ISOLATION AND CHARACTERISATION OF MYCOBIOME IN

AGRICULTURAL FIELDS

BY

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ND/23/SLT/PT/0008

A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOL

OGY, INSTITUTE OF APPLIED SCIENCES (IAS),

KWARA STATE POLYTECHNIC, ILORIN,

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF NATIONAL DI PLOMA (ND) DEGREE IN SCIENCE LABORATORY TECHNOLOGY,INSTITUTE OF APPLIE D SCIENCES (IAS),MICROBIOLOGY UNIT.KWARA STATE POLYTECHNIC ILORIN

JULY,**2025.**

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, an d also to my parents Mr and Mrs Abdullahi Amuda Onisese, my pillar of suppo rt. May Allah continue to bless and protect you.

ACKNOWLEDGEMENTS

I am indeed thankful and grateful to Almighty Allah for giving me the opportunity, courag e, energy, grace, and assistance needed to successfully achieve one of my desired acade mic heights in life.

With humility and appreciation, my foremost acknowledgement goes to my amiable sup ervisor, Mrs. Ahmed Tawakalitul who, despite her busy schedule, found time to guide and mentor me in my quest to achieve excellence. Hence, He has played a pivotal role in brin ging this study to completion. I pray that Allah, in His boundless mercy, fulfil all his heart desires.

I extend my heartfelt gratitude to all my lecturers in the Department of Science Laborator y Technology , Kwara State Polytechnic, Ilorin, may Allah bless you all abundantly.

I would like to also appreciate myself, the little soul in me for not getting tired and relent t hrough this journey, and I thank God almighty for not leaving my side, I pray almighty All ah continue to guide and protect me in everything I lay my hands on.

My appreciation also goes to my sister's and my brother's thank you all for your guidanc e and support, to make this journey successful i pray almighty Allah will always be with u and reward u all for all your efforts on me. And also I'll like to give a big thanks to my fr iend, the one I always run to when everything is down, my good friend, Abdulmumeen Ab dulfatai Oluwadamilare , whose love, support, and encouragement have been a constant source of inspiration, may Allah perfect all that concerns you. And to everyone that sees me through this journey, bless you all.

Lastly, I extend my heartfelt thanks and blessings to my fellow students for their camara derie and support, I am deeply grateful. May Allah bless us abundantly on our individual journeys.

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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 ser vices, and fungi collectively known as the mycobiome play crucial roles in nutri ent cycling, plant symbiosis, and disease suppression (Banerjee *et al.*, 2020). Despite their ecological importance, soil fungi have been less studied compar ed to bacteria. Recent insights reveal that these fungi contribute significantly t o soil structure and fertility through organic matter decomposition and root int eractions (Liu *et al.*, 2021). Characterizing the native mycobiome is essential, as it may unveil beneficial strains that support sustainable agriculture and imp rove crop health.

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

ces plant productivity and soil resilience.

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

The isolation of fungi typically involves serial dilution and culturing on media li ke PDA or SDA, followed by colony observation and microscopic examination (Singh *et al.*, 2021). These traditional methods are cost-effective and still wide ly used, especially when combined with modern techniques like ITS sequencin g for accurate identification (Wu *et al.*, 2023). Although not all fungi are cultura ble, many agriculturally relevant ones can be studied this way. A combination of morphological and molecular tools ensures comprehensive characterizatio n 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) supportin g different fungal communities (Zhang *et al.*, 2020; Huang *et al.*, 2021). Comp aring fungi across different farming systems helps reveal crop-specific interac tions and informs targeted soil management strategies. This ecological under standing is vital for improving food security and achieving sustainable agricult ure.

Fungi rarely act alone; they engage in complex interactions with other microbe s and plant roots, affecting plant health and nutrient acquisition (Sun *et al.*, 20 22). Beneficial fungi improve growth and resistance, while pathogens can hind er root function. Functional characterization of isolates through tests on nutrie nt solubilization, biocontrol, or growth promotion—can inform development of bioinoculants. These native microbial formulations offer environmentally frien dly 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 contr ibutes to global microbial biodiversity and may uncover fungi with unique enzy matic 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 un der various farming practices to assess microbial balance. The isolation and id entification of these fungi help determine whether beneficial symbiotic relatio nships are being supported. A balanced mycobiome plays a pivotal role in enh ancing 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 bot h culture-dependent methods and metagenomics. Their study highlighted that although culture-based techniques are useful for isolating common fungal gen era such as *Aspergillus*, *Penicillium*, and *Trichoderma*, many fungal species re main 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 characteriza tion 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 arbus cular mycorrhizal fungi can enhance nutrient absorption and protect plants fro m pathogens. They argued that isolating native strains is more effective than using foreign commercial inoculants due to environmental adaptability. Their r eview stressed that characterization must precede application, to ensure the selected fungal strains are ecologically compatible and functionally potent. Th is underlines the need for field-based isolation and screening of fungal commu nities. 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 influenc e soil fungal diversity and function. Their research compared organic and conv entional farming systems and found that organically managed soils host a hig her abundance of fungi involved in nutrient cycling, such as phosphate-solubili zing and cellulose-degrading fungi. These beneficial fungi enhance soil health and plant nutrition. The study suggests that different agricultural practices sh ape distinct fungal communities. Consequently, isolating fungi from organicall y managed fields could yield strains with valuable ecological functions. Unders tanding 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 s oil ecosystems, categorizing fungi as symbionts, saprophytes, or pathogens. T hey emphasized that fungal roles are dynamic and may shift depending on en vironmental factors such as moisture, temperature, and plant stress. Some en dophytes can promote plant growth under ideal conditions but become opport unistic pathogens when conditions deteriorate. Therefore, accurate isolation a nd 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 agricultu re.

1.2 Statement of problem

- Despite the critical role soil fungi play in nutrient cycling, plant health, an d soil fertility, there is limited region-specific data on the diversity and fu nctional characteristics of fungal communities in many agricultural field s, 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 b iofertilizers and biocontrol agents, hindering efforts to reduce chemical i nput 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 applicati ons in sustainable agriculture.**

1.4 Objectives

- To isolate and identify fungal species present in agricultural field soils us ing culture-dependent methods and morphological characterization.

- To evaluate the diversity and abundance of the isolated fungal communi ties 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, distille d water, inoculating loop, Petri dishes, test tube racks, forceps, sterile syringe s, cotton wool, spirit, and sealing tape. Microscopic slides, cover slips, lactoph enol cotton blue stain, hand lens, microscope, and permanent markers were al so 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 Ag ar (PDA) powder, streptomycin (an antibiotic), and lactophenol cotton blue stai n. All chemicals were of analytical grade and used according to the manufactu rer's specifications. The PDA medium was used to support fungal growth, whil e 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 P olytechnic, including the Agric Tech Commercial Farm, Tourism Community, B ank Area, Yankari, and Ibos Area. Each soil sample was collected using sterile containers and immediately transported to the laboratory under sterile conditi ons to avoid contamination. Upon arrival, the pH of each soil sample was mea sured and recorded using a calibrated pH meter. The samples were then store d appropriately prior to further processing.

2.3 Preparation of Media

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

2.4 Isolation of Fungi

Serial dilution of the soil samples was performed using six test tubes, each co ntaining 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. Subsequen t 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 mix ed 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 isolat ion of pure fungal strains. The sub cultured plates were incubated under the s ame conditions until adequate growth was achieved for further analysis. Follo wing 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 disti nct, countable colonies (30–300 colonies) were considered for accurate enum eration. Colony counts were recorded, and representative pure fungal colonies were selected for further characterization, including macroscopic and microsc opic 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 m ounted 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, f ollowed 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