ISOLATION OF FUNGAL SPECIES FROM SELECTED AGRICULTURAL FARMLAND SOIL

BY

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE UNIVERSITY OF BENIN, BENIN CITY, AWARD OF BACHELOR OF SCIENCE (B.Sc HONS) DEGREE

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CERTIFICATION

This is to certify that this project work was carried out by Precious Osawonamen OSAYI (Miss) of the Department of Microbiology, Faculty of Life Science, University of Benin, Benin City.

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DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies throughout my period of study.

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ABSTRACT

This study focused on isolating and identifying fungal species associated with agricultural farmland soil in UNIBEN. Soil samples were collected in sterile plastic ziplock bags from selected agricultural farmlands in Benin City, Edo State, and were subsequently transported to the laboratory for fungal identification. The identification of fungal isolates was conducted using cultural and morphological methods. The results indicated that total fungal counts of soil samples collected from the selected farmlands ranged from 5.70±0.42 to 12.50±1.56. The highest fungal counts were observed in Farm 2 (12.50±1.56), followed by Farm 5 (8.70±0.71), while Farm 4 exhibited the lowest count at 5.70±0.42. The identified fungal isolates included *Aspergillus niger, Trichoderma* sp., *Penicillium* sp., *Rhizopus arrhizus*, and *Mucor mucedo*. The percentages of occurrence for these fungal isolates varied from 7.14% to 28.57%. *Aspergillus niger* had the highest occurrence rate at 28.57%, followed by *Trichoderma* sp. and *Penicillium* sp., each at 21.43%, while *Rhizopus arrhizus* had the lowest occurrence at 7.14%. This study emphasizes the importance of understanding the dynamics of fungal populations within these agricultural soils to inform management strategies aimed at enhancing soil health. By monitoring these microbial communities, farmers at these sites can adopt more effective agronomic practices that capitalize on beneficial fungi while minimizing the impact of pathogenic species.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

It is widely accepted that the continuously growing human population needs rapid solutions to respond to the increased global demand for high agricultural productivity. These efforts should, at the same time, follow the principles of sustainability and circular economy (Watanabe *et al.,* 2017). It is well known that a gram of undisturbed soil contains thousands of individual microbial taxa including bacteria, fungi, protists, oomycetes and viruses, which determine soil fertility and enhance plant growth and health. The best and most logical way to improve soil fertility and increase plant growth is to use and manage plant beneficial microorganisms. Until recently, chemical fertilization was widely used in soil–plant systems, but this resulted in a decrease in soil natural fertility, plant diversity and microbial richness. In addition to the overload of chemical fertilizers, an increasing number of stress factors, such as salinity, alkalinity/acidity, contamination, nutrient deficiency or drought, soil disturbance due to climate change, and various biotic factors, are affecting the overall soil plant characteristics (Weiss *et al.,* 2019).

The use of plant-beneficial microorganisms, including combinations of pro- and postbiotics, is now a common practice applied to manage and stimulate the existing beneficial microbiome to mitigate all these problems in plant production, bearing also in mind the principles of modern, sustainable agriculture in the conditions of the increasing world population and environmental and climate changes. During the last few years, a large number of plant-beneficial microorganisms have been isolated and tested in controlled and natural conditions (Winder and Shamoun, 2016). The results confirmed the beneficial effect of the selected microorganisms on plant growth and health, enhancing nutrient content and improving soil properties. Among soil microbes, fungal communities play important roles in agriculture and the soil environment. Many fungal microorganisms are known for their potential biotechnological applications including in agriculture as they synthesize functional bioactive compounds for plant growth promotion and serve as biocontrol agents (Wolińska *et al.,* 2017). Within the vast fungal diversity associated with plant systems, arbuscular mycorrhizal fungi (AMF) occupy a special place due to their almost universal soil occupation and role in plant nutrition and health, particularly in conditions of the changing climate and soils with very low fertility due to contamination or desertification.

Fungi are relatively understudied, and we know very little not only regarding specific fungal interactions with other soil organisms but also the factors that determine the fungal role in plant microbial interactions. We should study more and learn how to explore fungi more effectively in the frame of sustainable agriculture following the 3-P strategy (prebiotics, probiotics, and postbiotics). The main lines of study in the field of fungal microorganisms and their role in sustainable agricultural practice could be gathered into three groups (Yang *et al.,* 2017): AM fungi; P-solubilizing fungi, and fungi involved in bioremediation activities. The benefits of mycorrhizal fungi have gained significant attention in agricultural practices. The use of mycorrhizal and other fungal inoculants can improve crop yields, reduce the need for chemical fertilizers and pesticides, and enhance soil health but also facilitate plant resistance mechanisms against various stress conditions like salinity, drought, and temperature fluctuations and various types of toxicity. Thus, fungi also act as an essential part of the plant microbiome offering sustainable agriculture by ensuring ecosystem modulation and phytobiome engineering for successful crop production (Zhou *et al.,* 2019). Fungi play a vital role in improving plant nutrition, particularly phosphorus acquisition. Phosphorus is a crucial macronutrient required for various cellular processes and energy production. However, it is often present in the soil in forms that are poorly accessible to plants. Fungi like *A. niger* boost the growth of plants, particularly offering a promising bio-input for vegetable seedling production (Žifčáková *et al.,* 2019). The emphasis of the scientific activity in the field of microbial fungal inoculants is on developing environmentally friendly and efficient microbial formulations and analyze how the introduced fungi affect microbial community, diversity, and the specific plant–microbial interactions, which determine the plant holobiome functioning.

The significance of fungi in agricultural soil cannot be overstated, as they contribute to a myriad of ecological processes that underpin soil fertility and plant health. Fungi are not merely passive inhabitants of soil; rather, they engage in complex interactions with other soil organisms, including bacteria, plants, and invertebrates (Thomson *et al.,* 2018). Their roles range from decomposers of organic matter to mutualistic partners in symbiotic relationships with plants. Understanding the diversity of fungal species present in agricultural soils is essential for developing sustainable agricultural practices that enhance soil health and productivity. As agriculture faces increasing pressures from climate change, soil degradation, and the need for higher yields, the role of fungi becomes even more critical in fostering resilience and sustainability in farming systems (Tian *et al.,* 2020).

1.2 Aim and Objectives

The aim of this study is to isolate and identify fungi species associated with agricultural farmland soil.

The specific objectives of the study are to:

1. enumerate and isolate, the bacterial population associated with agricultural farm land soil.
2. determine the prevalence of bacterial species present in the agricultural farm land soil.

CHAPTER TWO

LITERATURE REVIEW

2.1 Soil

Soil, also commonly referred to as earth or dirt, is a mixture of organic matter, minerals, gases, liquids, and organisms that together support the life of plants and soil organisms. Some scientific definitions distinguish dirt from soil by restricting the former term specifically to displaced soil. Soil consists of a solid phase of minerals and organic matter (the soil matrix), as well as a porous phase that holds gases (the soil atmosphere) and water (the soil solution) (Treseder and Lennon, 2019). Accordingly, soil is a three-state system of solids, liquids, and gases. Soil is a product of several factors: the influence of climate, relief (elevation, orientation, and slope of terrain), organisms, and the soil's parent materials (original minerals) interacting over time. It continually undergoes development by way of numerous physical, chemical and biological processes, which include weathering with associated erosion. Given its complexity and strong internal connectedness, soil ecologists regard soil as an ecosystem. Most soils have a dry bulk density between 1.1 and 1.6 g/cm^3, though the soil particle density is much higher, in the range of 2.6 to 2.7 g/cm^3 (Turner *et al.,* 2023).

2.1.1 Soil Composition

A typical soil is about 50% solids (45% mineral and 5% organic matter), and 50% voids (or pores) of which half is occupied by water and half by gas. The percent soil mineral and organic content can be treated as a constant (in the short term), while the percent soil water and gas content is considered highly variable whereby a rise in one is simultaneously balanced by a reduction in the other (Uroz *et al.,* 2023). The pore space allows for the infiltration and movement of air and water, both of which are critical for life existing in soil.[34] Compaction, a common problem with soils, reduces this space, preventing air and water from reaching plant roots and soil organisms.

Given sufficient time, an undifferentiated soil will evolve a soil profile that consists of two or more layers, referred to as soil horizons. These differ in one or more properties such as in their texture, structure, density, porosity, consistency, temperature, color, and reactivity (van der Heijden *et al.,* 2018). The horizons differ greatly in thickness and generally lack sharp boundaries; their development is dependent on the type of parent material, the processes that modify those parent materials, and the soil-forming factors that influence those processes. The biological influences on soil properties are strongest near the surface, though the geochemical influences on soil properties increase with depth. Mature soil profiles typically include three basic master horizons: A, B, and C. The solum normally includes the A and B horizons. The living component of the soil is largely confined to the solum, and is generally more prominent in the A horizon (van West *et al.,* 2023). It has been suggested that the pedon, a column of soil extending vertically from the surface to the underlying parent material and large enough to show the characteristics of all its horizons, could be subdivided in the humipedon (the living part, where most soil organisms are dwelling, corresponding to the humus form), the copedon (in intermediary position, where most weathering of minerals takes place) and the lithopedon (in contact with the subsoil). The soil texture is determined by the relative proportions of the individual particles of sand, silt, and clay that make up the soil (Małecka *et al.,* 2019).

The interaction of the individual mineral particles with organic matter, water, gases via biotic and abiotic processes causes those particles to flocculate (stick together) to form aggregates or peds. Where these aggregates can be identified, a soil can be said to be developed, and can be described further in terms of color, porosity, consistency, reaction (acidity), etc.

Water is a critical agent in soil development due to its involvement in the dissolution, precipitation, erosion, transport, and deposition of the materials of which a soil is composed (Michielse and Rep, 2019). The mixture of water and dissolved or suspended materials that occupy the soil pore space is called the soil solution. Since soil water is never pure water, but contains hundreds of dissolved organic and mineral substances, it may be more accurately called the soil solution. Water is central to the dissolution, precipitation and leaching of minerals from the soil profile. Finally, water affects the type of vegetation that grows in a soil, which in turn affects the development of the soil, a complex feedback which is exemplified in the dynamics of banded vegetation patterns in semi-arid regions.

Soils supply plants with nutrients, most of which are held in place by particles of clay and organic matter (colloids) The nutrients may be adsorbed on clay mineral surfaces, bound within clay minerals (absorbed), or bound within organic compounds as part of the living organisms or dead soil organic matter (Małecka *et al.,* 2019). These bound nutrients interact with soil water to buffer the soil solution composition (attenuate changes in the soil solution) as soils wet up or dry out, as plants take up nutrients, as salts are leached, or as acids or alkalis are added.

Plant nutrient availability is affected by soil pH, which is a measure of the hydrogen ion activity in the soil solution. Soil pH is a function of many soil forming factors, and is generally lower (more acidic) where weathering is more advanced.

Most plant nutrients, with the exception of nitrogen (Michielse and Rep, 2019), originate from the minerals that make up the soil parent material. Some nitrogen originates from rain as dilute nitric acid and ammonia,but most of the nitrogen is available in soils as a result of nitrogen fixation by bacteria. Once in the soil-plant system, most nutrients are recycled through living organisms, plant and microbial residues (soil organic matter), mineral-bound forms, and the soil solution. Both living soil organisms (microbes, animals and plant roots) and soil organic matter are of critical importance to this recycling, and thereby to soil formation and soil fertility. Microbial soil enzymes may release nutrients from minerals or organic matter for use by plants and other microorganisms, sequester (incorporate) them into living cells, or cause their loss from the soil by volatilisation (loss to the atmosphere as gases) or leaching (Morrien *et al.,* 2017).

2.1.2 Soil biodiversity

Large numbers of microbes, animals, plants and fungi are living in soil. However, biodiversity in soil is much harder to study as most of this life is invisible, hence estimates about soil biodiversity have been unsatisfactory. A recent study suggested that soil is likely home to 59 ± 15% of the species on Earth. Enchytraeidae (worms) have the greatest percentage of species in soil (98.6%), followed by fungi (90%), plants (85.5%), and termites (Isoptera) (84.2%). Many other groups of animals have substantial fractions of species living in soil, e.g. about 30% of insects, and close to 50% of arachnids.While most vertebrates live above ground (ignoring aquatic species), many species are fossorial, that is, they live in soil, such as most blind snakes (Nguyen *et al.,* 2018).

2.2 Soil Fungi

A fungus is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as one of the traditional eukaryotic kingdoms, along with Animalia, Plantae and either Protista or Protozoa and Chromista. A characteristic that places fungi in a different kingdom from plants, bacteria, and some protists is chitin in their cell walls. Fungi, like animals, are heterotrophs; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi do not photosynthesize (Pal *et al.,* 2019). Growth is their means of mobility, except for spores (a few of which are flagellated), which may travel through the air or water. Fungi are the principal decomposers in ecological systems. These and other differences place fungi in a single group of related organisms, named the Eumycota (true fungi or Eumycetes), that share a common ancestor (i.e. they form a monophyletic group), an interpretation that is also strongly supported by molecular phylogenetics. This fungal group is distinct from the structurally similar myxomycetes (slime molds) and oomycetes (water molds). The discipline of biology devoted to the study of fungi is known as mycology (from the Greek μύκης mykes, mushroom). In the past, mycology was regarded as a branch of botany, although it is now known that fungi are genetically more closely related to animals than to plants (Phosri *et al.,* 2022).

Abundant worldwide, most fungi are inconspicuous because of the small size of their structures, and their cryptic lifestyles in soil or on dead matter. Fungi include symbionts of plants, animals, or other fungi and also parasites. They may become noticeable when fruiting, either as mushrooms or as molds. Fungi perform an essential role in the decomposition of organic matter and have fundamental roles in nutrient cycling and exchange in the environment. They have long been used as a direct source of human food, in the form of mushrooms and truffles (Porras-Alfaro *et al.,* 2021); as a leavening agent for bread; and in the fermentation of various food products, such as wine, beer, and soy sauce. Since the 1940s, fungi have been used for the production of antibiotics, and, more recently, various enzymes produced by fungi are used industrially and in detergents. Fungi are also used as biological pesticides to control weeds, plant diseases, and insect pests. Many species produce bioactive compounds called mycotoxins, such as alkaloids and polyketides, that are toxic to animals, including humans. The fruiting structures of a few species contain psychotropic compounds and are consumed recreationally or in traditional spiritual ceremonies. Fungi can break down manufactured materials and buildings, and become significant pathogens of humans and other animals (Porras-Alfaro *et al.,* 2014). Losses of crops due to fungal diseases (e.g., rice blast disease) or food spoilage can have a large impact on human food supplies and local economies.

The fungus kingdom encompasses an enormous diversity of taxa with varied ecologies, life cycle strategies, and morphologies ranging from unicellular aquatic chytrids to large mushrooms. However, little is known of the true biodiversity of the fungus kingdom, which has been estimated at 2.2 million to 3.8 million species. Of these, only about 148,000 have been described, with over 8,000 species known to be detrimental to plants and at least 300 that can be pathogenic to humans. Ever since the pioneering 18th and 19th century taxonomical works of Carl Linnaeus, Christiaan Hendrik Persoon, and Elias Magnus Fries, fungi have been classified according to their morphology (e.g., characteristics such as spore color or microscopic features) or physiology (Geiser *et al.,* 2024). Advances in molecular genetics have opened the way for DNA analysis to be incorporated into taxonomy, which has sometimes challenged the historical groupings based on morphology and other traits. Phylogenetic studies published in the first decade of the 21st century have helped reshape the classification within the fungi kingdom, which is divided into one subkingdom, seven phyla, and ten subphyla.

2.2.1 Diversity of Fungi in Agricultural Soil

Fungal diversity in agricultural soils is vast, encompassing various taxonomic groups, including Ascomycota, Basidiomycota, Zygomycota, and Glomeromycota. Each of these groups contains numerous species that exhibit distinct ecological roles and adaptations to soil environments. The richness of fungal diversity is not only a testament to their evolutionary success but also highlights their importance in maintaining ecological balance within agricultural ecosystems (Girisha *et al.,* 2023). Fungi are very successful inhabitants of soil, due to their high plasticity and their capacity to adopt various forms in response to adverse or unfavorable conditions. Due to their ability to produce a wide variety of extracellular enzymes, they are able to break down all kinds of organic matter, decomposing soil components and thereby regulating the balance of carbon and nutrients. Fungi convert dead organic matter into biomass, carbon dioxide, and organic acids. Many species of fungi possess the ability to act as an effective biosorbent of toxic metals such as cadmium, copper, mercury, lead, and zinc, by accumulating them in their fruiting bodies. Though these elements may inhibit their growth and affect their reproduction (Gonzalez *et al.,* 2021). The diversity and activity of fungi is regulated by various biotic (plants and other organisms) and abiotic (soil pH, moisture, salinity, structure, and temperature) factors. Fungi can be found in almost every environment and can live in wide range of pH and temperature.

Soil fungi can be classified into three functional groups including: (1) biological controllers, (2) ecosystem regulators, and (3) species participating in organic matter decomposition and compound transformations. Ecosystem regulators are responsible for soil structure formation and modification of habitats for other organisms by regulating the dynamics of physiological processes in the soil environment. Biological controllers can regulate diseases, pests, and the growth of other organisms (Goss and deVarennes, 2022). For example, the mycorrhizal fungi improve plant growth by increasing the uptake of nutrients and protect them against pathogens. Fungal populations are strongly influenced by the diversity and composition of the plant community and in return affect plant growth through mutualism, pathogenicity and their effect on nutrient availability and cycling. Moreover, fungi participate in nitrogen fixation, hormone production, biological control against root pathogens and protection against drought. They also play an important role in stabilization of soil organic matter and decomposition of residues (Gweon *et al.,* 2019).

2.2.2 Ascomycota

Ascomycota, commonly referred to as sac fungi, are characterized by their unique reproductive structures known as asci. This phylum includes a wide range of species, some of which are significant decomposers in agricultural soils. For instance, species such as *Aspergillus niger* and *Penicillium chrysogenum* are known for their ability to break down complex organic materials, thereby facilitating nutrient cycling. Furthermore, certain Ascomycota species form mycorrhizal associations with plants, enhancing nutrient uptake and improving plant resilience to environmental stressors. These fungi can also produce secondary metabolites that have applications in biotechnology and medicine, showcasing their multifaceted importance beyond agriculture (Hannula *et al.,* 2017).

Ascomycota or Endomycopsis, is a phylum of the kingdom Fungi that, together with the Basidiomycota, forms the subkingdom Dikarya. Its members are commonly known as the sac fungi or ascomycetes. It is the largest phylum of Fungi, with over 64,000 species. The defining feature of this fungal group is the "ascus", a microscopic sexual structure in which nonmotile spores, called ascospores, are formed. However, some species of Ascomycota are asexual and thus do not form asci or ascospores. Familiar examples of sac fungi include morels, truffles, brewers' and bakers' yeast, dead man's fingers, and cup fungi. The fungal symbionts in the majority of lichens (loosely termed "ascolichens") such as Cladonia belong to the Ascomycota (Högberg and Högberg, 2022).

Ascomycota is a monophyletic group (containing all of the descendants of a common ancestor). Previously placed in the Basidiomycota along with asexual species from other fungal taxa, asexual (or anamorphic) ascomycetes are now identified and classified based on morphological or physiological similarities to ascus-bearing taxa, and by phylogenetic analyses of DNA sequences (Hannula and van Veen, 2016).

Ascomycetes are of particular use to humans as sources of medicinally important compounds such as antibiotics, as well as for fermenting bread, alcoholic beverages, and cheese. Examples of ascomycetes include Penicillium species on cheeses and those producing antibiotics for treating bacterial infectious diseases.

Many ascomycetes are pathogens, both of animals, including humans, and of plants. Examples of ascomycetes that can cause infections in humans include Candida albicans, Aspergillus niger and several tens of species that cause skin infections (Hesse *et al.,* 2019). The many plant-pathogenic ascomycetes include apple scab, rice blast, the ergot fungi, black knot, and the powdery mildews. The members of the genus Cordyceps are entomopathogenic fungi, meaning that they parasitise and kill insects. Other entomopathogenic ascomycetes have been used successfully in biological pest control, such as Beauveria. Several species of ascomycetes are biological model organisms in laboratory research. Most famously, Neurospora crassa, several species of yeasts, and Aspergillus species are used in many genetics and cell biology studies.

2.2.3 Basidiomycota

Basidiomycota, or club fungi, are another prominent group found in agricultural soils. This phylum includes mushrooms, puffballs, and shelf fungi, many of which play critical roles in decomposing lignin and cellulose in plant materials. Species such as *Ganoderma lucidum* and

Trametes versicolor are notable for their ligninolytic capabilities, which contribute to the breakdown of woody debris and enhance soil organic matter (Huhe *et al.,* 2017). Additionally, some Basidiomycota species form ectomycorrhizal relationships with trees, facilitating nutrient exchange and improving soil structure. The fruiting bodies of these fungi, often visible above ground, also serve as important food sources for various wildlife, further integrating fungi into the broader ecosystem.

Basidiomycota is one of two large divisions that, together with the Ascomycota, constitute the subkingdom Dikarya (often referred to as the "higher fungi") within the kingdom Fungi. Members are known as basidiomycetes. More specifically, Basidiomycota includes these groups: agarics, puffballs, stinkhorns, bracket fungi, other polypores, jelly fungi, boletes, chanterelles, earth stars, smuts, bunts, rusts, mirror yeasts, and Cryptococcus, the human pathogenic yeast (Watanabe *et al.,* 2017).

Basidiomycota are filamentous fungi composed of hyphae (except for basidiomycota-yeast) and reproduce sexually via the formation of specialized club-shaped end cells called basidia that normally bear external meiospores (usually four). These specialized spores are called basidiospores. However, some Basidiomycota are obligate asexual reproducers. Basidiomycota that reproduce asexually (discussed below) can typically be recognized as members of this division by gross similarity to others, by the formation of a distinctive anatomical feature (the clamp connection), cell wall components, and definitively by phylogenetic molecular analysis of DNA sequence data (Weiss *et al.,* 2019).

Basidiomycota tend to have mutually indistinguishable, compatible haploids which are usually mycelia being composed of filamentous hyphae. Typically haploid Basidiomycota mycelia fuse via plasmogamy and then the compatible nuclei migrate into each other's mycelia and pair up with the resident nuclei. Karyogamy is delayed, so that the compatible nuclei remain in pairs, called a dikaryon. The hyphae are then said to be dikaryotic. Conversely, the haploid mycelia are called monokaryons. Often, the dikaryotic mycelium is more vigorous than the individual monokaryotic mycelia, and proceeds to take over the substrate in which they are growing. The dikaryons can be long-lived, lasting years, decades, or centuries (Winder and Shamoun, 2016). The monokaryons are neither male nor female. They have either a bipolar (unifactorial) or a tetrapolar (bifactorial) mating system. This results in the fact that following meiosis, the resulting haploid basidiospores and resultant monokaryons, have nuclei that are compatible with 50% (if bipolar) or 25% (if tetrapolar) of their sister basidiospores (and their resultant monokaryons) because the mating genes must differ for them to be compatible. However, there are sometimes more than two possible alleles for a given locus, and in such species, depending on the specifics, over 90% of monokaryons could be compatible with each other.

The maintenance of the dikaryotic status in dikaryons in many Basidiomycota is facilitated by the formation of clamp connections that physically appear to help coordinate and re-establish pairs of compatible nuclei following synchronous mitotic nuclear divisions (Wolińska *et al.,* 2017). Variations are frequent and multiple. In a typical Basidiomycota lifecycle the long lasting dikaryons periodically (seasonally or occasionally) produce basidia, the specialized usually club-shaped end cells, in which a pair of compatible nuclei fuse (karyogamy) to form a diploid cell. Meiosis follows shortly with the production of 4 haploid nuclei that migrate into 4 external, usually apical basidiospores. Variations occur, however. Typically the basidiospores are ballistic, hence they are sometimes also called ballistospores. In most species, the basidiospores disperse and each can start a new haploid mycelium, continuing the lifecycle. Basidia are microscopic but they are often produced on or in multicelled large fructifications called basidiocarps or basidiomes, or fruitbodies, variously called mushrooms, puffballs, etc (Yang *et al.,* 2017). Ballistic basidiospores are formed on sterigmata which are tapered spine-like projections on basidia, and are typically curved, like the horns of a bull. In some Basidiomycota the spores are not ballistic, and the sterigmata may be straight, reduced to stubs, or absent. The basidiospores of these non-ballistosporic basidia may either bud off, or be released via dissolution or disintegration of the basidia.

2.2.4 Zygomycota

Zygomycota, although less diverse than Ascomycota and Basidiomycota, includes important soil fungi such as *Rhizopus stolonifer*, which is known for its role in decomposing organic matter. These fungi are typically saprophytic, thriving on decaying plant material and contributing to nutrient cycling within the soil ecosystem. Their ability to rapidly colonize organic substrates makes them vital players in the decomposition process, ensuring that nutrients are recycled efficiently back into the soil for plant use (Zhou *et al.,* 2019).

Zygomycota, or zygote fungi, is a former division or phylum of the kingdom Fungi. The members are now part of two phyla: the Mucoromycota and Zoopagomycota. Approximately 1060 species are known. They are mostly terrestrial in habitat, living in soil or on decaying plant or animal material. Some are parasites of plants, insects, and small animals, while others form symbiotic relationships with plants. Zygomycete hyphae may be coenocytic, forming septa only where gametes are formed or to wall off dead hyphae. Zygomycota is no longer recognised as it was not believed to be truly monophyletic (Žifčáková *et al.,* 2019).

The zygomycetes are able to grow in a wide range of environments. Most of them are mesophilic (growing at 10–40 °C with an optimum 20–35 °C), but some, like Mucor miehei or Mucor pusillus, are thermophilic with a minimum growth temperature of about 20 °C and maximum extending up to 60 °C. Others like Mucor hiemalis can grow at temperatures below 0 °C.

Some species of the order Mucorales are able to grow under anaerobic conditions, while most of them require aerobic conditions. Furthermore, while the majority of the zygomycetes only grow at high water activities, some of them are able to grow in salt concentrations of at least 15%. Most species of Mucor grow rapidly on agar at room temperature filling the Petri dish in 2–3 days with their coarse aerial mycelium (Thomson *et al.,* 2018). When incubated in liquid culture under semi-anaerobic conditions, several species grow in yeast like state. Zygospore formation may be stimulated at higher temperatures of incubation (30–40 °C).

Growth of Zygomycota in solid agar can produce low or very high fibrous colony that rapidly fills the entire Petri dish. Its color may range from pure white to shades of gray or brown. In old cultures, dark pigmented sporangia are observed. Everything depends on the species and the media used. In liquid culture, Zygomycota usually form a bland mass and do not produce spores. This is because they cannot grow aerial hyphae. Zygomycetes grow well on most standard fungal culture medium such as Sabouraud dextrose agar. They can also grow on both selective and non-selective media (Tian *et al.,* 2020). Minimal media, supplementary media and induction media can also be used. Most zygomycetes are sensitive to cycloheximide (actidione) and this agent should not be used in culture media.

2.2.5 Glomeromycota

Glomeromycota is a unique phylum that includes arbuscular mycorrhizal fungi (AMF), which form symbiotic relationships with the roots of most terrestrial plants. These fungi are essential for enhancing plant nutrient uptake, particularly phosphorus, and improving soil structure through the formation of hyphal networks. The presence of AMF in agricultural soils is associated with increased crop yields and improved soil health. Research has shown that crops grown in association with AMF exhibit enhanced drought tolerance and nutrient efficiency, making these fungi indispensable allies in sustainable agriculture (Treseder and Lennon, 2019).

Glomeromycota are one of eight currently recognized divisions within the kingdom Fungi, with approximately 230 described species. Members of the Glomeromycota form arbuscular mycorrhizas (AMs) with the thalli of bryophytes and the roots of vascular land plants. Not all species have been shown to form AMs, and one, *Geosiphon pyriformis*, is known not to do so. Instead, it forms an endocytobiotic association with Nostoc cyanobacteria. The majority of evidence shows that the Glomeromycota are dependent on land plants (Nostoc in the case of Geosiphon) for carbon and energy, but there is recent circumstantial evidence that some species may be able to lead an independent existence (Turner *et al.,* 2023). The arbuscular mycorrhizal species are terrestrial and widely distributed in soils worldwide where they form symbioses with the roots of the majority of plant species (>80%). They can also be found in wetlands, including salt-marshes, and associated with epiphytic plants.

According to multigene phylogenetic analyses, this taxon is located as a member of the phylum Mucoromycota. Currently, the phylum name Glomeromycota is invalid, and the subphylum Glomeromycotina should be used to describe this taxon.

New colonization of AM fungi largely depends on the amount of inoculum present in the soil. Although pre-existing hyphae and infected root fragments have been shown to colonize the roots of a host successfully, germinating spores are considered to be the key players in new host establishment (Uroz *et al.,* 2023). Spores are commonly dispersed by fungal and plant burrowing herbivore partners, but some air dispersal capabilities are also known. Studies have shown that spore germination is specific to particular environmental conditions such as right amount of nutrients, temperature or host availability. It has also been observed that the rate of root system colonization is directly correlated to spore density in the soil. In addition, new data also suggests that AM fungi host plants also secrete chemical factors that attract and enhance the growth of developing spore hyphae towards the root system (van der Heijden *et al.,* 2018).

The necessary components for the colonization of Glomeromycota include the host's fine root system, proper development of intracellular arbuscular structures, and a well-established external fungal mycelium. Colonization is accomplished by the interactions between germinating spore hyphae and the root hairs of the host or by the development of appressoria between epidermal root cells. The process is regulated by specialized chemical signaling and changes in gene expression of both the host and AM fungi. Intracellular hyphae extend up to the cortical cells of the root and penetrate the cell walls but not the inner cellular membrane creating an internal invagination (van West *et al.,* 2023). The penetrating hyphae develop a highly branched structure called an arbuscule, which has low functional periods before degradation and absorption by the host's root cells. A fully developed arbuscular mycorrhizal structure facilitates the two-way movement of nutrients between the host and mutualistic fungal partner. The symbiotic association allows the host plant to respond better to environmental stresses, and the non-photosynthetic fungi to obtain carbohydrates produced by photosynthesis.

2.3 Ecological Functions of Fungi in Agricultural Soil

The ecological functions of fungi in agricultural soils are multifaceted and encompass several critical processes that contribute to soil health and fertility. These functions include decomposition, nutrient cycling, soil structure formation, and disease suppression. Each of these processes is interconnected, highlighting the importance of fungi in maintaining a balanced and productive soil ecosystem (Małecka *et al.,* 2019).

2.3.1 Decomposition

Fungi are among the primary decomposers in terrestrial ecosystems, breaking down complex organic materials such as dead plant matter, animal remains, and other organic residues. Through the secretion of extracellular enzymes, fungi can degrade lignin, cellulose, and other polymers, thereby releasing essential nutrients back into the soil. This process not only enriches the soil but also promotes the formation of humus, which is vital for maintaining soil structure and fertility. The efficiency of fungi in decomposition is crucial for nutrient cycling, as it ensures that organic matter is recycled and made available for plant uptake, thereby supporting healthy crop growth (Michielse and Rep, 2019).

2.3.2 Nutrient Cycling

Fungi play a pivotal role in nutrient cycling, particularly in the mobilization of macronutrients such as nitrogen, phosphorus, and potassium. By decomposing organic matter, fungi release nutrients that are subsequently available for plant uptake. Moreover, mycorrhizal fungi enhance nutrient availability by extending their hyphal networks into the soil, effectively increasing the surface area for nutrient absorption. This symbiotic relationship is particularly beneficial in nutrient-poor soils, where plants may struggle to acquire sufficient resources. The ability of fungi to solubilize nutrients and make them accessible to plants is a key factor in promoting soil fertility and crop productivity (Morrien *et al.,* 2017).

Soil microorganisms, including fungi are an important component of grassland ecosystems due to their biochemical activity and engagement in nutrient cycling (Dengler et al., 2014). Grasslands provide many forms of ecosystem services including: supporting, provisioning, regulatory, and cultural services. Importantly, the role of biodiversity has been established as fundamental in ensuring the performance of ecosystem functioning. Grazing activities influence soil fungal community structure by changing edaphic conditions and the vegetation biodiversity in plant communities. It has been proven that moderate grazing sustains plants diversity while heavy grazing results in species loss. Furthermore, plant-fungal interactions can inhibit biodiversity in grasslands due to the production of different root exudates such as enzymes, organic compounds, and polysaccharides (Nguyen *et al.,* 2018).

Plant pathogenic fungi also have a large impact on plant diversity in grasslands by limiting the abundance of their hosts, affecting biomass production. The study by Phosri *et al.* (2022) suggests that fungal pathogens could affect nutrient cycling in grasslands reducing the abundance of dominant grasses and enhancing the growth of legumes. Soil fungal communities in grasslands can also be influenced by human activities and the components of long-term fertilization and other treatments (Pal *et al.,* 2019). Unlike in agricultural soils, where ascomycetes dominate, in grasslands, basidiomycetes are major decomposers of dead organic matter.

2.3.3 Soil Structure Formation

The hyphal networks formed by fungi contribute significantly to soil structure. These networks bind soil particles together, promoting the formation of aggregates that enhance soil aeration, water retention, and root penetration. Improved soil structure is essential for maintaining healthy plant growth and resilience against erosion and compaction. Additionally, the presence of fungal hyphae can create microhabitats for other soil organisms, fostering biodiversity and enhancing overall soil health (Porras-Alfaro *et al.,* 2021).

2.3.4 Disease Suppression

Fungi can also play a role in suppressing soil-borne diseases through various mechanisms, including competition for resources, production of antimicrobial compounds, and induction of plant defense responses. Certain fungal species, such as *Trichoderma*, are well-known for their biocontrol properties and are utilized in sustainable agriculture to manage plant pathogens. By promoting beneficial fungi in the soil, farmers can reduce the incidence of diseases, leading to healthier crops and reduced reliance on chemical fungicides (Porras-Alfaro *et al.,* 2014).

2.4 Interactions between Fungi and Plants

The interactions between fungi and plants are complex and can be categorized into mutualistic, pathogenic, and saprophytic relationships. Understanding these interactions is crucial for optimizing agricultural practices and enhancing crop productivity. The balance between these different types of interactions can significantly influence the health of both the soil and the plants growing within it.

Knowledge of the soil chemical and physical properties has always been of interest to foresters to evaluate the capacity of sites and to increase forest productivity (Geiser *et al.,* 2024). Forest soils (including humus, litter, and coarse woody debris) are an important reservoir of microorganisms and soil biota that in turn influence carbon storage, soil structure, fertility, productivity, and plant/tree growth.

Ectomycorrhizal associations are created by a specific group of plant families that includes the Pinaceae, Fabaceae, Betulaceae, and Fagaceae. The results of research obtained by Girisha *et al.* (2002), indicate a significant contribution by ectomycorrhizal mycelium to forest soil microbial biomass and by ectomycorrhizal roots to the production of extractable dissolved organic carbon, which is a carbon source for other microbes (Gonzalez *et al.,* 2021).

During the processes of thinning, the transfer of nutrients from aboveground biomass to forest soil takes place. A higher concentration of nutrients comes from the green litter of thinned trees than litter returned to the forest floor after senescence or from the woody residue left on the ground after harvesting. Consequently, the quality and quantity of organic substrates presented to the soil fungal community by thinned and non-thinned forests may vary to a great extent. The community of soil microorganisms depends highly on organic matter as it provides a suitable environment and energy sources for them that are critical to maintain the nutritional quality and water-retaining capacity of forest soils (Goss and deVarennes, 2022). Soil organic matter is of key relevance in maintaining soil resistance and stability, although it is uncertain how deterioration of soil properties and changes in fungal communities affect the functional stability of soils. Degradation of soil properties followed by deforestation may lead to decreases in soil fungal diversity and functional stability.

2.4.1 Mutualistic Relationships

Mutualistic relationships between fungi and plants, particularly through mycorrhizal associations, are among the most beneficial interactions in agricultural ecosystems. Mycorrhizal fungi enhance nutrient uptake, particularly phosphorus, while plants provide carbohydrates to the fungi in return. This symbiotic relationship is essential for the growth and health of many crops, particularly in nutrient-deficient soils. The benefits of mycorrhizal associations extend beyond nutrient acquisition; they also improve plant resilience to environmental stresses such as drought and soil salinity, making them invaluable in the face of climate change (Gweon *et al.,* 2019).

2.4.2 Pathogenic Relationships

Conversely, some fungi can act as pathogens, causing diseases that adversely affect plant health and agricultural productivity. Fungal pathogens such as *Fusarium*, *Rhizoctonia*, and *Phytophthora* can lead to significant crop losses. Understanding the dynamics of these pathogenic interactions is vital for developing effective disease management strategies and minimizing the impact of fungal diseases on agricultural systems. Integrated pest management approaches that incorporate biological control methods can help mitigate the effects of these pathogens while promoting a healthier soil ecosystem (Hannula *et al.,* 2017).

2.4.3 Saprophytic Relationships

Saprophytic fungi, which decompose organic matter, also interact with plants by recycling nutrients back into the soil. This process is essential for maintaining soil fertility and supporting healthy plant growth. The balance between saprophytic and pathogenic fungi is crucial for sustaining agricultural productivity. By fostering a diverse community of saprophytic fungi, farmers can enhance nutrient availability and improve soil health, ultimately leading to more resilient agricultural systems (Hannula and van Veen, 2016).

2.5 Implications for Sustainable Agriculture

The understanding of fungal diversity and their ecological roles in agricultural soils has significant implications for sustainable agricultural practices. By leveraging the beneficial aspects of fungi, farmers can enhance soil health, improve crop yields, and reduce reliance on chemical fertilizers and pesticides. The integration of fungal management strategies into agricultural practices can lead to more sustainable and resilient farming systems (Hesse *et al.,* 2019).

2.5.1 Enhancing Soil Health

Promoting fungal diversity in agricultural soils can enhance soil health by improving nutrient cycling, soil structure, and disease suppression. Practices such as cover cropping, reduced tillage, and organic amendments can foster a diverse fungal community, leading to improved soil fertility and resilience. Additionally, the use of compost and other organic materials can introduce beneficial fungi into the soil, further enhancing its health and productivity (Högberg and Högberg, 2022).

2.5.2 Integrated Pest Management

Utilizing beneficial fungi in integrated pest management strategies can reduce the need for chemical pesticides. By introducing biocontrol agents such as *Trichoderma* or other antagonistic fungi, farmers can manage soil-borne diseases and enhance plant health, ultimately leading to more sustainable agricultural systems. The incorporation of fungal biocontrol agents into pest management programs not only helps in disease suppression but also promotes a healthier soil microbiome.

2.5.3 Crop Rotation and Diversity

Implementing crop rotation and promoting plant diversity can enhance fungal diversity in agricultural soils. Different crops can support various fungal species, leading to a more resilient soil ecosystem. This practice not only improves soil health but also reduces the risk of disease outbreaks associated with monoculture systems. By rotating crops and incorporating cover crops, farmers can create a dynamic environment that supports a diverse array of fungi, ultimately benefiting overall agricultural productivity (Huhe *et al.,* 2017).

The diversity of fungi found in agricultural soils is vast and encompasses a range of species that play critical ecological roles. From decomposition and nutrient cycling to forming symbiotic relationships with plants, fungi are integral to maintaining soil health and agricultural productivity. Understanding these complex interactions and the implications for sustainable agriculture is essential for developing practices that enhance soil fertility and crop resilience. As the agricultural sector continues to face challenges related to soil degradation and climate change, the role of fungi in promoting sustainable practices will undoubtedly become increasingly significant. By embracing the ecological functions of fungi, farmers can work towards a more sustainable future, ensuring food security while preserving the health of our planet's soils (Watanabe *et al.,* 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area/Sample Collection

The study was carried out in the Benin metropolis, from selected agricultural farmlands (Soils) in UNIBEN, Edo state. Samples were transported to the laboratory for analysis within three hours of collection in sterile plastic Ziplock bags.

3.2 Sterilization of Materials

Materials such as Petri dishes, pipettes, glass containers (conical flask, round bottom flask), and bottles were washed, drained, and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160^{o}C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of a Bunsen burner flame and the disinfection of work surfaces with alcohol.

3.3 Preparation and Sterilization of Media

All media used were obtained from Oxoid and were prepared according to manufacturers' instructions. The media used in this study include Potato dextrose agar (PDA), Spirit blue, and Tryptone soya agar (TSA)

3.4 Enumeration and Isolation of Bacterial from Samples

A one thousand fold dilution was used for the analysis where the samples were diluted by mixing 10 g of soil with 40 ml of sterile saline water (SSW) and further diluted serially. After that, an inoculum volume of 0.1 ml from the 3rd tube was transferred to sterile Petri dishes, to which potato dextrose agar was added (supplemented with 1% chloramphenicol). All samples were diluted using a 5-fold, and a volume of 0.1 ml was plated. The formula employed for the dilution factor is given below in equation (1)

$$Dilution\ factor = \frac{final\ volume}{aliquot\ volume} \qquad (1)$$

Enumeration of the bacterial isolates was carried out using the formula delineated by Willey *et al*. (2008), and it is shown in equation (2) below.

$$\frac{cfu}{g} = \frac{number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ inoculum} \qquad (2)$$

3.5 Microscopic Identification of fungal isolates

After successful enumeration, the fungal isolates were subcultured on PDA and the moulds and yeasts were morphologically characterized after being stained with Lactophenol cotton blue. The results obtained were then compared with standard references for proper identification of the isolates.

A drop of lactophenol blue stain was placed on a clean grease-free sterilized glass slide and after this, a sterile inoculating wire loop was used to pick the mycelium onto the glass slide from the mould culture. The mycelium was spread evenly on the slide and then covered with coverslips gently and allowed to stay for some seconds before being observed under x40 under the microscope. The colonial and morphological characteristics of each isolate were determined, appearance of special structures including the nature of spore/ascospores if produced. The growth and the appearance of the colony from the initial to the time of maturity were also taken into consideration as well as the presence or absence of septate hyphae.

3.6 Lipase Solubilization Test

Spirit blue agar was used to identify lipolytic microorganisms capable of producing the enzyme lipase. Lipase is secreted, hydrolyzing triglycerides to glycerol and three long-chain fatty acids. These monomers are small enough to pass through the bacterial cell wall. Spirit blue medium powder was dissolved and autoclaved at 121°C for 15 minutes. Cooled and aseptically added lipoidal substrate, 5% of sterile olive oil was mixed thoroughly. The mixture was poured into Petri dishes, inoculated with isolated pure inoculums and incubated at 37°C for 24 hours. Plates were examined; colonies of the lipolytic organisms developed a cleared zone and/or a deep blue colour around and under each colony (Georgescu *et al*., 2016).

3.7 Antifugal Sensitivity Test

Voriconazole: Voriconazole (VFEND, Pfizer Ireland Pharmaceuticals, Ringaskiddy, Ireland) is a triazole antifungal agent that inhibits fungal ergosterol biosynthesis. It is structurally related to fluconazole, with the major difference being the substitution of a fluoropyrimidine grouping in place of a triazolemoiety(Hoffman *et al*.,2002).

Voriconazole in the form of antifungal discs, were placed in plates with isolated colonies streaked on PDA. The distance of visible growth around the disc determine the microorganism's sensitivity to Voriconazole

Nystatin: Nystatin is in a class of antifungal medications called polyenes. It works by stopping the growth of fungi that cause infection. Nystatin in the form of antifungal discs, were placed in plates with isolated colonies streaked on PDA. The distance of visible growth around the disc determine the microorganism's sensitivity to Nystatin.

3.7.1 Protease Test

Proteases are enzymes whose catalytic function is to hydrolyze peptide bonds of proteins into amino acids and peptides. They are part of a large group of enzymes belonging to the class of hydrolase enzymes(Vojcicv *et al*., 2015).

The agar used was prepared from a combination of Tryptone Soya Agar and Tween 80 oil. Colonies from cultures are you sreaked on the agar and incubated at 37°c for 48 hours. After 4 hours the presence of a clear zone around the colonies indicates a positive protease result and the absence of a clear zone indicates a negative protease result.

CHAPTER FOUR

RESULTS

Table 1 shows the total fungal counts of soils samples collected from different farmlands in UNIBEN. The total fungal counts of soils samples collected from selected farmlands ranged from 5.70±0.42 to 12.50±1.56. The highest fungal counts was obtained from Farm 2(12.50±1.56), follow by farm 5 (8.70±0.71), while the lowest value was seen in farm 4 (5.70±0.42) respectively. Table 2 shows the cultural, morphological and microscopic characteristics of fungal isolates from different farmlands soils in UNIBEN. The cultural characteristics used to identify the fungi isolate were margin, colour, shape and size. The morphological characteristics used were nature of hyphae, colour of spore, type of spore and appearance of special structure. The probable fungi isolates were *Aspergillus niger, Trichoderma* sp., *Penicillium* sp., *Rhizopus arrhizus* and *Mucor mucedo* respectively.

Table 3 shows the distribution of fungi species from different farmlands in UNIBEN. *Mucor mucedo* had the highest distribution frequency of 5, followed by *Aspergillus niger, Trichoderma* sp., *Penicillium* sp., *Rhizopus arrhizus* which had the less frequency distribution of 3 each respectively.

Figure 1 shows the percentage of occurrence of fungal isolates from different farmlands soil in UNIBEN. The percentage of occurrence of fungal isolates ranged from 7.14% to 28.57%. The highest percentage of occurrence was obtained from *Aspergillus niger* (28.57%) follow by *Trichoderma* sp., *Penicillium* sp. and *Mucor mucedo* having 21.43% each. While the lowest percentage of occurrence was seen in *Rhizopus arrhizus* (7.14%) respectively.

Table 1: Total fungal counts of soils from different farmlands in UNIBEN

Farmlands	Fungal Counts in Standard form (x10^4 CFU/ml)
Farm 1	8.20±0.28
Farm 2	12.50±1.56
Farm 3	6.10±0.14
Farm 4	5.70±0.42
Farm 5	8.70±0.71

Key: data presented were mean±SD from duplicate determinations

Table 2: Cultural, morphological and microscopic characteristics of fungal isolates

Cultural Morphology	**1**	**2**	**3**	**4**	**5**
The colour of mycelium on the agar plate	Dark colored growth	Green mycelium	Army green and entire, non-luxuriant with concentric ring	Initially white, with age turning gray and developing black dots	grey to off-white or white
colour of plate culture reverse	Dark	Pale yellow	Orange	light gray	black
Microscopic characteristics					
Nature of hyphae	Septate	Septate	Septate	Non- septate	Non- septate
Type of Spore	Conidiospore	Conidiospore	Conidiospore	Sporangiophores	Sporangiophores
Spore structure/Attachment	*A. niger* consists of a smooth and colourless conidiophores and spores.	Conidia size and shape are similar to *Penicillium* but *Trichoderma* forms sticky clumps of conidia with a distinctive green pigment. Typical green spore clumps are identified as Trichoderma.	clear (not pigmented) hyphae with smooth-walled conidiophores, stipes are rather long and are bi-verticillate	single and unbranched sporangiophore	sporangiospores
Rhizoids	Absent	Absent	Absent	Present	Absent
Appearance of special structure	Conidial heads radiate, becoming columnar when mature; conidiophores are long and smooth-walled; biseriate; two rows of phialides cover the entire cyst.	Conidiophores are hyaline and loosely branched at right angles. Phialides are flask-shaped and inflated at the base, with very short collarettes	Conidiophore stipes smooth-walled; phialides mono- or bi-verticillate, flask-shaped. Phialides do not show long pointed extensions at the tips	Rhizoids occur at the junction of stolon and sporangiophore	sporangia and are produced on the tips of sporangiophores. Sporangia contain spores, which are the reproductive units of Mucor
Class of fungi	Ascomycetes	Ascomycetes	Ascomycetes	Zygomycetes	Zygomycetes
Possible Identity	*Aspergillus niger*	*Trichoderma* sp.	*Penicillium* sp.	*Rhizopus arrhizus*	*Mucor mucedo*

Table 3: Distribution of Fungal species from different farmlands in UNIBEN

Sample code	*Aspergillus niger*	*Trichoderma* sp.	*Penicillium* sp.	*Rhizopus arrhizus*	*Mucor mucedo*
Farm 1	+	+	-	-	+
Farm 2	-	-	-	-	+
Farm 3	+	+	-	-	+
Farm 4	-	+	+	+	+
Farm 5	-	-	+	+	+

Key: + = present, - = absent

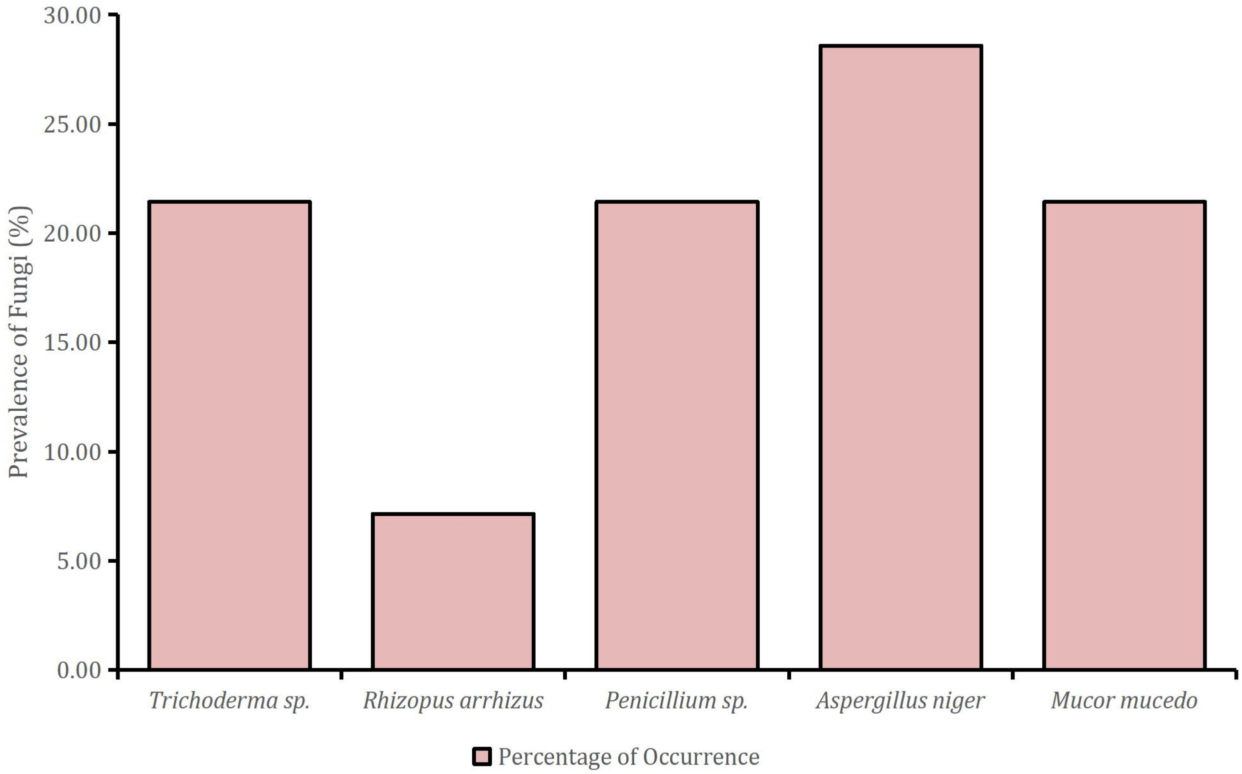

Figure 1: Percentage of occurrence of fungal isolates

CHAPTER FIVE

DISCUSSION

Fungi play a vital role in the health and sustainability of agricultural farmland soils, contributing significantly to nutrient cycling and organic matter decomposition. The biodiversity of fungal species in these environments is crucial for maintaining soil structure and fertility (Goss and deVarennes, 2022). The results obtained from the present study revealed that the total fungal counts of soils samples collected from UNIBEN farmlands ranged from 5.70±0.42 to 12.50±1.56. The highest fungal counts was obtained from Farm 2(12.50±1.56), follow by farm 5 (8.70±0.71), while the lowest value was seen in farm 4 (5.70±0.42). This is also in line with the work of Huhe *et al.* (2017) who reported fungi count of 8.65 to 16.20 x10^4 Cfu/g in soils of agricultural rice field. Phosri *et al.* (2022) reported that the prevalence of fungal diversity may largely differ depending on the soil physiochemical properties of soil and the cultivated crops. According to the research of Porras-Alfaro *et al.* (2021) agricultural practices directly influence the composition of soil fungal communities. For instance, a study by Thomson *et al.* (2018) demonstrated that specific management strategies, such as zero tillage combined with pasture/agriculture rotation, favor saprophytic fungi organisms essential for breaking down organic matter and enhancing soil health. This study highlights the importance of sustainable farming practices in promoting beneficial fungal populations while mitigating harmful pathogens that threaten crop yields (Tian *et al.,* 2020). Despite the knowledge surrounding the functional roles of various fungi within farmland soils, specific species associated with these ecosystems remain underexplored (Uroz *et al.,* 2023).Treseder and Lennon (2019) examined fungal count of 10.68 x10^4 Cfu/g in abandoned agricultural lands compared to active agricultural soils with a count of 17.90 x10^4 Cfu/g.

The results also showed that the identify fungi isolates from UNIBEN agricultural farmland soils were *Aspergillus niger, Trichoderma* sp.*, Penicillium* sp.*, Rhizopus arrhizus* and *Mucor mucedo*. The percentage of occurrence of fungal isolates ranged from 7.14% to 28.57%. The highest percentage of occurrence was obtained from *Aspergillus niger* (28.57%) follow by *Trichoderma* sp.*, Penicillium* sp. and *Mucor mucedo* having 21.43% each. While the lowest percentage of occurrence was seen in *Rhizopus arrhizus* (7.14%) respectively. This is also in accordance with the work of Wolińska *et al.* (2017) who stated that fungal isolates such as *Saccharomyces* spp., *Candida* spp., *Mucor* spp., *Penicillium* spp. and *Aspergillus niger* were isolated from mixed farming agricultural soils. According to Yang *et al.* (2017) the most prevalent fungi species isolated from agricultural soils were *Aspergillus niger* (32.1%) and *Mucor mucedo* (28.3%) respectively. These findings could be probably due to the type of soil which favors the growth of fungi and also due to fungal spores in the environment, soil and possible exposure to organic manure. Žifčáková *et al.* (2019) confirmed that *Aspergillus niger*, is a prevalent mold found in various environments, particularly in soil and decaying organic matter. While most species within this genus are harmless, certain strains can pose significant health risks to humans, especially those with compromised immune systems. The study of Zhou *et al.* (2019) shows that exposure to airborne molds like *Aspergillus niger* can lead to severe respiratory conditions such as invasive pulmonary aspergillosis and allergic reactions, its presence in soil highlights the potential for human exposure through environmental pathways.

Winder and Shamoun (2016) also reported that *Trichoderma* spp., a genus of fungi prevalent in soil ecosystems, plays a pivotal role in enhancing agricultural sustainability and indirectly influencing human health through improved food security. These fungi are recognized for their multifaceted biological functions, including acting as biocontrol agents against plant pathogens and promoting plant growth (Weiss *et al.,* 2016). The application of *Trichoderma* spp. can significantly improve soil health by increasing nutrient availability and enhancing microbial diversity, which is essential for the cultivation of healthy crops. As highlighted by van West *et al.* (2023), the adoption of *Trichoderma* as an alternative to chemical fertilizers not only supports environmental sustainability but also mitigates potential risks associated with synthetic inputs that can adversely affect human health.

According to Uroz *et al.* (2023) *Penicillium* species are a diverse and prevalent group of fungi found in various environments, particularly in soil rich in organic matter. With over 350 known species, they thrive predominantly in cool, temperate climates (Treseder and Lennon, 2019). Their ability to produce numerous tiny spores enables them to become airborne easily, which poses significant health risks when inhaled (Turner *et al.,* 2023). Inhalation of these spores can lead to a range of health issues, from allergies and respiratory infections to more severe conditions associated with mycotoxin production. The implications of pathogenic *Penicillium* strains have been documented in clinical cases where individuals with compromised immune systems developed severe infections such as pneumonia caused by *Penicillium digitatum* (Geiser *et al.,* 2024). Such instances emphasize the necessity for increased awareness regarding fungal pathogens originating from environmental sources like agricultural soils.

Girisha *et al.* (2023) stated that *Rhizopus arrhizus* and *Mucor mucedo* are ubiquitous fungi commonly found in soil and decaying organic matter. They belong to the Mucorales order, which is characterized by its ability to thrive in various environments, including compost heaps and stored grains (Gonzalez *et al.* 2021). These fungi play a significant role in the decomposition of organic materials, contributing to nutrient cycling within ecosystems. However, their ecological presence poses potential health risks, particularly for immunocompromised individuals who may be more susceptible to invasive infections. Mucormycosis is a severe fungal infection primarily associated with *Rhizopus arrhizus*. This disease can lead to significant morbidity and mortality among affected individuals (Goss and deVarennes, 2022). Although mucormycosis predominantly affects those with compromised immune systems such as patients with diabetes or undergoing immunosuppressive therapy it can also impact immunocompetent hosts under certain conditions. The inhalation of spores from these fungi or direct contact with contaminated surfaces can serve as pathways for infection. Therefore, understanding the environmental habitats of these organisms is crucial for mitigating exposure risks (Hannula *et al.,* 2016).

5.1 Conclusion

The results of this present study had showed that fungal species such as *Aspergillus niger, Trichoderma* sp.*, Penicillium* sp.*, Rhizopus arrhizus* and *Mucor mucedo* are prevalent UNIBEN agricultural farmland soils. Understanding the dynamics of fungal populations within these agricultural soils can inform management strategies aimed at enhancing soil health. By monitoring these microbial communities, farmers at these various sites can implement more effective agronomic practices that leverage beneficial fungi while mitigating the effects of pathogenic species. Overall, further empirical studies focusing specifically on Uniben’s farmland soils would provide invaluable data to support sustainable agriculture initiatives within the region.

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