



**ISOLATION AND CHARACTERISATION OF MYCOBIOME IN
AGRICULTURAL FIELDS**

BY

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CERTIFICATION

This is certify that this project is the original work carried out and reported by **ABDULLAHI KEHINDE RIHANAT** with matric number **ND/23/SLT/PT/0008** to t he Department of Science Laboratory Technology, Microbiology unit, Institute of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been appro ved In partial fulfillment of the requirements for the Award of National Diplom a (ND) In Science Laboratory Technology

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DEDICATION

This project is dedicated to Almighty Allah, the provider and sustainer of life, Whose grace has made this journey of my National Diploma (ND) possible, and also to my parents Mr and Mrs Abdullahi Amuda Onisese, my pillar of support. May Allah continue to bless and protect you.

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ABSTRACT

*Soil fungi, collectively referred to as the mycobiome, are essential components of agricultural ecosystems, playing key roles in nutrient cycling, plant health, and biological control. This study aimed to isolate and characterize fungal communities from different agricultural fields within Kwara Polytechnic using culture-dependent and morphological methods. Soil samples were collected from five distinct locations and analyzed for pH, followed by serial dilution, inoculation on Potato Dextrose Agar (PDA), and incubation. Pure fungal isolates were obtained through sub culturing and identified based on macroscopic and microscopic features. A total of eight pure fungal cultures were obtained, representing six distinct species: *Aspergillus niger*, *Penicillium* sp., *Rhizopus stolonifer*, *Fusarium* sp., *Mucor* sp., and *Trichoderma* sp. The highest fungal diversity was observed in the Ibas Area, while soil pH ranged from 5.2 to 5.6 across all sites, supporting fungal growth. These findings highlight the rich fungal biodiversity in agricultural soils and underscore their potential application in sustainable farming practices such as biofertilization and biocontrol. Understanding and leveraging native fungal communities could lead to reduced chemical input and improved crop productivity.*

CHAPTER ONE

1.0 INTRODUCTION

Agricultural soils harbor diverse microbial communities vital for ecosystem services, and fungi collectively known as the mycobiome play crucial roles in nutrient cycling, plant symbiosis, and disease suppression (Banerjee *et al.*, 2020). Despite their ecological importance, soil fungi have been less studied compared to bacteria. Recent insights reveal that these fungi contribute significantly to soil structure and fertility through organic matter decomposition and root interactions (Liu *et al.*, 2021). Characterizing the native mycobiome is essential, as it may unveil beneficial strains that support sustainable agriculture and improve crop health.

Soil fungi perform varied ecological functions, from decomposers breaking down organic matter to mutualistic species like arbuscular mycorrhizal fungi (AMF) that enhance nutrient uptake (Chen *et al.*, 2022; Zhou *et al.*, 2021). Some fungi, such as *Trichoderma*, also offer biocontrol benefits, while pathogenic species like *Fusarium* and *Alternaria* threaten crop yields. Identifying and isolating these fungi enables early detection and guides the implementation of biological control strategies. Thus, the balance of fungal populations directly influen

ces plant productivity and soil resilience.

Anthropogenic activities intensive tillage, pesticides, and monocultures—disrupt soil microbial balance, often favoring pathogens while diminishing beneficial fungi (Ma *et al.*, 2020). Characterizing the soil mycobiome helps assess these impacts and design ecological interventions. For instance, enhancing beneficial fungi through cover cropping or reduced chemical inputs aligns with regenerative agriculture goals. Fungal diversity supports nutrient cycling and carbon retention, ultimately reducing reliance on synthetic fertilizers and promoting soil health (Choudhary *et al.*, 2021).

The isolation of fungi typically involves serial dilution and culturing on media like PDA or SDA, followed by colony observation and microscopic examination (Singh *et al.*, 2021). These traditional methods are cost-effective and still widely used, especially when combined with modern techniques like ITS sequencing for accurate identification (Wu *et al.*, 2023). Although not all fungi are culturable, many agriculturally relevant ones can be studied this way. A combination of morphological and molecular tools ensures comprehensive characterization of field fungal communities.

Environmental factors like soil pH, organic matter, temperature, and moisture

greatly affect fungal diversity. Additionally, crop type and agricultural practices shape fungal assemblages, with different crops (e.g., maize vs. rice) supporting different fungal communities (Zhang *et al.*, 2020; Huang *et al.*, 2021). Comparing fungi across different farming systems helps reveal crop-specific interactions and informs targeted soil management strategies. This ecological understanding is vital for improving food security and achieving sustainable agriculture.

Fungi rarely act alone; they engage in complex interactions with other microbes and plant roots, affecting plant health and nutrient acquisition (Sun *et al.*, 2022). Beneficial fungi improve growth and resistance, while pathogens can hinder root function. Functional characterization of isolates through tests on nutrient solubilization, biocontrol, or growth promotion—can inform development of bioinoculants. These native microbial formulations offer environmentally friendly alternatives to synthetic agrochemicals (Jiang *et al.*, 2020).

Regional differences in fungal populations remain understudied, particularly in tropical and subtropical zones (Ali *et al.*, 2021). Isolation of native strains contributes to global microbial biodiversity and may uncover fungi with unique enzymatic or stress-resilient traits. Some isolates can degrade pollutants or produc

e bioactive compounds with pharmaceutical potential (Li *et al.*, 2023; Zhou *et al.*, 2022). Thus, fungal research not only supports agriculture but also enables industrial and environmental applications, underscoring the multifaceted value of the mycobiome.

Lastly, while traditional isolation techniques are foundational, they have limitations many fungi are non-culturable or require specific growth conditions (Ahmed *et al.*, 2021). Metagenomic approaches can complement culture methods to uncover hidden diversity. Seasonal fluctuations and farming practices further influence fungal populations (Gao *et al.*, 2023; Zhang *et al.*, 2021). A combined approach involving taxonomy, physiology, and environmental monitoring is necessary to fully understand and harness the agricultural potential of soil fungi for sustainable innovation.

1.1 Literature Review

Fungal communities in agricultural soils are crucial for nutrient cycling, decomposition of organic matter, and maintaining plant health. Banerjee et al. (2020) reported that agricultural intensification significantly reduces microbial network complexity, particularly diminishing keystone fungal taxa in the root zone. This decrease in diversity may negatively affect the stability and functionality of

agroecosystems. Their findings support the need to study soil mycobiomes under various farming practices to assess microbial balance. The isolation and identification of these fungi help determine whether beneficial symbiotic relationships are being supported. A balanced mycobiome plays a pivotal role in enhancing crop resilience and productivity. Hence, soil fungal community studies are vital for sustainable agricultural development.

Ahmed *et al.* (2021) investigated fungal diversity in agricultural soils using both culture-dependent methods and metagenomics. Their study highlighted that although culture-based techniques are useful for isolating common fungal genera such as *Aspergillus*, *Penicillium*, and *Trichoderma*, many fungal species remain undetectable without molecular tools. The combined approach provided a more comprehensive understanding of soil fungal diversity. They found that soil conditions, such as organic content and pH, significantly influence fungal prevalence and type. This integrated methodology offers a deeper insight into fungal biodiversity and supports better ecological interpretation. Isolation and sequencing, therefore, complement each other for reliable fungal characterization in agricultural soils.

Jiang *et al.* (2020) discussed the role of fungal bioinoculants in sustainable ag

riculture, emphasizing that strains such as *Trichoderma harzianum* and arbuscular mycorrhizal fungi can enhance nutrient absorption and protect plants from pathogens. They argued that isolating native strains is more effective than using foreign commercial inoculants due to environmental adaptability. Their review stressed that characterization must precede application, to ensure the selected fungal strains are ecologically compatible and functionally potent. This underlines the need for field-based isolation and screening of fungal communities. The work suggests that local mycobiome characterization is a key step in building effective microbial products tailored to specific regions.

According to Huang *et al.* (2021), organic farming practices positively influence soil fungal diversity and function. Their research compared organic and conventional farming systems and found that organically managed soils host a higher abundance of fungi involved in nutrient cycling, such as phosphate-solubilizing and cellulose-degrading fungi. These beneficial fungi enhance soil health and plant nutrition. The study suggests that different agricultural practices shape distinct fungal communities. Consequently, isolating fungi from organically managed fields could yield strains with valuable ecological functions. Understanding these relationships enables targeted use of fungi for organic amendm

ents and biological soil management.

Sun *et al.* (2022) presented a detailed review of plant–fungus interactions in soil ecosystems, categorizing fungi as symbionts, saprophytes, or pathogens. They emphasized that fungal roles are dynamic and may shift depending on environmental factors such as moisture, temperature, and plant stress. Some endophytes can promote plant growth under ideal conditions but become opportunistic pathogens when conditions deteriorate. Therefore, accurate isolation and identification of fungi are necessary to classify them correctly and manage their functions effectively. The review underlines the importance of thorough mycobiome characterization to optimize plant–fungal interactions in agriculture.

1.2 Statement of problem

- Despite the critical role soil fungi play in nutrient cycling, plant health, and soil fertility, there is limited region-specific data on the diversity and functional characteristics of fungal communities in many agricultural fields, particularly in tropical and developing regions.
- Inadequate characterization of indigenous soil fungi has limited their ap

plication in sustainable agricultural practices, such as the development of biofertilizers and biocontrol agents, hindering efforts to reduce chemical input and improve crop productivity.

1.3 Aim

To isolate and characterize the fungal communities (mycobiome) present in agricultural fields using both culture-dependent and morphological methods, in order to understand their diversity, ecological roles, and potential applications in sustainable agriculture.

1.4 Objectives

- To isolate and identify fungal species present in agricultural field soils using culture-dependent methods and morphological characterization.
- To evaluate the diversity and abundance of the isolated fungal communities across different agricultural field conditions.

CHAPTER TWO

2.0 Materials and Methods

2.1 Materials

The materials used for this study included sterile sample collection containers, measuring balance, sterile test tubes, conical flasks, beakers, pipettes, distilled water, inoculating loop, Petri dishes, test tube racks, forceps, sterile syringes, cotton wool, spirit, and sealing tape. Microscopic slides, cover slips, lactophenol cotton blue stain, hand lens, microscope, and permanent markers were also utilized. All glassware and equipment were properly sterilized before use to prevent contamination.

2.1.1 Media and Reagents

The reagents and chemicals used included distilled water, Potato Dextrose Agar (PDA) powder, streptomycin (an antibiotic), and lactophenol cotton blue stain. All chemicals were of analytical grade and used according to the manufacturer's specifications. The PDA medium was used to support fungal growth, while streptomycin was added to inhibit bacterial growth. Lactophenol cotton blue was used for staining fungal structures for microscopic examination.

2.2 Preparation of Sample

Soil samples were collected aseptically from various locations within Kware Polytechnic, including the Agric Tech Commercial Farm, Tourism Community, Bank Area, Yankari, and Ibos Area. Each soil sample was collected using sterile containers and immediately transported to the laboratory under sterile conditions to avoid contamination. Upon arrival, the pH of each soil sample was measured and recorded using a calibrated pH meter. The samples were then stored appropriately prior to further processing.

2.3 Preparation of Media

Potato Dextrose Agar (PDA) was prepared following the manufacturer's instructions. The media was sterilized by autoclaving at 121°C for 20 minutes. After sterilization, the medium was allowed to cool under sterile conditions. Streptomycin was then added to the medium to inhibit bacterial contamination. The prepared media was poured into sterile Petri dishes and allowed to solidify before inoculation.

2.4 Isolation of Fungi

Serial dilution of the soil samples was performed using six test tubes, each containing 9 ml of sterile distilled water. An initial suspension was prepared by m

ixing 1 gram of each soil sample with 1 ml of sterile distilled water. Subsequent serial dilutions were carried out, and 0.1 ml from each dilution was aseptically inoculated onto solidified Potato Dextrose Agar (PDA) plates (two plates per dilution per sample). The plates were incubated at room temperature for 3 to 5 days. After incubation, fungal growth was observed, and plates showing mixed fungal colonies were identified. To obtain pure cultures, individual colonies with mixed growth were sub cultured aseptically onto fresh PDA plates. Each morphologically distinct colony was transferred separately to ensure the isolation of pure fungal strains. The sub cultured plates were incubated under the same conditions until adequate growth was achieved for further analysis. Following incubation, the number of fungal colonies on each plate was counted to estimate the colony-forming units (CFU) per gram of soil. Only plates with distinct, countable colonies (30–300 colonies) were considered for accurate enumeration. Colony counts were recorded, and representative pure fungal colonies were selected for further characterization, including macroscopic and microscopic examination.

2.6 Macroscopic and Microscopic Characterization

Macroscopic identification involved observing and recording the physical featu

res of fungal colonies, such as texture, color, shape, and growth patterns. For microscopic examination, small portions of fungal colonies were picked and mounted on clean microscopic slides containing a drop of distilled water. Two to three drops of lactophenol cotton blue stain were added, and the preparation was covered with a cover slip. The slide was first observed under low power, followed by high power magnification to examine spore structure, hyphae type, and other microscopic features.

CHAPTER THREE

3.0 RESULT

3.1 Soil pH at Different Locations

Table 1: Soil pH at Different Locations

Sample Location	Soil pH
Distilled Water	6.28
Agric Tech Farm	5.6
Tourism Village	5.4
Bank Area	5.4
Yankari	5.3
Ibas Area	5.2

3.2 Colony Count from Mixed Cultures (after Serial Dilution)

Table 2: Colony Count from Mixed Cultures (after Serial Dilution)

Sample Location	Dilution Level	Number of Colonies	Culture Type
Agric Tech Farm	10^{-2}	9	Mixed culture
Agric Tech Farm	10^{-4}	4	Mixed culture
Tourism community	10^{-2}	11	Mixed culture
Tourism community	10^{-4}	13	Mixed culture
Bank Area	10^{-2}	76	Mixed culture
Bank Area	10^{-4}	No growth	–
Yankari	10^{-2}	10	Pure culture
Yankari	10^{-4}	No growth	–
Ibas Area	10^{-2}	43	Mixed culture

Ibas Area

10^{-4}

15

Mixed cultur
e