

EFFECT OF FUNGICIDE'S APPLICATION ON SOIL MYCOBIOME

BY

DAVID GOODNEWS UKOIMA

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CERTIFICATION

This is certify that this project is the original work carried out and report ed by **DAVID GOODNEWS UKOIMA** with matric number **HND/23/SLT/FT /0174** to the Department of Science Laboratory Technology, Microbiolog y unit, Institute of Applied Sciences (IAS) Kwara State Polytechnic Ilorin and it has been approved In partial fulfillment of the requirements for th e Award of Higher National Diploma (HND) In Science Laboratory Techn ology

MISS. AHMED T.
(PROJECT SUPERVISOR)

DATE

MISS. AHMED T.
(HOU MICROBIOLOGY)

DATE

DR. USMAN A.
(HEAD OF DEPARTMENT)

DATE

External Examiner

DATE



DEDICATION

This project is dedicated to the sake of God, my creator, and my master, whose grace has made this journey of my (hnd) possible and also to my great sponsor Mr and Mrs David, Miss grace my pillar of support. may almighty continue to bless and protect them all.

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ABSTRACT

*This study investigates the impact of fungicide application on soil mycobiome composition, diversity, and function in selected agricultural environments. Soil samples collected from various sites were treated with commercial fungicides, including Red Force, Black Force, Total Force, Stampede, and Ultimax Plus, and compared to untreated controls. Results revealed that fungicide application led to notable changes in soil pH ranging from 4.93-5.70, generally increasing it across all treated samples. Fungal diversity and abundance were significantly affected, with suppression of key genera such as *Aspergillus*, *Penicillium*, and *Fusarium* in treated soils. Conversely, an unexpected proliferation of yeast species was observed, especially in samples from Akuo and ARA, suggesting that fungicides may create ecological niches for resistant or opportunistic fungi. These findings align with previous studies that have highlighted fungicide-induced shifts in soil microbial communities and reduced ecological function. The study underscores the ecological consequences of fungicide use, suggesting the need for more sustainable practices such as integrated pest management, biocontrol agents, and reduced chemical input to preserve soil microbial balance and health.*

CHAPTER ONE

1.0 INTRODUCTION

Soil ecosystems host a diverse range of microbial communities essential for nutrient cycling, organic matter decomposition, and plant health. Among these microbes, fungi play key ecological roles, forming symbiotic associations, decomposing organic residues, and suppressing pathogens. The collective fungal community in soil is referred to as the *soil mycobiome*. These communities can be sensitive to chemical disturbances, including the application of fungicides. The increasing use of fungicides in modern agriculture, although beneficial for controlling crop diseases, raises 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 harmful pathogens but also affect beneficial and neutral fungi. Mycorrhizal fungi, for example, are crucial partners for most plants, aiding in nutrient uptake and stress tolerance. Studies have shown that repeated or excess

ive fungicide use can reduce mycorrhizal colonization and spore production, thereby impairing plant–fungal symbiosis (Li *et al.*, 2020). Furthermore, fungicides may alter the abundance and richness of fungal taxa, leading to a shift in community structure and potential dominance of resistant strains or opportunistic pathogens (Qi *et al.*, 2024).

The soil mycobiome is known for its resilience, yet prolonged exposure to chemical agents may lead to functional loss and long-term degradation. For instance, fungicide residues may accumulate in soil and affect fungal enzyme activity, which in turn disrupts nutrient cycling processes like nitrogen mineralization and phosphorus solubilization. This chemical disturbance may also indirectly affect bacterial populations through fungal–bacterial interactions, potentially reducing overall soil microbial diversity and function (Zhang *et al.*, 2022). Thus, while fungicides offer immediate protection for crops, their broader ecological consequences require careful 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 effects than contact fungicides (Liu *et al.*, 2021).

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

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

t (IPM), crop rotation, use of biological control agents, and reduced reliance on chemical fungicides can help maintain a balanced soil mycobiome. 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 (Gao *et al.*, 2020). While fungicides are indispensable tools in modern agriculture, their non-target effects on the soil mycobiome warrant greater attention. Disruptions in fungal diversity and function can have cascading impacts on soil health, plant productivity, and ecosystem sustainability. Future agricultural practices must strike a balance between disease control and microbial conservation. Continuous monitoring, adoption of environmentally friendly alternatives, and awareness of fungicide–mycobiome interactions are essential steps toward resilient and ecologically sound food production systems.

1.1 Literature Review

According to Guo *et al.* (2020), fungicides are extensively applied in modern agriculture to combat fungal pathogens; however, their impact extends beyond target organisms to non-target soil fungal communities. The study revealed that chlorothalonil, a commonly used broad-spectrum fungicide, led to a significant reduction in fungal diversity and altered community composition in treated soils. Sensitive species were suppressed, while resistant strains became more prevalent. Additionally, key enzymatic activities involved in nutrient cycling, such as β -glucosidase and urease, were impaired, indicating a disruption in essential soil biochemical processes 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 applications in agricultural fields reduced AMF colonization and spore density, thereby impairing phosphorus uptake and contributing to a decline in crop yield. The disruption of the mutualistic root-fungal relationship further u

underscores the indirect negative influence of chemical fungicides on plant 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 findings showed that systemic fungicides had a more profound and long-lasting effect compared to contact fungicides. Notably, systemic fungicides suppressed the richness of *Ascomycota* and *Basidiomycota*, which play essential roles in organic matter decomposition. These effects could lead to a decline in soil organic matter breakdown, hinder carbon cycling, and negatively affect soil fertility over time.

In the context of fungicide types commonly used in agricultural practices, products such as Red Force, Black Force, and Ultimax Plus have gained popularity due to their broad-spectrum antifungal activity. Red Force typically contains difenoconazole, which interferes with fungal sterol biosynthesis, 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 disease control, continuous application of these fungicides has been associated with the suppression of beneficial soil fungi, emergence of resistant fungal strains, and alterations in the natural microbial equilibrium of the soil.

Chen *et al.* (2020) emphasized the significance of soil characteristics in modulating fungicide effects. For example, soil pH and organic matter content influence the persistence and toxicity of fungicides. Acidic soils tend to retain fungicides longer, enhancing their adverse impact on fungal populations, whereas soils rich in organic matter can adsorb fungicide residues, mitigating their toxicity. Moreover, soil texture and color are critical in determining microbial activity and fungicide interactions. Sandy soils, with larger pore spaces and low organic content, facilitate leaching of fungicides and reduce their retention time. In contrast, loamy and clay

soils, which have finer particles and higher moisture retention, can accumulate more fungicide residues, increasing potential toxicity to non-target organisms. Darker-colored soils are often associated with higher organic content and microbial biomass, which could either buffer or amplify fungicide effects depending on the compound involved.

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

To mitigate these adverse effects, Gao *et al.* (2020) recommended environmentally sustainable alternatives such as the use of biocontrol agents (*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 management (IPM) frameworks, along with careful soil monitoring and dose reduction, is essential to preserve soil biodiversity and sustain long-term agricultural 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 agricultural 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

impact on non-target soil fungal communities that are essential for nutrient cycling, organic matter decomposition, and plant–microbe interactions. 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 fungicides affect key fungal groups, such as mycorrhizal and saprophytic 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 diversity and abundance of fungal species in agricultural soil.
- To determine the changes in community composition and functional 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 manufacturer's dosage guidelines (González-Pérez *et al.*, 2020).

2.2 Preparation of Sample

Soil samples were collected from five distinct locations within Kwara State Polytechnic, Ilorin: ARA, Akuo, Tourism, Village, and Yankari. Using a sterile hand trowel, samples were taken at a depth of 5–15 cm. About 200 grams of soil was collected from each site and transported to the microbiology laboratory in sterile polythene bags. Each soil sample was thoroughly mixed and divided into two equal portions of 100 grams: one labeled 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 Force, 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. Control 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 soil sample was measured using a calibrated digital pH meter. The readings were taken by inserting the electrode into a soil-water slurry prepared in 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 (Wu *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 ($27 \pm 2^\circ\text{C}$), 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 flasks and incubated at ambient room temperature ($27 \pm 2^{\circ}\text{C}$) for 7 days. To ensure even distribution of the fungicides and promote aeration, distilled water was added to the samples and they were shaken gently once daily to prevent dryness. This incubation period allowed for the fungicides to interact with the soil fungal communities (Kavamura & Esposito, 2020).

2.7 Identification of Fungi

Fungal colonies that emerged on the SDA plates were first evaluated based on their macroscopic characteristics such as colony color, texture, and growth rate. Distinct colonies were subcultured for purification. Microscopic 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 treated

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

