EFFECT OF FUNGICIDE'S APPLICATION ON SOIL MYCOBIOME

BY

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CERTIFICATION

This is certify that this project is the original work carried out and report ed by **EKUNDAYO BISOLA CHRISTIANA** with matric number **HND/23/SL T/FT/0618** to the Department of Science Laboratory Technology, Micro biology unit, Institute of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been approved In partial fulfillment of the requirements for the Award of Higher National Diploma (HND) In Science Laboratory T echnology

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DEDICATION

This project is dedicated to Almighty God, the provider and sustainer of l ife, Whose grace has made this journey of my Higher National Diploma (HND) possible, and also to my lovely sister MRS FOLASHADE BABATU NDE and my mom Mrs Omoyeni Ekundayo ,my pillars of support. May G od continue to bless and protect you all.

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I would like to also appreciate myself, the little soul in me for not getting

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ABSTRACT

This study investigates the impact of fungicide application on soil myco biome composition, diversity, and function in selected agricultural enviro nments. Soil samples collected from various sites were treated with co mmercial fungicides, including Red Force, Black Force, Total Force, Sta mpede, and Ultimax Plus, and compared to untreated controls. Results r evealed that fungicide application led to notable changes in soil pH rang ing from 4.93-5.70, generally increasing it across all treated samples. Fu ngal diversity and abundance were significantly affected, with suppressi on of key genera such as Aspergillus, Penicillium, and Fusarium in treate d soils. Conversely, an unexpected proliferation of yeast species was ob served, especially in samples from Akuo and ARA, suggesting that fungi cides may create ecological niches for resistant or opportunistic fungi. These findings align with previous studies that have highlighted fungici de-induced shifts in soil microbial communities and reduced ecological function. The study underscores the ecological consequences of fungici de use, suggesting the need for more sustainable practices such as inte grated pest management, biocontrol agents, and reduced chemical inpu t to preserve soil microbial balance and health.

CHAPTER ONE

1.0 INTRODUCTION

Soil ecosystems host a diverse range of microbial communities essentia l for nutrient cycling, organic matter decomposition, and plant health. A mong these microbes, fungi play key ecological roles, forming symbiotic associations, decomposing organic residues, and suppressing pathogen s. The collective fungal community in soil is referred to as the *soil myco biome*. These communities can be sensitive to chemical disturbances, i ncluding the application of fungicides. The increasing use of fungicides i n modern agriculture, although beneficial for controlling crop diseases, r aises concerns about potential disruptions to the natural fungal balance in soil ecosystems (Guo *et al.*, 2020).

Fungicides, particularly broad-spectrum types, may not only target harm ful pathogens but also affect beneficial and neutral fungi. Mycorrhizal fu ngi, for example, are crucial partners for most plants, aiding in nutrient u ptake and stress tolerance. Studies have shown that repeated or excess

ive fungicide use can reduce mycorrhizal colonization and spore product ion, thereby impairing plant–fungal symbiosis (Li *et al.*, 2020). Furtherm ore, fungicides may alter the abundance and richness of fungal taxa, lea ding to a shift in community structure and potential dominance of resist ant strains or opportunistic pathogens (Qi *et al.*, 2024).

The soil mycobiome is known for its resilience, yet prolonged exposure t o chemical agents may lead to functional loss and long-term degradatio n. For instance, fungicide residues may accumulate in soil and affect fun gal enzyme activity, which in turn disrupts nutrient cycling processes like nitrogen mineralization and phosphorus solubilization. This chemical dis turbance may also indirectly affect bacterial populations through fungal –bacterial interactions, potentially reducing overall soil microbial diversit y and function (Zhang *et al.*, 2022). Thus, while fungicides offer immedia te protection for crops, their broader ecological consequences require ca reful evaluation.

Fungal diversity is a key indicator of soil health. Reduction in fungal richness due to fungicide application can limit ecosystem functions and resilience to stressors. For example, saprophytic fungi involved in breaking down complex organic matter may be suppressed, leading to slower decomposition rates and reduced soil fertility over time. Some fungicides have been found to disproportionately suppress Ascomycota and Basidiomycota phyla, which are central to decomposition and nutrient transformation processes (Whitaker *et al.*, 2025). Such shifts in fungal community structure may also affect carbon sequestration and greenhouse gas emissions.

Environmental factors such as soil type, organic matter content, pH, and moisture influence the extent of fungicide impact on mycobiomes. For example, soils with high organic matter may bind fungicides and reduce their bioavailability, whereas sandy soils may allow for deeper fungicide penetration and wider fungal disruption. Moreover, the mode of fungicide application (e.g., foliar spray vs. soil drench) and the active ingredients

used can produce different outcomes in fungal populations. Fungicides with systemic action tend to have more prolonged and far-reaching effe cts than contact fungicides (Liu *et al*., 2021).

Monitoring and assessing soil mycobiome responses to fungicide expos ure require modern molecular tools. High-throughput sequencing of fun gal internal transcribed spacer (ITS) regions has enabled researchers to unravel complex community shifts and detect even low-abundance tax a. Metagenomics and transcriptomics further offer insight into functiona l changes and gene expression patterns under chemical stress (Sun *et a l*., 2023). These tools have revealed that fungicide exposure may reduce not only taxonomic diversity but also functional potential, impacting ligni n degradation, secondary metabolite production, and stress tolerance in fungal communities.

Given the crucial roles of fungi in plant health and soil fertility, sustainabl e fungicide practices must be emphasized. Integrated pest managemen

t (IPM), crop rotation, use of biological control agents, and reduced relia nce on chemical fungicides can help maintain a balanced soil mycobiom e. Additionally, selecting fungicides with lower persistence and narrower target ranges may mitigate off-target effects on non-pathogenic fungi. A growing body of research supports the use of biofungicides and natural antifungal compounds that pose minimal risks to beneficial microbes (G ao *et al.*, 2020). While fungicides are indispensable tools in modern agric ulture, their non-target effects on the soil mycobiome warrant greater at tention. Disruptions in fungal diversity and function can have cascading i mpacts on soil health, plant productivity, and ecosystem sustainability. F uture agricultural practices must strike a balance between disease contr ol and microbial conservation. Continuous monitoring, adoption of envir onmentally friendly alternatives, and awareness of fungicide–mycobiom e interactions are essential steps toward resilient and ecologically soun d food production systems.

1.1 Literature Review

According to Guo *et al.* (2020), fungicides are extensively applied in mod ern agriculture to combat fungal pathogens; however, their impact exten ds beyond target organisms to non-target soil fungal communities. The study revealed that chlorothalonil, a commonly used broad-spectrum fu ngicide, led to a significant reduction in fungal diversity and altered com munity composition in treated soils. Sensitive species were suppressed, while resistant strains became more prevalent. Additionally, key enzyma tic activities involved in nutrient cycling, such as β-glucosidase and urea se, were impaired, indicating a disruption in essential soil biochemical pr ocesses that support ecosystem stability.

Similarly, Li *et al.* (2020) highlighted the detrimental effects of fungicide application on arbuscular mycorrhizal fungi (AMF), which are integral to plant nutrient absorption and soil structural integrity. Repeated applicati ons in agricultural fields reduced AMF colonization and spore density, th ereby impairing phosphorus uptake and contributing to a decline in crop yield. The disruption of the mutualistic root-fungal relationship further u

nderscores the indirect negative influence of chemical fungicides on pla nt productivity and soil health.

Wang *et al.* (2021) conducted a comparative study on different fungicide classes and their influence on soil fungal diversity in rice paddies. The fi ndings showed that systemic fungicides had a more profound and long-l asting effect compared to contact fungicides. Notably, systemic fungicid es suppressed the richness of *Ascomycota* and *Basidiomycota*, which pl ay essential roles in organic matter decomposition. These effects could lead to a decline in soil organic matter breakdown, hinder carbon cyclin g, and negatively affect soil fertility over time.

In the context of fungicide types commonly used in agricultural practice s, products such as Red Force, Black Force, and Ultimax Plus have gaine d popularity due to their broad-spectrum antifungal activity. Red Force ty pically contains difenoconazole, which interferes with fungal sterol biosy nthesis, while Black Force often combines mancozeb and metalaxyl, dis

rupting fungal metabolism and nucleic acid synthesis. Ultimax Plus is a multi-action systemic fungicide used for preventive and curative control of a wide range of fungal diseases. Despite their effectiveness in diseas e control, continuous application of these fungicides has been associate d with the suppression of beneficial soil fungi, emergence of resistant fu ngal strains, and alterations in the natural microbial equilibrium of the so il.

Chen *et al.* (2020) emphasized the significance of soil characteristics in modulating fungicide effects. For example, soil pH and organic matter c ontent influence the persistence and toxicity of fungicides. Acidic soils t end to retain fungicides longer, enhancing their adverse impact on funga l populations, whereas soils rich in organic matter can adsorb fungicide r esidues, mitigating their toxicity. Moreover, soil texture and color are criti cal in determining microbial activity and fungicide interactions. Sandy so ils, with larger pore spaces and low organic content, facilitate leaching o f fungicides and reduce their retention time. In contrast, loamy and clay

soils, which have finer particles and higher moisture retention, can accu mulate more fungicide residues, increasing potential toxicity to non-targ et organisms. Darker-colored soils are often associated with higher orga nic content and microbial biomass, which could either buffer or amplify f ungicide effects depending on the compound involved.

According to Sun *et al.* (2023), modern molecular approaches such as m etagenomics and transcriptomics have unveiled detailed insights into ho w fungicides influence soil fungal communities. Their study showed a s uppression of genes involved in lignin degradation, nitrogen cycling, and oxidative stress response after fungicide exposure, indicating a decline i n the functional capacity of the soil mycobiome. This reduction may hin der plant-soil interactions and essential ecosystem services.

To mitigate these adverse effects, Gao *et al.* (2020) recommended envir onmentally sustainable alternatives such as the use of biocontrol agent s (*e.g.*, *Trichoderma* spp., *Bacillus subtilis*) and biofungicides. These opti

ons effectively control pathogens with minimal disturbance to beneficial microbes. The integration of such strategies into integrated pest manag ement (IPM) frameworks, along with careful soil monitoring and dose re duction, is essential to preserve soil biodiversity and sustain long-term a gricultural productivity.

1.2 Aim

The aim of this study is to evaluate the effect of fungicide application on the diversity, composition, and functional roles of soil mycobiome in agri cultural environments.

1.3 Statement of problem

- Despite the widespread use of fungicides in agriculture to control plant diseases, there is limited understanding of their long-term i

mpact on non-target soil fungal communities that are essential for nu trient cycling, organic matter decomposition, and plant–microbe i nteractions. This knowledge gap raises concerns about potential disruptions to soil health and ecosystem sustainability.

- Many conventional fungicides not only suppress pathogenic fungi but may also unintentionally alter the composition and diversity of beneficial soil fungi. The lack of sufficient data on how different fu ngicides affect key fungal groups, such as mycorrhizal and saprop hytic fungi, hinders the development of sustainable management practices that protect soil microbial biodiversity.

1.4 Objectives

- To assess the impact of different fungicide treatments on the dive rsity and abundance of fungal species in agricultural soil.

- To determine the changes in community composition and functio nal roles of soil fungi following fungicide application.

CHAPTER TWO

2.0 Materials and Methods

2.1 Materials

The materials used for this study included sterile hand trowels for soil collection, sterile polythene bags for sample transport, 250 mL conical flasks for soil incubation, sterile pipettes and test tubes for serial dilution, and Petri dishes for fungal culture. Additional materials included a digital pH meter for measuring soil pH, laboratory incubators maintained at appropriate temperatures for fungal growth, and microscope slides and cover slips for microscopic examination (Choudhary *et al.*, 2023).

2.1.1 Media and Reagents

The main culture medium used was Sabouraud Dextrose Agar (SDA), which was supplemented with chloramphenicol to inhibit bacterial contamination. Reagents used for fungal staining included lactophenol cotton blue, while sterile distilled water was used for serial dilution. Commercial fungicides applied to soil samples included Stampede, Total Force, Ulti

max Plus, Black Force, and Red Force, all applied according to manufact urer's dosage guidelines (González-Pérez *et al.*, 2020).

2.2 Preparation of Sample

Soil samples were collected from five distinct locations within Kwara Sta te Polytechnic, Ilorin: ARA, Akuo, Tourism, Village, and Yankari. Using a s terile hand trowel, samples were taken at a depth of 5–15 cm. About 20 0 grams of soil was collected from each site and transported to the micr obiology laboratory in sterile polythene bags. Each soil sample was thor oughly mixed and divided into two equal portions of 100 grams: one lab eled as "treated" and the other as "control"

2.2.1 Preparation and Application of Fungicide

One hundred grams (100 g) of soil from each location was treated with a specific fungicide as follows: Akuo with Stampede, ARA with Total For ce, Tourism with Ultimax Plus, Village with Black Force, and Yankari with Red Force. Each fungicide was prepared by weighing 1 gram and dissolv

ing it in 100 mL of distilled water in a beaker. From this solution, 10 mL was measured and added to the respective treated soil samples. Contro l samples received no fungicide treatment but were incubated under the same conditions to serve as a baseline for comparison (Wu *et al.*, 2021).

2.3 Measurement of soil pH

After the 7-day incubation period, the pH of each treated and control soi l sample was measured using a calibrated digital pH meter. The reading s were taken by inserting the electrode into a soil-water slurry prepared i n a 1:2.5 soil-to-water ratio. These pH values were recorded to evaluate changes in soil acidity or alkalinity as a result of fungicide application (W u *et al.*, 2021).

2.4 Preparation of Media

Sabouraud Dextrose Agar (SDA) was prepared according to the manufacturer's specifications by dissolving the required amount in distilled water, followed by sterilization in an autoclave at 121°C for 15 minutes. After cooling to about 45°C, chloramphenicol was added aseptically to suppress bacterial growth. The medium was then poured into sterile Petri dishes and allowed to solidify before inoculation (Zhao *et al.*, 2022).

2.5 Isolation of Fungi

Following a 7-day incubation period at room temperature (27 ± 2°C), 1 gram of each soil sample was aseptically transferred into 9 mL of sterile distilled water to initiate serial dilution. Appropriate dilutions were made, and 0.1 mL from each was inoculated onto SDA plates. The plates were incubated at 28°C for 3–5 days, after which fungal colonies were observed, counted, and recorded as colony-forming units per gram (cfu/g) of soil (Wu *et al.*, 2021).

2.6 Incubation of Soil Samples

All treated and control samples were placed in sterile 250 mL conical fla sks and incubated at ambient room temperature (27 ± 2°C) for 7 days. T o ensure even distribution of the fungicides and promote aeration, distill ed water was added to the samples and they were shaken gently once daily to prevent dryness. This incubation period allowed for the fungicid es to interact with the soil fungal communities (Kavamura & Esposito, 2 020).

2.7 Identification of Fungi

Fungal colonies that emerged on the SDA plates were first evaluated ba sed on their macroscopic characteristics such as colony color, texture, a nd growth rate. Distinct colonies were subcultured for purification. Micro scopic examination was carried out using lactophenol cotton blue stain, and fungal structures such as spores and hyphae were observed under the microscope to identify the fungi to the genus level. This allowed for comparative analysis of fungal diversity and abundance between treate

d and control samples (Choudhary *et al.*, 2023).

CHAPTER THREE

3.0 Results

3.1 Soil pH of Treated and Control Samples

Table 1: Soil pH of Treated and Control Samples

Sample Location	Treated Soil pH	Control Soil pH
Tourism	5.43	4.93
Yankari	5.32	4.96
ARA	5.18	5.14
Village	5.70	5.53
Akuo	5.43	4.99