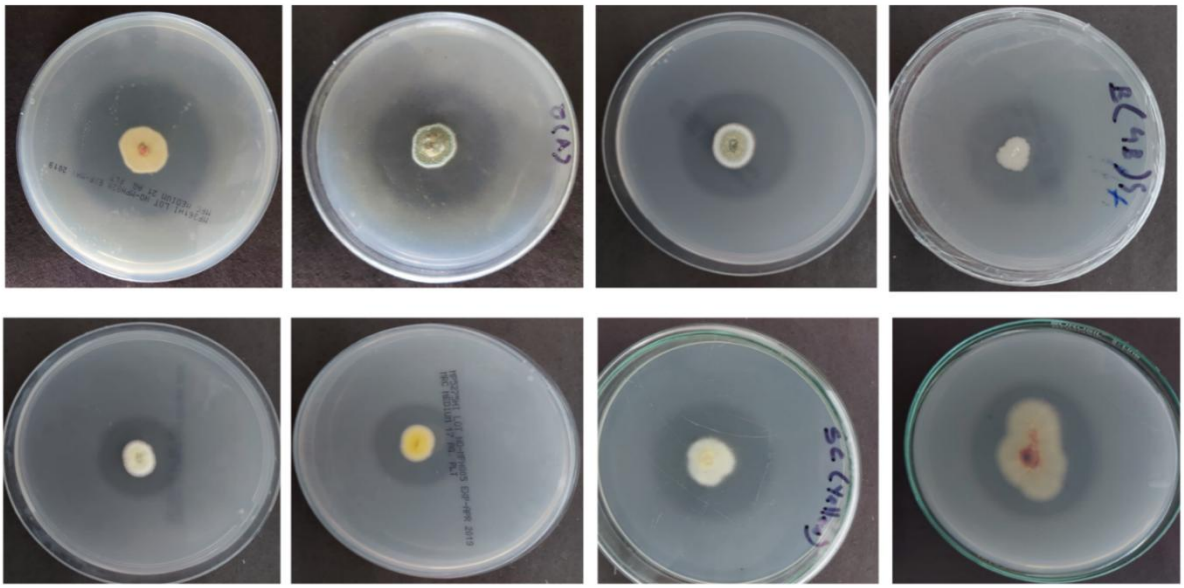

**Figure 3.1: Phosphate solubilization by the fungal isolates in PVK agar indicated by halo zone formation**

**Table 3.1: PSF Screened from Jhum fields**

| Sl. No | Genus | Species |
|---|---|---|
| 1 | *Aspergillus* | *Aspergillus* sp 1,<br>*Aspergillus* sp 2,<br>*Aspergillus flavus,*<br>*Aspergillus niger* |
| 2 | *Penicillium* | *P. sclerotiorum,*<br>*P. janthinellum* |
| 3 | *Trichoderma* | *Trichoderma harzianum* |
| 4 | *Talaromyces* | *Talaromyces* |

**Table 3.2 Quantitative estimation of phosphate solubilization by PSF isolates.**

| Sl. No | Species | 3rd day (mean±SD) | 5th day (mean±SD) | 7th day (mean±SD) |
|---|---|---|---|---|
| 1 | *Aspergillus sp 1* | 1.06±0.12 | 1.42±0.02 | 1.84±0.23 |
| | *Aspergillus sp 2* | 1.02±0.23 | 1.42±0.13 | 1.53±0.22 |
| | *Aspergillus flavus* | 1.05±0.53 | 2.65±0.02 | 2.71±0.31 |
| | *Aspergillus niger* | 3.20±0.25 | 3.50±0.24 | 3.54±0.22 |
| 2 | *P. sclerotiorum* | 2.12±0.05 | 2.64±0.22 | 3.10±0.09 |
| | *P. janthinellum* | 1.14±0.14 | 2.58±0.25 | 2.94±0.18 |
| 3 | *Trichoderma harzianum* | 1.02±0.23 | 1.42±0.13 | 1.53±0.22 |
| 4 | *Talaromyces* | 1.81±0.13 | 2.22±0.36 | 2.52±0.17 |

**Note:** *Values are mean ± SD of three replicates for each incubation day*.

**Table 3.3. Isolates showing PSF on PVK medium and on IAA production test**

| SL. NO | Fungal species | PSF | IAA |
|---|---|---|---|
| 1 | *Aspergillus niger* | + | + |

| | | | |
|---|---|---|---|
| 2 | *Aspergillus flavus* | + | ± |
| 3 | *Aerophialophora levis* | + | _ |
| 4 | *Penicillium sp* | + | _ |
| 5 | *Penicillium spinulosum* | + | + |
| 6 | *Penicillium brefeldianum* | + | _ |
| 7 | *Penicillium sclerotiorum* | + | _ |
| 8 | *Paecilomyces* sp | ± | _ |
| 9 | *Talaromyces amestolkiae* | _ | _ |
| 10 | *Talaromyces purpureogenus* | _ | _ |

**Note:** "+" indicates a positive result (activity observed); "±" indicates weak or variable activity; "–" indicates no activity observed.

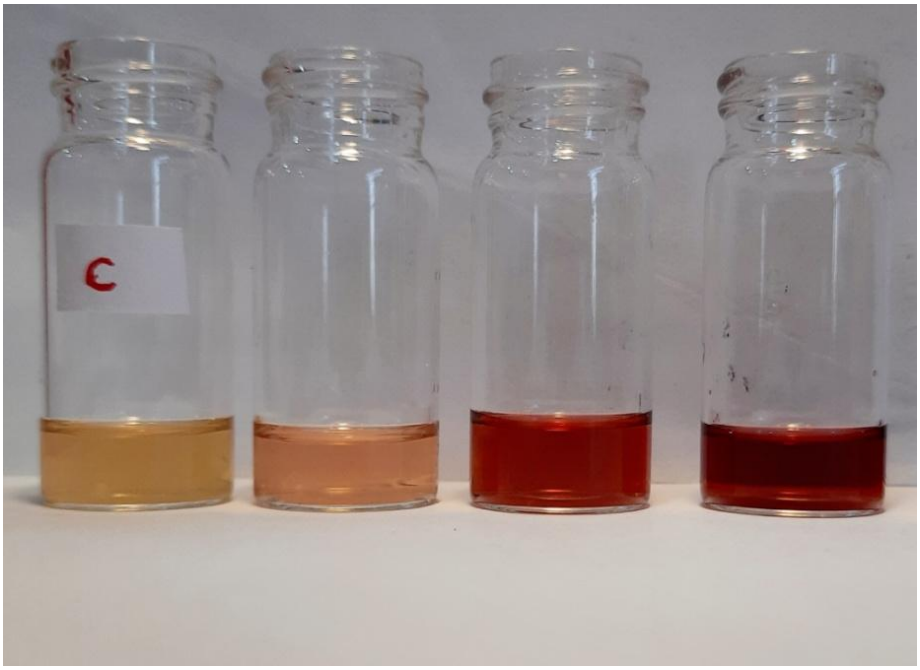


**Figure 3.2: IAA production test by the fungal isolates**

The main isolates belonged to genera *Aspergillus, Penicillium*, and *Talaromyces*, exhibiting high sequence similarity (≥97%) with reference strains. The GenBank accession numbers for these isolates were obtained after sequence submission.

The isolates clustered predominantly within three genera: *Aspergillus, Penicillium*, and *Talaromyces*. Specifically:Isolates identified as *Aspergillus niger* showed 97.20% to 100% sequence identity with reference strains (GenBank accession numbers PP733973 and PP733983).

Several *Penicillium* isolates clustered within species such as *Penicillium spinulosum, Penicillium brefeldianum, and Penicillium sclerotiorum*, with sequence identities ranging from 99.19% to 100% (accessions PP734277, PP734310, and PP734419 respectively). *Talaromyces purpureogenus* and *Talaromyces amestolkiae* isolates exhibited high similarity with known strains, sharing over 99% sequence identity.

**Table 3.4. Molecular Identification and GenBank Accession Details of Fungal Isolates Based on ITS-rDNA Sequencing**

| Sl. No | Fungal Isolate ID | Closest Species Identified | % Identity | GenBank Accession No. |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 1 | AN1 | *Aspergillus niger* | 99.7% | PP733983 |
| 2 | AN2 | *Aspergillus niger* | 97.20% | PP733973 |
| 3 | PN1 | *Penicillium spinulosum* | 100% | PP734277 |
| 4 | PN2 | *Penicillium* sp. | 99.19% | PP734288 |
| 5 | TP1 | *Talaromyces purpureogenus* | 99.81% | PP734297 |
| 6 | TP2 | *Talaromyces purpureogenus* | 99.47% | PP734299 |
| 7 | TA1 | *Talaromyces amestolkiae* | 100% | PP734305 |
| 8 | TA2 | *Talaromyces amestolkiae* | 99.62% | PP734307 |
| 9 | AL1 | *Aerophialophora levis* | 99.80% | PP734415 |
| 10 | AL2 | *Aerophialophora levis* | 99.79% | PP734417 |
| 11 | AF1 | *Aspergillus flavus* | 100% | PP734308 |
| 12 | AF2 | *Aspergillus flavus* | 99.60% | PP734309 |
| 13 | PB1 | *Penicillium brefeldianum* | 100% | PP734310 |
| 14 | PS1 | *Paecilomyces* sp. | 100% | PP734417 |
| 15 | PSC1 | *Penicillium sclerotiorum* | 100% | PP734419 |

(AN = *Aspergillus niger* isolate, PN = *Penicillium notatum/spinulosum* isolate, TP = *Talaromyces purpureogenus* isolate, TA = *Talaromyces amestolkiae* isolate, AL = *Aerophialophora levis* isolate, AF = *Aspergillus flavus* isolate, PB = *Penicillium brefeldianum,* PS = *Paecilomyces* sp., PSC = *Penicillium sclerotiorum*)

**Phylogenetic Analysis**

Phylogenetic trees constructed using the neighbor-joining method in MEGA11 software reinforced the BLASTn-based taxonomic identifications. Isolates grouped into well-supported clusters corresponding to their respective genera and species with bootstrap values exceeding 90%. This reinforced the genetic distinctiveness and evolutionary relationships among isolates. The phylogenetic clustering underscored the genetic diversity of PSF isolated from diverse rhizospheric soils and supported their robust taxonomic placement within ecologically and functionally important fungal groups.

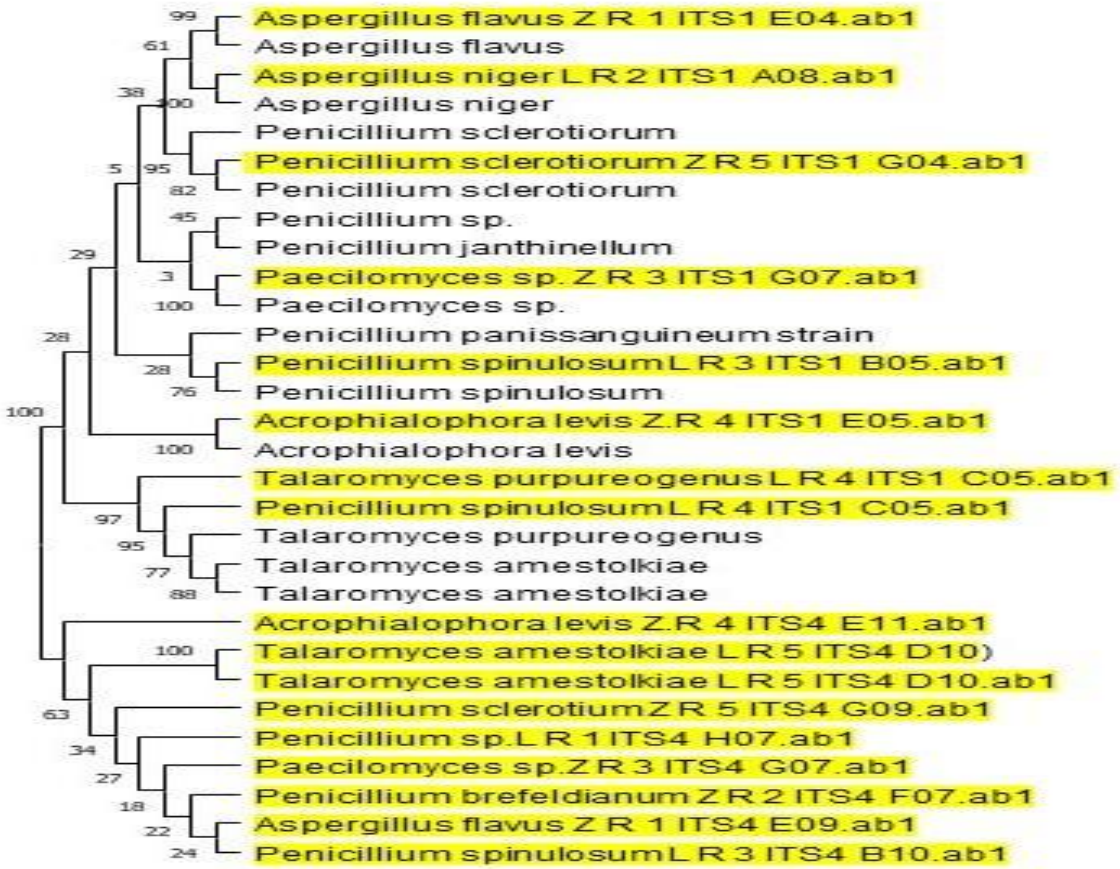


**Figure 4.3: Phylogenetic tree of fungal isolates based on Its rRNA gene sequence showing the evolutionary position of *Aspergillus flavus, A. niger, Penicillum sclerotiorum, Paecilomyces, P. spinulosum, Aerophialophora levis, Taloromyces amestolkiae, P. brefeldianum and P. spinulosum* with other related fungal species sequence, which were retrived from databases.**

# Chapter – 4

# Analysis of PSF Strains Efficacy in Pot Experiment.

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**Introduction**

Modern agricultural systems have achieved remarkable short-term increases in productivity through the use of synthetic inputs such as chemical fertilizers, herbicides, and pesticides. However, the excessive reliance on these agrochemicals has come at a significant cost, including soil degradation, disruption of native microbial communities, reduced soil fertility, environmental pollution, and, paradoxically, the decline of crop productivity over time (Tilman *et al*., 2002; Savci, 2012; Sharma *et al*., 2013). As the sustainability of intensive agriculture is increasingly called into question, there is a pressing need to develop alternative strategies that sustain yields while preserving ecosystem health.

One promising solution is the use of plant growth-promoting fungi (PGPF), a diverse group of beneficial fungi that establish close associations with plant roots, predominantly within the rhizosphere. These fungi are attracting interest not only for their ability to enhance nutrient acquisition but also for their roles in improving plant stress tolerance, suppressing soil-borne diseases, and contributing to overall soil health (Vassilev *et al*., 2006; Verma *et al*., 2017). Among the many traits exhibited by PGPF, phosphate solubilization stands out due to phosphorus's critical yet often limited availability in agricultural soils.

In most acidic or calcareous soils , a large proportion of total phosphorus exists in insoluble mineral forms such as iron phosphate, aluminum phosphate, or tri calcium phosphate (Rodríguez & Fraga, 1999; Sharma *et al*., 2013). This renders phosphorus inaccessible to plants, severely constraining crop growth and yield. PSF address this challenge by secreting organic acids and enzymes capable of mobilizing insoluble phosphorus into plant-available forms, thus directly enhancing phosphorus nutrition

(Vassilev *et al*., 2006; Khan *et al*., 2010). Additionally, many PSF strains confer further benefits by producing phytohormones such as IAA, generating siderophores that facilitate iron uptake, and exhibiting a degree of tolerance to various abiotic stresses including salinity and heavy metal toxicity (Egamberdieva *et al*., 2017).

Various genera of fungi such as *Aspergillus*, *Penicillium*, *Trichoderma*, and *Talaromyces* have been identified as efficient phosphate solubilizers and plant growth promoters. For example, inoculation of wheat and chickpea with *Aspergillus* sp. Studies have demonstrated that PSF can also ameliorate the adverse effects of salinity and heavy metal toxicity, further underlining their potential in sustainable agriculture (Zhu *et al*., 2011; Verma *et al*., 2017).

Given these diverse and important roles, this chapter focuses on the screening, characterization, and evaluation of various fungal isolates for their phosphate-solubilizing activity and their effects on the growth and development of three agriculturally significant crops: tomato (*Solanum lycopersicum* L), wheat (*Triticum aestivum* L), and chilli (*Capsicum annuum* L). Through systematic analysis, this work aims to expand the repertoire of effective PSF strains and lay the groundwork for their application in environmentally resilient and productive agricultural systems.

Given these capabilities, this chapter focuses on screening, characterizing, and evaluating selected fungal isolates for their P solubilizing abilities and their impact on the growth of three important crops: *Solanum lycopersicum* L, *Oryza sativa* L, and *Capsicum annuum* L..

**Materials and Methods:**

**Inoculation of Phosphate Solubilizing Fungi**

To explore the plant growth-promoting potential of PSF, two well-documented fungal species — *A. niger* and *Trichoderma* — were selected. These fungi were evaluated for their ability to enhance growth in three economically significant crops: *S. lycopersicum* L, *O.sativa* L, and *C. annuum* L. The study focused on traits of PSF: their P solubilization potential

**1. Seed Sterilization and Preparation for Inoculation**

Healthy seeds of tomato, rice, and Chilli were surface-sterilized using a 2% sodium hypochlorite solution for 2–3 minutes, followed by multiple washes with sterile distilled water to remove any residual chlorine. The sterilized seeds were then air-dried in a laminar flow hood and stored in sterile petri dishes until use.

**2. Preparation of Fungal Inoculum**

Fungal inoculants were prepared by inoculating mycelial plugs of selected fungal strains into 50 ml of Potato Dextrose Agar broth, followed by a 3-day incubation in a shaking incubator at 28±2ºC. Subsequently, each fungal isolate was introduced into individual pots containing 3 plantlets each of the selected plants. The pots were regularly watered with sterile water. A control treatment without fungal inoculation received only autoclaved tap water. Selected isolates were evaluated for their impact on the growth and development of the three crop plants. After one month, plants were harvested, and various growth parameters such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight were measured.

**3. Inoculation Procedure and Pot Experiments**

Pre-germinated seeds of each plant species were transferred to plastic pots filled with sterile soil-sand-coco peat mixture (2:1:1). Each pot received one of the following treatments:

- T1: Control (no fungal inoculum)
- T2: Inoculation with *Aspergillus niger*
- T3: Inoculation with *Trichoderma sp*

Approximately 10 ml of fungal spore suspension was applied directly to the rhizosphere region of each seedling during transplanting. The seedlings were sown 1cm depth. Each treatment was replicated three times, and the pots were kept under laboratory conditions. Plants were watered regularly, and no additional fertilizer was applied to avoid interference with phosphorus dynamics.

**4. Sporulation of Fungal Cultures for Pot Experiments**

To ensure consistent inoculum quality and viability, *A. niger* and *Trichoderma* sp were first sub-cultured on fresh Potato Dextrose Agar (PDA) plates. PDA is a nutrient-rich

medium that promotes rapid mycelial growth and abundant sporulation in filamentous fungi.

Plates were incubated at 28 ± 2°C for 5–7 days under dark conditions to encourage spore formation. Once a dense spore mat had developed, the spores were harvested by adding 1ml sterile water containing to the plate surface and gently scraping with a sterile loop. The resulting suspension was filtered through sterile muslin cloth to remove mycelial debris, and spore concentration was standardized to $1\times10^7$ spores/ml using a hemocytometer.

These sporulated cultures were then used as the inoculum source for all pot culture experiments, ensuring uniform application across all replicates and treatments.

**5. Statistical Analysis**

All descriptive statistics analysis was performed in SPSS (24). All the reported values are the means of three replicates and deviations which were entered as the standard error of the mean (SEM). When the *p* value was ≤0.05, differences were deemed significant.

**Results**

The present study evaluated the growth-promoting potential of two phosphate-solubilizing fungi (PSF), *A. niger* and *Trichoderma* sp, on three agriculturally important plant species: *S. lycopersicum*, *O.sativa*, and *C. annuum*. The experiments were designed to assess the impact of fungal inoculation on plant growth parameters such as root length, shoot length, shoot and root fresh weight, and dry weight. P solubilization ability of both fungi were evaluated using standard broth culture assays.


**Figure 4.1: Effect of fungal inoculation of *Capsicum annuum* L.**

**Figure 4.2: Effect of fungal inoculation on the growth and development of (*Solanum lycopersicum* L.**

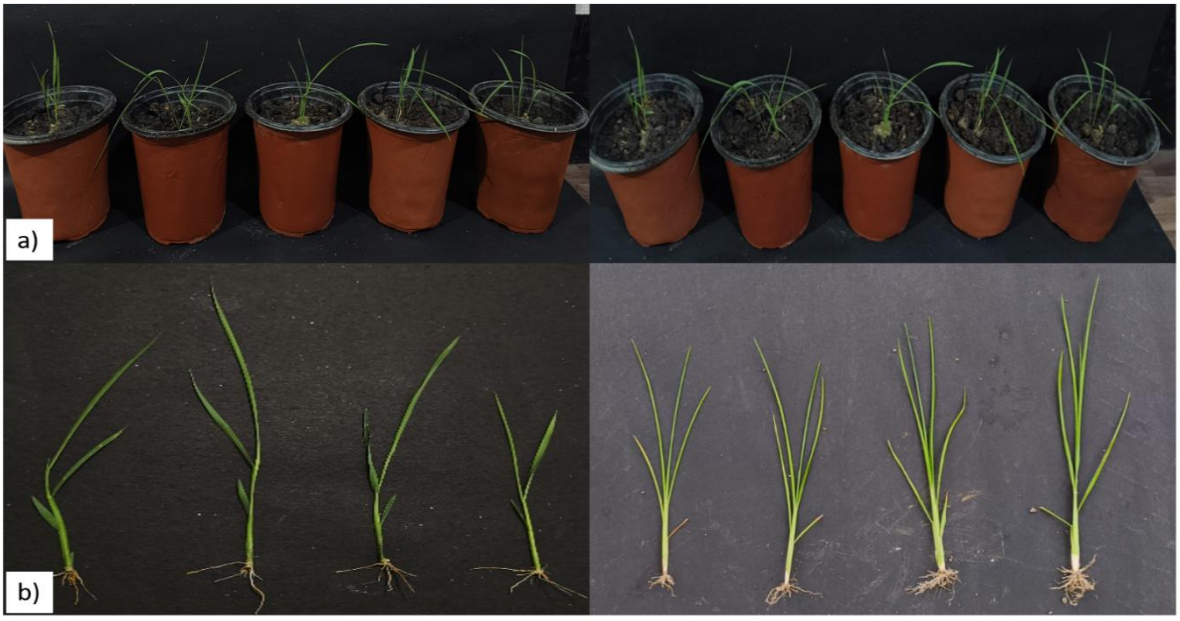


**Figure 4.3: Effect of fungal inoculation of the *O. sativa* L**

**Effect of PSF Inoculation on *S. lycopersicum* L**

Tomato plants treated with *A. niger* displayed significantly improved growth across all parameters compared to the uninoculated control. Root length increased from $5.21 \pm 0.32$ cm in the control to $7.63 \pm 0.41$ cm in the *A. niger* treatment. Similarly, shoot length increased from $11.12 \pm 0.45$ cm to $14.55 \pm 0.67$ cm. Fresh and dry biomass also increased, with shoot fresh weight reaching $1.04 \pm 0.10$ g and shoot dry weight $0.41 \pm 0.03$ g, compared to $0.62 \pm 0.08$ g and $0.19 \pm 0.02$ g in the control. Root biomass was also enhanced, with root fresh and dry weights measuring $0.22 \pm 0.02$ g and $0.07 \pm 0.01$ g, respectively.

In tomato plants inoculated with *Trichoderma* sp, improvements were also observed though slightly lower than those seen in the *A. niger* treatment. Root and shoot lengths were $6.78 \pm 0.37$ cm and $13.87 \pm 0.51$ cm, respectively. Shoot fresh weight reached

0.95 ± 0.07 g and dry weight 0.36 ± 0.02 g. Root fresh and dry weights were 0.20 ± 0.01 g and 0.06 ± 0.01 g, respectively.

**Effect of PSF Inoculation on *O. sativa***

In rice, both PSF strains positively influenced plant development. *A. niger* inoculation led to a root length of 6.92 ± 0.45 cm and shoot length of 25.71 ± 0.74 cm, a significant increase over the control values of 4.35 ± 0.29 cm and 19.88 ± 0.53 cm, respectively. Shoot and root biomass also showed marked increases: fresh shoot weight was 1.23 ± 0.11 g, and dry weight was 0.49 ± 0.04 g; root fresh and dry weights reached 0.20 ± 0.01 g and 0.06 ± 0.01 g.

Rice plants inoculated with *Trichoderma* demonstrated similar, albeit slightly lower, enhancements. Root length increased to 6.20 ± 0.38 cm and shoot length to 24.35 ± 0.69 cm. Shoot fresh and dry weights were 1.12 ± 0.10 g and 0.45 ± 0.03 g, while root biomass recorded values of 0.19 ± 0.01 g and 0.05 ± 0.01 g.

**Effect PSF Inoculation on *C. annum* L**

Among the three crops, chilli plants exhibited the lowest absolute growth parameters but still showed significant improvements upon fungal inoculation. In *A. niger*–treated plants, root and shoot lengths reached 6.04 ± 0.41 cm and 13.97 ± 0.52 cm, compared to 3.89 ± 0.30 cm and 10.22 ± 0.38 cm in the control. Shoot biomass was 0.81 ± 0.06 g (fresh) and 0.34 ± 0.02 g (dry), while root biomass measured 0.18 ± 0.01 g (fresh) and 0.05 ± 0.01 g (dry).

*Trichoderma* sp inoculated chilli plants also demonstrated improvements, with shoot length increasing to 13.41 ± 0.48 cm and root length to 5.57 ± 0.36 cm. Shoot and root biomass values were 0.72 ± 0.07 g and 0.31 ± 0.02 g (fresh and dry shoot), and 0.17 ± 0.01 g and 0.04 ± 0.01 g (fresh and dry root), respectively.

**Table 4.1: Effects of PSF inoculation on growth parameters of *S. lycopersicum* L.**

| Treatment | Root Length (cm) | Shoot Length | Shoot Fresh | Shoot Dry Weight (g) | Root Fresh Weight (g) | Root Dry Weight (g) |
|---|---|---|---|---|---|---|

| | ± SE | (cm) ± SE | Weight (g) ± SE | ± SE | ± SE | ± SE |
|---|---|---|---|---|---|---|
| Control | 5.21 ± 0.32 | 11.12 ± 0.45 | 0.62 ± 0.08 | 0.19 ± 0.02 | 0.14 ± 0.01 | 0.04 ± 0.01 |
| *A. niger* | 7.63 ± 0.41 | 14.55 ± 0.67 | 1.04 ± 0.10 | 0.41 ± 0.03 | 0.22 ± 0.02 | 0.07 ± 0.01 |
| *P. notatum* | 6.78 ± 0.37 | 13.87 ± 0.51 | 0.95 ± 0.07 | 0.36 ± 0.02 | 0.20 ± 0.01 | 0.06 ± 0.01 |

***Note: Values represent the mean of three replicates ± Standard Error (SE).***

**Table 4.2: Effects of PSF inoculation on growth parameters of *O. sativa* L.**

| Treatment | Root Length (cm) ± SE | Shoot Length (cm) ± SE | Shoot Fresh Weight (g) ± SE | Shoot Dry Weight (g) ± SE | Root Fresh Weight (g) ± SE | Root Dry Weight (g) ± SE |
|---|---|---|---|---|---|---|
| Control | 4.35 ± 0.29 | 19.88 ± 0.53 | 0.48 ± 0.05 | 0.18 ± 0.03 | 0.11 ± 0.01 | 0.03 ± 0.01 |
| *A. niger* | 6.92 ± 0.45 | 25.71 ± 0.74 | 1.23 ± 0.11 | 0.49 ± 0.04 | 0.20 ± 0.01 | 0.06 ± 0.01 |
| *P. notatum* | 6.20 ± 0.38 | 24.35 ± 0.69 | 1.12 ± 0.10 | 0.45 ± 0.03 | 0.19 ± 0.01 | 0.05 ± 0.01 |

***Note: Values represent the mean of three replicates ± Standard Error (SE).***

**Table 4.3: Effects of PSF inoculation on growth parameters of *C. annum* L.**

| Treatment | Root Length (cm) ± SE | Shoot Length (cm) ± SE | Shoot Fresh Weight (g) | Shoot Dry Weight (g) ± SE | Root Fresh Weight (g) ± SE | Root Dry Weight (g) ± SE |
|---|---|---|---|---|---|---|

| | | | ± SE | | | |
|---|---|---|---|---|---|---|
| Control | 3.89 ± 0.30 | 10.22 ± 0.38 | 0.38 ± 0.03 | 0.11 ± 0.01 | 0.09 ± 0.01 | 0.02 ± 0.01 |
| *A. niger* | 6.04 ± 0.41 | 13.97 ± 0.52 | 0.81 ± 0.06 | 0.34 ± 0.02 | 0.18 ± 0.01 | 0.05 ± 0.01 |
| *P. notatum* | 5.57 ± 0.36 | 13.41 ± 0.48 | 0.72 ± 0.07 | 0.31 ± 0.02 | 0.17 ± 0.01 | 0.04 ± 0.01 |

***Note: Values represent the mean of three replicates ± Standard Error (SE).***

## Discussion

The significant enhancements in plant growth observed following fungal inoculation provide compelling evidence for the effectiveness of *Aspergillus niger* and *Trichoderma* sp as potent plant growth-promoting fungi (PGPF). These fungi appear to contribute to plant development through multiple, synergistic mechanisms, principally phosphate solubilization and indole-3-acetic acid (IAA) production, as well as through their positive influence on rhizospheric nutrient dynamics and overall soil biological activity. Such multifunctional benefits mark *A. niger* and *P. notatum* as promising bio-inoculants in sustainable agriculture aimed at reducing chemical fertilizer usage.

Phosphorus (P) plays a quintessential role in plant physiology, acting as a key macronutrient involved in energy transfer via adenosine triphosphate (ATP), nucleic acid synthesis, membrane integrity, and signal transduction pathways (Marschner, 2012; Rodríguez & Fraga, 1999). However, despite its abundance in soil matrices, the majority of phosphorus exists as insoluble mineral complexes such as tricalcium phosphate, iron phosphate, or aluminum phosphate, depending on soil *p*H and composition. These mineral forms are largely unavailable to plants, limiting phosphorus nutrition and thus constraining growth and yield potential. The direct consequence of this phosphorus mobilization is reflected in improved root elongation and biomass accumulation observed across all inoculated plants. Particularly, rice exhibited a robust response, likely due to its inherent fibrous root system and increased phosphorus demand during critical

growth phases such as tillering and panicle initiation. This finding is supported by Marschner, (2012) on efficient phosphorus uptake during these stages is crucial for optimizing grain development and overall yield. The significant improvements in root length provide a morphological basis for enhanced nutrient and water absorption, setting the foundation for improved shoot growth and biomass accumulation.

The augmented root systems not only facilitate increased nutrient uptake including phosphorus but also improve water absorption, thereby enhancing plant physiological status and growth, especially under early seedling conditions where root vigour dictates subsequent developmental trajectories (Glick, 2012). Similarly, enhanced hormonal stimulation by fungal-derived IAA may also prime plants for better stress resilience, indirectly mitigating deleterious effects of environmental stressors such as salinity and temperature extremes.

The consistent outperformance of *A. niger* compared to *Trichoderma* sp across almost all growth parameters signifies possible differences in fungal metabolic activity, colonization efficiency, and compatibility with host plant rhizosphere environments. The higher organic acid production and IAA secretion by *A. niger* likely contribute to its stronger biofertilizer efficacy, as indicated in previous studies highlighting its aggressive colonization and phosphate solubilization traits as also reported Vassilev *et al*., (2006) and Khan *et al*., (2010).

Nevertheless, *Trichoderma* showed improved plant growth significantly compared to uninoculated controls (table 4.3**)** demonstrating its value as an alternative phosphate solubilizer. The variability in growth promotion magnitude among the three crops — tomato, rice, and chilli may be attributed to intrinsic differences in root system architecture, nutrient requirements, and growth rates. Tomato and rice, characterized by rapid growth and greater nutrient demand, exhibited more pronounced responses, while chilli displayed comparatively modest growth enhancements, possibly due to slower early-stage biomass accumulation and inherently less expansive root systems (Ahmad et al., 2016).

The data presented on the table highlight the significant impact of phosphate-solubilizing fungal inoculation on the growth of tomato, rice, and chilli plants. Across all six measured parameters-root length, shoot length, shoot and root fresh weight, and shoot and root dry weight plants (Table 4.1, 4.2 and 4.3) treated with both *Aspergillus niger*

and *Penicillium notatum* consistently outperformed the uninoculated controls. This indicates that both fungi effectively promoted plant growth, likely through mechanisms such as improved phosphate availability and phytohormone (IAA) production. Among the two fungi, *A. niger* showed a more pronounced effect, this is particularly evident in shoot dry weight and total biomass accumulation. For instance, tomato shoot dry weight increased from $0.19 \pm 0.02$ g in the control to $0.41 \pm 0.03$ g with *A. niger*, more than doubling the plant's aboveground tissue mass. Similarly, rice (table 4.3) treated with *A. niger* exhibited the highest shoot length ($25.71 \pm 0.74$ cm) and shoot fresh weight ($1.23 \pm 0.11$ g), highlighting the species' responsiveness to phosphorus enrichment. Chilli plants, though smaller overall, still it demonstrated strong improvements, particularly in root biomass, suggesting that even slow-growing crops benefit substantially from fungal inoculation. The consistent trends across different crops confirm that both fungal strains can enhance plant performance in a species-independent manner, with *A. niger* showing slightly superior efficacy. These findings support the role of phosphate-solubilizing fungi as effective bioinoculants for improving early plant growth, reducing dependence on chemical fertilizers, and enhancing sustainability in crop production systems.

The crop-specific differential responses also underscore the importance of selecting compatible fungal inoculants tailored to target host species for maximizing biofertilizer efficacy. Understanding the plant-fungal interactions at a physiological and molecular level can further optimize inoculum formulations and application methods.

**Conclusion**

The present research confirms the potential of *Aspergillus niger* and *Trichoderma* as effective biofertilizers, capable of significantly enhancing early growth parameters in tomato, rice, and chilli through mechanisms involving phosphate solubilization and IAA-mediated root development. The superior efficacy of *A. niger* suggests it may be preferable under many agronomic scenarios, although *Trichoderma* remains a valuable candidate for further exploration.

Future research should focus on validating these promising results under field conditions, examining the long-term impacts on crop yield and soil health, and exploring formulation techniques to enhance fungal viability and colonization efficiency. Additionally, elucidating the molecular basis of stress tolerance and colonization traits,

PSF will be instrumental in developing diverse agroecological zones and given the global concerns over the environmental impact and economic costs associated with synthetic phosphorus fertilizers, these findings advocate for integrating phosphate solubilizing fungi such as *A. niger* and *P. notatum* into biofertilizer strategies. Such bio-inoculants can reduce fertilizer dependence, improve phosphorus use efficiency, and simultaneously enhance crop productivity, especially in soils with insoluble P or limited fertility.

# Chapter - 5

# Summary and Conclusion

Phosphorus is a vital nutrient for plant growth, yet its availability in soil is often limited due to its presence in insoluble forms. While chemical fertilizers are commonly used to supplement phosphorus, much of it becomes fixed in the soil and remains inaccessible to plants. Phosphate-solubilizing fungi (PSF) offer a sustainable solution by converting insoluble phosphate into forms that plants can readily absorb. These fungi also contribute to plant health through the production of growth-promoting substances such as indole-3-acetic acid (IAA), making them valuable agents in sustainable agriculture as PSF are recognized for their dual role in nutrient cycling and plant growth promotion through secretion of organic acids, phosphatase enzymes, and phytohormones.

The use of chemical phosphorus fertilizers presents several challenges, particularly for smallholder farmers in developing regions due to high costs and limited accessibility. Improper dosages can disrupt soil nutrient balance and lead to environmental issues such as eutrophication from runoff. Additionally, prolonged exposure to these chemicals can pose health risks to farm workers and surrounding communities. Chemical inputs also negatively impact soil microbial diversity, reducing long-term fertility and sustainability. The use of chemical phosphorus fertilizers presents several challenges, particularly for smallholder farmers in developing regions due to high costs and limited accessibility. Improper dosages can disrupt soil nutrient balance and lead to environmental issues such as eutrophication from runoff. These concerns are further compounded in site-specific contexts like Zunheboto, Nagaland, where a lack of localized data on PSF underscores the urgent need for region-specific research to harness native microbial potential for sustainable agriculture.

This thesis explores the diversity, efficiency, and application potential of PSF isolated from the jhum fields of Nagaland, Northeast India. Jhum agriculture, while culturally significant, is known to deplete soil fertility through repeated slash-and-burn cycles. Harnessing indigenous fungal resources from these soils provides an opportunity to restore nutrient balance and improve crop productivity in a sustainable manner.

Soil samples were collected from crop rhizospheres in Lumami and Sumi Settsu villages during different seasons. The fungi were isolated and screened on PVK agar and broth media to assess their phosphate-solubilizing ability, as indicated by halo formation (Solubilization Index) and quantitative measurement of soluble P release. The isolates represented a diverse group of fungi, with *Aspergillus* and *Penicillium* as dominant genera, followed by *Trichoderma*, *Talaromyces,* and others. Seasonal variation in fungal occurrence was observed, with certain isolates appearing preferentially after the monsoon season.

Screening revealed that while all isolates demonstrated P solubilization capacity, *A niger* consistently showed superior performance, exhibiting the largest halo diameters (SI ≈ 3.2–3.5) and the highest quantitative P release in broth assays. Several isolates, including *A. niger* and *P. spinulosum*, also produced IAA, suggesting additional mechanisms of plant growth promotion. Molecular characterization using amplification and sequencing of the ITS rDNA region confirmed the accurate identification of selected isolates, aligning them with reference sequences of known PSF species.

To validate the functional significance of these isolates, pot experiments were conducted using tomato (*Solanum lycopersicum* L), rice (*Oryza sativa* L), and chilli (*Capsicum annuum* L) under controlled conditions without chemical fertilizer input. Seedlings inoculated with *A. niger* or *Trichoderma* sp. showed significantly enhanced growth compared to uninoculated controls. *A. niger* proved most effective, leading to substantial increases in shoot length, root length, fresh and dry biomass, particularly in tomato and rice. Even in chilli, a slower-growing crop, inoculation resulted in marked improvements in plant vigor.

The findings confirm that indigenous PSF, especially *A. niger*, are highly effective in mobilizing soil phosphorus and stimulating crop growth. By demonstrating both

laboratory efficiency and plant growth-promoting effects in pot trials, this study establishes a strong basis for the development of PSF based bio fertilizers tailored to regional farming systems. The work underscores the potential of reducing reliance on chemical fertilizer inputs while enhancing crop productivity in phosphorus-limited soils.

While highly promising, the study is limited by its short-term, controlled environment experiments and the small number of isolates tested in pot trials. Field-scale, long-term investigations are essential to validate the performance of PSF under real agricultural conditions. Nonetheless, this work highlights the ecological and agronomic value of native microbial resources and provides a framework for their integration into sustainable agricultural practices.

In vitro screening revealed that both *Aspergillus niger* and *Trichoderma* exhibited phosphate-solubilizing activity, as evidenced by clear zones on Pikovskaya's medium and increased soluble phosphate in broth culture. Quantitatively, *A. niger* outperformed *Trichoderma*, achieving a solubilization index (SI) of up to 2.10 and releasing higher phosphate levels. Similarly, both strains produced IAA in tryptophan-supplemented media, demonstrating a potential role in enhancing plant root development. Molecular characterization confirmed the identity of the isolates within the *Aspergillus* and *Penicillium* clades using ITS-based phylogenetic analysis. This step was crucial in confirming morphological identifications and provides a baseline for future genetic manipulation or strain improvement.

**Conclusion**

The findings of this research underscore the promising potential of *A. niger* and *Trichoderma* as effective phosphate-solubilizing fungi (PSF) with supplementary plant growth-promoting traits such as IAA production. Among the two, *A. niger* consistently exhibited superior performance across P solubilization and auxin synthesis assays. Given their dual functional traits and adaptability, these fungi could serve as eco-friendly bioinoculants to improve phosphorus uptake and plant productivity in phosphorus-deficient or acidic soils, such as those commonly found in jhum-cultivated regions. The results also suggest a path forward for integrating native fungal strains into sustainable

agriculture programs, reducing reliance on chemical fertilizers, and improving soil health.

**Future Scope**

This study was comprehensive in scope, encompassing everything from field sampling of indigenous microbes to controlled pot trials. Geographically, it focused on the rhizosphere soils of jhum cultivation fields in Nagaland, India – sites representative of traditional shifting agriculture with minimal chemical inputs. Functionally, the research spanned the full pipeline of biofertilizer development: Chapter 2 covered the isolation of fungi from soil; Chapter 3 involved in-depth screening of those isolates' biochemical and genetic characteristics; Chapter 4 tested the practical effects of applying the fungi on plant growth. The study thus bridges fundamental and applied research, starting from exploring biodiversity of PSF in a unique agro-ecosystem and culminating in evaluating their utility in promoting crop growth. The selection of three distinct crop species (tomato, rice, and chili) for the pot experiments also broadened the scope, demonstrating the fungi's effects across a vegetable, a cereal, and a spice crop. This multi-crop aspect provides insight into how different plants respond to PSF inoculation.

In summary, the unified conclusion of this work is that PSF represent a viable and promising tool for sustainable agriculture, capable of enhancing plant growth and nutrient uptake in an environmentally friendly way. The key findings across all chapters reinforce the concept that harnessing soil microbial resources, especially PSF, can significantly improve phosphorus use efficiency and crop productivity, which is an essential strategy in the face of finite phosphate reserves and the need for eco-friendly farming practices.

Rhizospheric soil hosts a diverse and effective range of PSF and these fungi have demonstrated significant potential in converting insoluble phosphorus into forms that are readily available to plants, making them valuable for improving soil fertility in phosphorus-deficient and acidic soils. By isolating and characterizing these PSFs, this research lays a solid foundation for their application in developing low-cost, eco-friendly biofertilizers. This is especially important for the region's traditional and often resource-limited farming systems. Integrating PSFs into agricultural practices could reduce dependency on chemical fertilizers, improve nutrient cycling, and support more resilient

cropping systems, all while preserving the delicate soil microbiome. They offer a path forward for more sustainable, productive, and ecologically sound agriculture that aligns with both traditional values and modern challenges.

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# List of Publications

1. Jamir, I., Temjen, W., and Tali, A. (2024). Diversity and Phosphate Solubilization Potential of Rhizospheric Fungi from different Land-use of Mokokchung district, Nagaland, India. Indian Journal of Ecology 51(5):1049-1053. https://doi.org/10.55362/IJE/2024/4350

2. Jamir, I., Tali, A., and Temjen, W. (2021). Phosphate Solubilizing Fungal Population From Soil Rhizosphere at Lumami, Zunheboto, India.

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# Phosphate Solubilizing Fungal Population From Soil Rhizosphere at Lumami, Zunheboto, India

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**Abstract:** Phosphate is a key element in soil as per the quantitative plant requirements. It is vital for plants in its early phases of development. As chemical fertilizers have long term negative effects on soil, phosphate solubilizing fungi (PSF) is a potent alternative. The information on such PSF however is lacking at Zunheboto. The present study therefore aims to document the various Phosphate solubilizing fungi located in the district. During the study, the PSF were collected from rhizospheric region of the study site. The dominant genus was observed to be *Aspergillus* followed by *Penicillium*. Such findings will be vital in initiating a development of sustainable farming in Nagaland with emphasis on PSF as an alternative to chemical fertilizers.

**Keywords:** Phosphate, Soil, Rhizospheric Fungi, Nagaland

## I. INTRODUCTION

Phosphorus (P) is a vital macronutrient for plants. It is essential for their metabolic processes and developmental growth of plants. Phosphorus in soil, however are mostly immobile and approximately only 0.1% of the total phosphorus exist in a soluble form which is readily available for plant uptake [1]. The traditional method for increasing phosphorus in the soil is by the application of fertilizers containing phosphorus, however plants are able to utilize only a small amount and a considerable amount becomes immobilized and unavailable to plants [2].

Phosphate-solubilizing fungi (PSF) are one possible solution to this very problem. PSF are capable of solubilizing insoluble phosphate in the soil and increase the plant-available phosphorus. Further, soil fungi has been reported to have a greater insoluble phosphate solubilizing ability than bacteria [3, 4]. Some PSF stimulate plant growth by secreting indole-3-acetic acid (IAA) and siderophore and have enormous potential in enhancing the release of phosphorous from fertilizer [5, 6]. A wide range of soil fungi such as *Aspergillus niger* and *Penicillium* sp. are the most common fungi capable of phosphate

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# Diversity and Phosphate Solubilization Potential of Rhizospheric Fungi from different Land-use of Mokokchung district, Nagaland, India

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***Abstract***: This study investigates the rhizospheric fungal communities in soil samples from Mokokchung, Nagaland, India, collected from five land-use types: natural forest (NF), tree plantation (TP), bamboo plantation (BP), jhum fallow (JF), and shifting cultivation (SC). Various soil parameters, including temperature, pH, organic carbon, moisture, available nitrogen, phosphorus, and potassium, were analyzed. Soil fungal species were isolated in rose Bengal agar (RBA) and potato dextrose agar (PDA), and the diversity, richness, evenness, and abundance were estimated using diversity indices such as Shannon, Simpson, Berger-Parker, and Pielou's evenness. Results depict significant variations in soil properties influencing fungal populations across the different land-use types. NF exhibited the highest fungal diversity, taxa, and richness, while SC had the lowest, as reflected by the diversity indices. *Aspergillus* and *Penicillium* were the predominant genera. Additionally, eight fungal isolates demonstrated the ability to solubilize phosphate, with *Penicillium* emerging as a particularly promising candidate for further evaluation due to its high solubilization potential. This study highlights the impact of land-use on fungal diversity and the potential of phosphate-solubilizing fungi for sustainable agriculture.

***Keywords***: Land-use, Fungal diversity, Phosphate solubilizing fungi, Diversity indices, North East India

The plant root system influences the soil rhizosphere and micro-organisms present in the soil are subsequently influenced by the plant-root metabolites (Sharma and Shrivastava 2017). Therefore, depending on the land-use, different fungal flora is present. The fungal population regulates the ecosystem, stabilizes habitat, and controls various soil processes (Frąc et al 2015). However, reports on the loss of biodiversity globally, which ultimately lowers ecosystem functionality, extinctions, and even ecosystem collapse in extreme situations are reported (Dunne and Williams 2009). The primary driver of this rapid loss of fungal biodiversity is converting forest land to agricultural systems and land-uses characterized by regular anthropogenic disturbances (Temjen et al 2021). Therefore, inventory is a valuable resource and ensures natural resource efficiency and sustainable utilization to prevent further biodiversity loss. The main instrument for ensuring ecological monitoring and addressing the biodiversity crisis is the use of diversity indices, which enable measurement of the two essential aspects of an ecosystem, i.e., richness and evenness (Stirling and Wilsey 2001, Morris et al 2014).

Phosphorus constitutes about 0.2% of the plant's dry weight and is essential for plant growth and metabolism (Widawati and Suliasih 2006). However, plants can only utilize a trace amount of the chemical P and a substantial amount is immobilized and left inaccessible to plants (El Attar et al 2022). The economically and environmentally beneficial solution would be a microbial inoculant capable of dissolving sparingly soluble inorganic soil P (Alori et al 2017). Phosphate solubilizing fungi (PSF) can solubilize insoluble phosphate in the soil. Fungi, in particular, have a more remarkable ability to solubilize insoluble phosphate than bacteria (Zhang et al 2018). Mokokchung district is a hilly region in Nagaland state, North-East India. The ever-increasing population in the state has amplified the pressure on the land. There is a trend of rapid conversion of natural forests into different land-use systems, which negatively affects the fungal community (Miah et al 2010, Temjen et al 2021). Therefore, the present work aims to study the diverse rhizospheric fungal populations from various land-use sites in Mokokchung district, Nagaland, India and estimate those fungi with the capacity to solubilize P for sustainable agriculture.

## MATERIAL AND METHODS

**Site selection:** Five land-use sites under Mokokchung district, Nagaland, India, were selected (Table 1). The major type of soil in the region have alluvial soil, non-laterite red soil, and forest soil, with an average temperature of 27°C and 2500 mm of rainfall annually (Temjen et al 2022).

**Soil sample**: Composite soil layers at 0-30 cm were collected from the rhizospheric region of each site during

# List of Seminars, Webinars, Workshops Attended and Paper Presented

**Presented Papers:**

RTU TEQIP – III sponsored International Conference on 'Recent Trends in Engineering and Technology' organized by Rajasthan Technical University, Kota and Arya College of Engineering and Research Centre, Jaipur on paper titled, 'Phosphate Solubilizing Fungal Population from Soil Rhizosphere at Lumami, Zunheboto, India' held on March 12$^{th}$- 13$^{th}$, 2021.

National seminar on 'Sustainable Emerging Approach in Agri-Business Development' organized by Department of Agriculture of Economics, Nagaland University, School of Agriculture Sciences, Medziphema Campus, Nagaland on paper titled 'Isolation of Phosphate solubilizing potential from the Rhizospheric Fungi in Jhum Rice fields of Zunheboto, Nagaland, India' held on 01$^{st}$ to 03$^{rd}$ November 2023.

**Webinars and conferences attented:**

Hands-on International training on Application of SPSS for Data Analysis organized by World Statistical Data Analysis Research Association (WSDA) powered by Eudoxia Research Centre from October 5th-11th, 2021

International Conference on Bioresources & Bioeconomy organized by Department of Botany, Nagaland University, Lumami in collaboration with Nagaland Forest Management Project, Department of Environment, Forest and Climate Change, Govt. Nagaland from September 19th-21st, 2022.