EMPTY FRUIT BUNCH APPLICATIOINS AND ITS EFFECTS ON FUNGAL COMMUNITY AND DIVERSITY IN OIL PALM (*Elaeis guineensis* L.) RHIZOSPHERES

BY

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FACULTY OF AGRICULTURE
UNIVERSITY OF BENIN

NOVEMBER, 2025

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PROJECT SUBMITTED TO THE DEPARTMENT OF SOIL SCIENCE AND LAND MANAGEMENT, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN. IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHELOR OF AGRICULTURE (B. AGRIC).

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CERTIFICATION

This is to certify that this project work was carried out by **Precious Oluchukwu OKOH** with matriculation number **AGR2004420** of the department of Soil Science and Land management, Faculty of Agriculture, University of Benin, Benin city, Nigeria.

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DATE

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DEDICATION

This project work is dedicated to Almighty God, who saw me through, sustained, favoured and strengthened me all through this study period. I also want to dedicate this project to my parents, for their love care and encouragement.

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ABSTRACT

The study was conducted to investigate the impact of oil palm empty fruit bunches (EFB) on the fungal community structure and diversity within the rhizosphere of *Elaeis guineensis* L. at the Nigerian Institute for Oil Palm Research (NIFOR), Edo State. EFB, a lignocellulosic by product of palm oil processing, was applied at varying rates (0, 50, 100, and 150 kg) to assess its effects on soil physicochemical properties and fungal dynamics. Standard laboratory analyses were used to evaluate soil parameters, while fungal enumeration and identification were conducted through morphological and microscopic characterization. Results revealed that moderate EFB application (100 kg) significantly ($P \geq 0.05$) improved soil properties, while enhancing fungal abundance and diversity. Identified fungi species were *Aspergillus niger*, *Trichoderma sp., Penicillium sp*., *Rhizopus arrhizus*, and *Mucor mucedo*, with *Trichoderma sp*. exhibiting notable phosphate-solubilizing and nitrogen-fixing properties. However, excessive EFB application (150 kg) led to nutrient immobilization and reduced fungal proliferation. In conclusion, moderate EFB incorporation at 100kg optimizes soil health and promotes beneficial fungal communities, offering a sustainable approach for enhancing oil palm productivity in tropical soils.

Chapter One

1.0 Introduction

1.1 Background of Study

Oil palm (*Elaeis Guineensis L.*) is a major tropical crop in tropical regions, notably in Nigeria, where it significantly contributes to the economy and rural livelihoods. The Oil Palm is a monocotyledonous evergreen plant belonging to the palm family Arecaceae. It has been described as the tree of life, not only because of its usefulness to man but because of its longevity. (Okolo *et al.*, 2019).

The oil palm industry is a major contributor to global vegetable oil production, especially in Nigeria, and it generates significant quantity of waste including the empty fruit bunches of oil palm which are rich in lignocellulosic materials that represent a substantial waste stream that can pose environmental challenges if not properly managed (Awogbemi *et al.,* 2020). However, this lignocellulosic content in empty fruit bunches play a huge role in improving the soil structure and fertility when applied at an optimum level.

Empty fruit bunches (EFB) in recent times have been applied to plantations as mulch or compost to improve the fertility status, enhance the water retention capacity of the soil and other functional purposes due to its richness in organic matter. Onyia *et al.,* (2023) extracted cellulolytic fungi species from empty fruit bunches highlighting their importance in breaking down complex organic material and facilitating nutrient cycling in the soil. Also, Kawata *et al.,* (2023) identified humic acid producing fungi in soil associated with empty fruit bunches, highlighting the role in boosting soil organic matter and enhancing the fungal community diversity.

The rhizosphere is the region of the soil influenced by the plant roots and is the environment where microbial interactions significantly contribute to plant health and productivity. The fungi within the rhizosphere greatly plays a part in the soil health and the application of the empty fruit bunch may alter the fungal community structure and diversity in the rhizosphere due to the changes in organic carbon, organic matter content, and other factors, potentially affecting the growth and yield of the Oil Palm (Sukri *et al.,* 2021).

The understanding of how these empty fruit bunches affect the fungal community is essential for maximising its use and maintaining a healthy rhizosphere for oil palm productivity. The use of empty fruit bunches in oil palm plantations, like NIFOR, has been adopted to improve the fertility status of the soil.

The research hitherto has been limited to the improvement of fertility status, fungal community changes and other beneficial effects such as, the beneficial effects of the fungi associated with EFB which included Aspergillus and Trichoderma species (Onyia *et al.,* 2023; Kawata *et al.,* 2023). The adverse effects of the application of the empty fruit bunches, most especially the pathogenic studies have been left unexplored, hence hindering the effective use of the empty fruit bunches in enhancing the soil health and oil palm productivity.

Despite the positive agronomic effects of the application of empty fruit bunches, little is known as regards the pathogenic impact of the fungal community and diversity on the soil health or the plant health following the application of the empty fruit bunches.

The effective understanding of the effects of the application of empty fruit bunches to the soil on the oil palm rhizosphere's fungal community is important in the development of sustainable oil palm cultivation practices.

Additionally, leveraging locally available organic waste such as empty fruit bunch aligns with environmentally friendly and cost-effective agricultural practices for farmers in Nigeria (Aderolu *et al.,* 2018).

1.2 Objective of study

The objective of this study specifically aimed at:

I. Accessing the impact of empty fruit bunch application on fungal community structure and diversity in the rhizosphere of oil palm (*Elaeis Guineensis L.*)

II. To evaluate the pathogenic impact of the fungal community on the Oil Palm.

Chapter Two

2.0 Literature Review

2.1 Oil palm waste management

Due to the expansion of the oil palm industry and being a major industry, there has been a substantial increase in the generation of various oil palm waste products which can lead to environmental pollution if not properly managed. Developing and implementing effective waste management strategies is essential in mitigating environmental impacts and maximizing the utilization of valuable waste products derived from oil palm processing and production (Izah *et al.,* 2016).

The wastes obtained from oil palm processing are high in lignocellulosic content and organic matter and nutrients. As useful as these wastes are, when not properly managed, they can pollute the environment.

There are various waste management strategies that can be applied in combating the growing generation of oil palm waste products, maximizing the use and reducing its pollusive effects on the environment. Some of these waste products include;

Mulch and soil amendments: Oil palm biomass wastes like pruned fronds and EFBs can improve soil health by increasing organic matter, adding structure, improving water retention, and enriching nutrient content. Utilizing oil palm wastes as soil amendments and mulch supports environmental stewardship by recycling biomass, reducing pollution, and improving soil carbon storage. Economically, it lowers chemical fertilizer costs and enhances crop yields, contributing to more resilient oil palm production systems (Supriatna *et al.,* 2022).

Composting: Composting is a viable solution to the issue of using agricultural waste directly for land and agricultural production. This is due to the fact that the structures of these wastes are often unknown and can contain harmful contaminants, such as pathogens, weed seeds, heavy metals, and unpleasant odours (Vakili *et al.,* 2015). By composting these wastes, we can ensure that they are safe to use and will not negatively impact the environment or human health.

Composting is the controlled biological process that involves breaking down and stabilizing organic materials and wastes, converting them to safe nutrient rich materials that can be used to enhance soil health and fertility. As a strategy for oil palm waste management, composting is commonly employed for oil palm residues, especially EFB and POME. studies have proven that composting EFB and POME together can result in valuable organic fertilizers (Egbe *et al.,* 2023). The breakdown of these wastes is significantly facilitated by the microorganisms, especially the lignocellulolytic fungi abundantly present in oil palm wastes, most especially the EFB (Awogbemi *et al.,* 2020). These fungi help breakdown lignin and cellulose, and the biomass is decomposed over several weeks or months.

Thermochemical conversions: Thermochemical conversion is the use of heat and chemical reactions to transform oil palm wastes into energy sources and other useful products. This is carried out with methods such as; pyrolysis, gasification, and liquefaction (Awalludin *et al.,* 2015).

Pyrolysis is a thermochemical process that involves heating biomass in an oxygen free environment resulting in the production of bio-oil, syngas, and biochar. Empty Fruit Bunches (EFB), and Palm Kernel shells (PKS) are mainly used for this process, due to their high lignin content with 20.4% lignin weight for EFB and 49.5% lignin weight for PKS (Safana *et al.,* 2018). This lignin is found in the cell wall of plants and it contributes to the formation of biochar during pyrolysis. Recent research has proven that PKS and EFB possess high lignin and volatile matter content and these findings implied that PKS and EFB are suitable sources for biochar production and the obtained chars portrayed significant biofuel potential (Lee *et al.,* 2017).

Gasification is a process that potentially oxidises organic materials at a high temperature (750°C - 1100°C), in an oxygen limited environment. This process converts biomass to syngas (Synthetic gas) and the conversion process serves as a versatile pathway for energy production and chemical synthesis (Mujtaba *et al.,* 2022). This syngas is a flammable mixture consisting of carbon monoxide (CO) and Hydrogen(H_2).

Liquefaction known as hydrothermal liquefaction is a thermochemical process involved in converting wet biomass into high energy density liquid fuel, typically referred to as bio crude. This process is done under moderate temperature (250°C- 400°C) and high pressure of 5-25 MPa (Thorson *et al.,* 2024). In this process, the organic content of the wet biomass is liquefied, it then produces a biocrude. This biocrude is the main product of liquefaction and it is in a dilute aqueous phase, and a solid residue that is rich in carbon and other minerals. This biocrude is traditionally used to produce liquid transport fuel. The liquefaction process is particularly effective for processing Palm oil mill effluent (POME) without the need for energy intensive drying steps (Nor *et al.,* 2019).

These technologies provide the combined effects of recovering energy and minimizing waste. Bio oil and syngas serve as renewable energy alternatives, while biochar offers potential as an enhancer and a means for long term carbon storage and sequestration. They are only mostly hindered by high operational costs.

Biotechnical application: This process involves the use of microorganisms or enzymes to convert oil palm wastes into valuable products. These methods are gaining attention currently because of their potential to convert lignocellulosic residues into biofuels, bio plastics and other valuable compounds. EFB for example serves as a vital source of lignocellulosic biomass which can be decomposed by these microbes or enzymes into fermentable sugars for bioethanol production (Tahir *et al.,* 2019). In addition, POME can be treated using microbial association to produce biogas and, in the process, reduce environmental pollution (Ahmed *et al.,* 2022).

These processes do not only reduce waste disposal issues but also contribute to the generation of renewable energy (Eze *et al.,* 2024). The implementation of these techniques in the oil palm sector illustrates a move toward circular economy principles, transforming wastes into valuable products, thereby improving both economic benefits and environmental sustainability.

2.2 Empty fruit bunches (EFB)

Empty fruit bunches are wastes obtained from the fresh fruit bunch of oil palm after removing the fruits. They are the largest solid waste compared to other solid waste from the palm oil industry (Windiastuti *et al.,* 2022). Empty fruit bunches (EFB) are one of the major waste products from palm oil processing and are largely discarded to rot. However, EFB can be repurposed into valuable products such as biofuels, fertilizers, and animal feed (Okereke and Ginikanwa 2020). This not only reduces waste but also promotes sustainability in the palm oil industry by utilizing resources more efficiently and minimizing environmental impact.

EFB fiber is generally made up from a complex matrix of three main polymers which are cellulose, hemicellulose and lignin. It is composed of around 44.2% cellulose, 33.5% hemicellulose and 20.4% of lignin (Nurul *et al.,* 2017). By decaying EFB, greenhouse gases are emitted into the atmosphere, posing a challenge to the environment and as a result, valuable energy cannot be extracted. (David *et al.,* 2023).

A ton of palm oil generates approximately one million tons of EFB, which is discarded and left to decompose in Nigeria every year and this decomposition results in CO_2 equivalent emission of approximately 0.23 tons per ton of CO_2 eq per ton per year therefore, alone contributes to liberating about 230,000 tons of carbon dioxide equivalent from N_2O and methane emissions, primarily due to improper handling and underutilization of EFB (Elbersen *et al.,* 2013).

Although EFB were once regarded merely as agricultural waste, they are increasingly being recognized for their substantial potential as a renewable biomass resource. Their effective use corresponds with the principles of sustainable development by contributing to eco agriculture, bioenergy production, and environmental restoration (Singh *et al.,* 2020). As research and technology advance, the strategic management and optimization of EFBs could play a transformative role in aiding a bio-based economy and facilitating eco-friendly practices across the agricultural and energy sectors (Nasution *et al.,* 2021).

2.2.1 EFB as a sustainable mulch material

EFB can be used in a variety of ways, including fresh, composted, mulched, or pelletized. In oil palm plantations, it is often used as a mulch by spreading it in between rows or around the base of the trees (Corley and Tinker, 2016). This not only helps to retain moisture in the soil, but also prevents the growth of weeds and adds organic matter over time. Using EFB as mulch, is especially advantageous in arid areas or sandy soils that have low capacity to retain water. It decreases the need for frequent irrigation, conserving water and reducing labor expenses (Rahman and Abdullah 2014).

The use of EFB mulch can serve as a protective barrier, shielding the soil surface from direct sunlight and wind to reduce evaporation. Not only does the fibrous structure of EFB provide this protective function, it also has the ability to absorb and gradually release moisture, promoting consistent soil hydration (Ahmed *et al.,* 2020). Research conducted highlighted that the use of EFB mulch in oil palm plantations resulted in a significant reduction of water loss by 30-40% in comparison to bare soil (Suhaimi and Ong, 2018).

It also helps suppress weeds. It has been proposed that a layer of EFB, measuring about 5–10 cm in thickness, can act as a physical barrier that blocks sunlight and prevents weed seeds from germinating (Khalid *et al.,* 2019). In addition, the decomposition of EFB may result in the release of compounds that have allelopathic effects, inhibiting the growth of some weeds (Ahmed *et al.,* 2020). These two mechanisms make EFB a promising option for weed control in agriculture and gardening.

The use of EFB mulch can provide essential benefits such as moisture retention, weed control, and soil enrichment. However, it is important to properly manage the EFB mulch through methods like shredding, N supplementation, and pest control (Supriatna *et al.,* 2022, Nyasapoh *et al.,* 2024).

2.2.2 EFB as an effective soil amendment

EFB has the potential to be utilized as a soil conditioner in order to enhance soil characteristics and boost agricultural production. By incorporating EFB into the soil, it can improve its texture, increase water retention, and provide essential nutrients for plant growth, ultimately resulting in higher crop yields (Adu *et al.,* 2022).

Incorporating EFB (empty fruit bunches) into soil is a beneficial practice that can greatly improve soil fertility and microbial activity. This is due to the increase in organic carbon levels, which is crucial for providing essential nutrients for plants and promoting healthy soil ecosystems. EFB can also aid in the reduction of nutrient leaching by acting as a gradual and steady supplier of nutrients and by enhancing the soil's ability to exchange cations (CEC). This is particularly advantageous in tropical soils that are highly weathered and often have poor nutrient retention (Moradi *et al.,* 2014).

Chemically, EFB contains essential nutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) (Asoka *et al.,* 2021). Although the carbon-to-nitrogen ratio and slow decomposition rate of EFB initially limit the immediate availability of nutrients, its long-term application has been shown to greatly enhance soil fertility through the gradual release of nutrients (Ishak *et al.,* 2021). Long-term use of EFB has been proven to have a positive impact on acidic tropical soils. One of its benefits is the increase in soil pH (from acidic to alkaline), which helps to reduce aluminium toxicity and improve the availability of nutrients (Teng *et al.,* 2015)

Some reviews have evaluated the potential of EFB as a soil amendment to improve soil fertility and increase yield (Adu *et al.,* 2022). Adu *et al.,* (2022) conducted an analysis to show the effect on crop growth and yield and how other factors moderate performance of soil amendment using different forms of EFB and applying EFB as mulch, biochar, or compost to soil resulted in a significant 49.2% increase in crop growth and yield, compared to soils that were not amended. Furthermore, when EFB was applied in conjunction with another material, such as mineral fertilizers, there was an additional 16.4% increase in crop growth and yield compared to unamended soils. This highlights the potential of using EFB in agriculture to improve crop production and overall soil health.

EFB composting typically yields bio-fertilisers that are rich in nutrients, making them effective in reducing the need for chemical fertilisers (Tan *et al.,* 2020). This not only helps to decrease the use of harmful chemicals, but also improves the overall health and sustainability of soil. Composting EFB also results in a more stable and nutrient-rich product, which allows for quicker nutrient release and reduces the risk of damaging plants (Sukri *et al.,* 2021).

Utilizing EFB as a soil amendment aligns with the principles of the circular economy, decreasing dependence on chemical fertilizers and diverting organic waste away from landfills or incineration. This practice also aids in carbon sequestration and mitigating greenhouse gas emissions by improving soil organic carbon levels and decreasing the need for open burning of biomass residues. From an economic perspective, the use of EFB application has the potential to significantly decrease input costs for fertilizers and enhance the long-term productivity of soil, particularly in smallholder settings. By incorporating EFB into sustainable land management practices, not only can it promote environmental stewardship, but also foster economic resilience in regions where oil palm is grown (Rudolf *et al.,* 2021).

2.2.3 Effects of EFB on Soil Microbial Biomass

Soil microbial biomass plays a crucial role in soil ecosystems, as it is responsible for organic matter decomposition, nutrient cycling, and plant health. By using organic mulches, such as Empty Fruit Bunch (EFB), the physicochemical properties of soil are modified, having a direct impact on the populations of microorganisms. This highlights the important relationship between soil health and the presence of microbial biomass.

EFB is a by-product of palm oil mills, made up of lignocellulosic waste, and is being used more frequently as mulch due to its plentiful supply and ability to improve soil quality. Nonetheless, the slow rate of decomposition and high carbon-to-nitrogen (C: N) ratio of approximately 80:1 present distinctive obstacle for microbial communities (Singh *et al.,* 2021). The high lignin and cellulose content of EFB, slow decomposition rate, and unique nutrient release dynamics can have a significant impact on microbial communities. Although EFB can enhance microbial activity in the long term, its initial decomposition phase may temporarily immobilize nitrogen, leading to changes in microbial biomass carbon and nitrogen (Oktaviani *et al.,* 2024)

The utilization of Empty Fruit Bunch (EFB) mulch, with its specific biochemical composition, results in the selective influence of various pressures that ultimately shape fungal communities (Zhang *et al.,* 2022). The components of EFB as said in 2.3 provide a favorable environment for fungi to thrive over bacteria, as fungi have a superior ability to break down complex polymers (Singh *et al.,* 2021).

During the first stage of EFB application to the soil, there is a high carbon to nitrogen ratio of EFB, estimated at approximately 80:1, which prompts microbes to sequester nitrogen, resulting in a temporary limitation of resources for certain fungi (Khalid *et al.,* 2019). During the early stages, Ascomycetes such as *Trichoderma* dominate due to their quick colonization of newly available organic matter (Zhang *et al.,* 2022). However, in soils with limited nitrogen, there may be an initial decrease of 5-15% in total fungal biomass (Ahmed *et al.,* 2020). During the lignin degradation phase, labile carbon decreases and white-rot fungi, such as *Phanerochaete*, become dominant (Singh *et al.,* 2021). This phase also leads to a 20-30% increase in fungal biomass compared to unmulched soils (Suhaimi and Ong, 2018), as well as a significant rise in the fungal-to-bacterial ratio (F: B) (Zhang *et al.,* 2022). Over the long-term (6-12 months), the fungal community remains stable due to continued organic matter input, with both saprotrophic fungi and mycorrhizal fungi, such as *Glomus*, increasing in association with plant roots (Khalid *et al.,* 2019).

EFB mulch aids in promoting decomposition pathways that are dominated by fungi, with a particular preference for ligninolytic species. Although there may be a temporary suppression of some fungi due to initial N immobilization, long-term application significantly increases the amount of fungal biomass.

2.3 Other organic soil amendments

Organic soil amendments are substances that are incorporated into soil in order to enhance its physical, chemical, and biological properties. Unlike fertilizers, which primarily aim to provide nutrients to plants, amendments work to improve soil structure, increase water retention, and promote microbial activity. These amendments can range from compost and manure to cover crops and biochar, all of which work towards creating a healthier and more productive soil environment. Organic soil amendments are gaining popularity as sustainable solutions for boosting soil fertility and ecosystem functionality. These amendments, which originate from plant, animal, or microbial materials, contribute to improved soil structure, nutrient regeneration, microbial diversity, and organic matter levels (Sharma *et al.,* 2018). Their significance is particularly crucial in regenerative and conservation agriculture, where maintaining long-term soil health is essential for productivity. The incorporation of organic amendments is suggested as a fundamental strategy for promoting soil sustainability and minimizing environmental impact. Major examples of these amendments include;

Compost: Compost is resulted from the aerobic decomposition of organic materials like crop residues, food scraps, and animal manure. The end result is a stable product that is rich in humus, essential nutrients, and beneficial microorganisms. Compost improves soil structure, increases porosity and water retention, and boosts microbial biomass along with enzyme activity. Additionally, it enhances cation exchange capacity and nutrient retention (Mengistu *et al.,* 2020). Well-matured compost mitigates phytotoxicity and reduces pathogen risks. Its use has been linked to improved soil aggregation and increased crop yields across various agro-ecological zones (Bernal *et al.,* 2017).

Animal manure: Animal manure, from sources such as cattle, poultry, and sheep, is a widely used organic amendment in agriculture. This is due to its high nutrient content, which includes nitrogen (N), phosphorus (P), potassium (K), and trace minerals in readily available forms. These nutrients are essential for plant growth and can enhance microbial activity and nutrient cycling in the soil. Additionally, animal manure can also supply both macro and micronutrients to plants, and it can build soil organic matter and improve moisture retention.

When properly composted, animal manure odour can be mitigated. It can also reduce the presence of pathogens, and decrease greenhouse gas emissions (Al-Kaisi and Guzman, 2019). However, it is important to note that over-application of animal manure can have negative impacts. Excessive amounts of nitrogen and phosphorus can lead to nitrate leaching and phosphorus accumulation in the soil, which can be harmful to both the environment and human health.

Green manuring: Plants grown for green manuring are leguminous crops that are specifically cultivated for use as fertilizer in the soil. Common legumes such as vetch, alfalfa, and clovers are often used as green manure crops due to their ability to fix atmospheric nitrogen through symbiotic relationships with Rhizobium bacteria (Blesh and Drinkwater, 2015). Not only do these legumes increase soil organic carbon and microbial activity, but they also suppress weed growth and minimize soil erosion. However, the effectiveness of green manure crops can vary depending on factors such as biomass quality, species selection, and climatic conditions.

Crop residues: Recycling crop residues, including maize stover, rice straw, and wheat husks, can have numerous benefits for the soil and its health. Not only does it improve nutrient cycling and increase organic matter, but it also helps to prevent erosion and encourages the growth of microbial populations. In addition, this process can aid in the sequestration of carbon in the soil. (Ramesh *et al.,* 2016).

Biochar: biochar a carbon-rich and porous substance created through the process of pyrolyzing organic biomass at high temperatures with limited oxygen, is known for its exceptional durability and ability to improve soil and environmental well-being. By integrating biochar into soil, it can help regulate pH levels, increase nutrient retention in acidic soils, foster microbial growth, minimize leaching, and serve as a long-term storage for carbon, making it a valuable tool for mitigating climate change (Lehmann *et al.,* 2015). Recent research has also shown that biochar works in synergy with compost and manure, further enhancing soil fertility and carbon storage (Kammann *et al.,* 2017).

Other organic amendments include;

Peat Moss: This material is commonly used in horticulture to improve the water-holding capacity and aeration of soil. However, the extraction of peat moss raises sustainability concerns. As a result, alternative materials such as coir and composted bark are being adopted (Alexander *et al.,* 2020).

Vermicompost: Also known as worm castings, this material is rich in microbial biomass and growth-promoting compounds. It has been shown to improve root development, nutrient uptake, and disease resistance (Domínguez and Gómez-Brandón, 2021).

Seaweed Extracts and Fish Emulsions: These natural substances provide plants with essential micronutrients and plant hormones such as cytokinins and auxins. This can enhance root vigor and help plants tolerate stress. Etc.

Strategies for implementing organic soil amendments must carefully consider factors such as material quality, application rates, and environmental impacts (Bhogal *et al.,* 2018). These amendments, including compost, animal manure, green manure, crop residues, and biochar, play a crucial role in restoring soil function and promoting sustainable agricultural practices (Omokaro *et al.,*2024). They contribute to soil health through physical, chemical, and biological improvements, reducing the need for synthetic fertilizers and supporting long-term productivity (Rashmi *et al.,* 2022) Additionally, the incorporation of organic soil amendments can enhance ecosystem services, making them an essential aspect of any successful agricultural operation (Bhogal *et al.,* 2018).

2.4 Fungi

The Kingdom Fungi, encompasses a diverse group of organisms with significant ecological and economic importance. From decomposers to symbionts, fungi play vital roles in maintaining the balance of ecosystems and providing essential services such as nutrient cycling. Economically, fungi are crucial in industries such as food, medicine, and biotechnology (Charya 2015). Fungi are a diverse group of eukaryotic organisms, encompassing microorganisms such as yeasts and molds, as well as larger, more recognizable organisms like mushrooms. They are classified as their own kingdom, distinct from plants, animals, protists, and bacteria. One key distinction is that fungi have cell walls made of chitin, unlike the cell walls of plants or bacteria (Naranjo-Ortiz *et al.,* 2019).

They are organisms that reproduce sexually or asexually under different conditions. When there is an abundance of nutrition, fungi tend to reproduce asexually. However, when conditions become unfavorable, they switch to sexual reproduction. This allows them to adapt and survive in changing environments (CK-12 foundation., 2021).

Fungi are organisms that obtain their energy by consuming chemicals and other organic matter. They can exist as parasites, feeding off their living hosts, or as saprophytes, consuming dead or decaying matter (Treseder and Lennon, 2015). Some fungi also have a symbiotic relationship with other organisms, where both parties benefit from the exchange of nutrients (Compant *et al.,* 2019, Smith and Smith, 2020).

Mycorrhizal fungi have a symbiotic relationship with over 80% of land plants, with the two most common types being Arbuscular Mycorrhizal Fungi (AMF) and Ectomycorrhizal Fungi. AMF penetrates plant roots to help with the absorption of phosphorus and micronutrients. Ectomycorrhizal Fungi, on the other hand, are more prevalent in forest ecosystems and form external networks around root tips. The presence of these fungi in agriculture has proven to have numerous benefits, including improved nutrient uptake, better drought and salinity tolerance, and increased resistance to pathogens, as shown in studies (Begum *et al.,* 2019).

Endophytes are fungi that inhabit the inner tissues of plants without causing harm. In fact, they can actually improve the overall health of the plant by producing special compounds that repel pests and diseases. For instance, Trichoderma spp. have been found to enhance plant growth and increase root biomass. Similarly, non-pathogenic strains of Fusarium oxysporum have been shown to aid in nitrogen fixation in legumes (White *et al.,*2019). Saprophytic fungi play a crucial role in decomposing complex organic matter, which in turn releases essential nutrients for plant growth and contributes to the overall amount of soil organic carbon (Six *et al.,* 2017).

Fungi play a vital role in the functioning of ecosystems by decomposing dead organic material and contributing to the nutrient cycle. Through their breakdown of organic matter, fungi release essential nutrients back into the soil, making them available for use by other living organisms. This process of decomposition is crucial for maintaining the balance and health of ecosystems. (Kirsten.,2018).

Fungi is essential for nutrient recycling and maintaining soil fertility, as well as forming symbiotic associations with plants (Charya 2015).

2.4.1 Ecological interaction of Soil Fungi and EFB

EFB is a rich source of organic matter when added to the soil. It is broken down by many types of soil microbes, especially fungi. They help break down tough plant materials, release nutrients and improve soil structure and the presence of EFB can alter the balance of carbon and nutrients in the soil, thereby affecting the type and population of these fungi (Mahmud and Chong.,2021).

When EFB is applied to the soil, it leads to several ecological changes. Fungi that break down tough materials like cellulose and lignin, especially the saprotrophic fungi in the groups; *ascomycota, zygomycota* and *basidiomycota,* multiply in number quickly, helping to speed up decomposition and nutrient recycling (Yang *et al.,* 2022). Also, some beneficial fungi like the ectomycorrhizal and arbuscular mycorrhizal fungi may also shift, depending on the amount of EFB applied, and may end up competing with these decomposers for space and nutrients in the root zone (Lebreton *et al.,*2021).

The population of fungal species within a given rhizosphere can be enhanced by introducing new substrates and microhabitats like EFB that support the colonization of diverse fungal guilds, including endophytes, saprotrophs and mycorrhizal fungi but excessive EFB application or overly rapid nutrient mineralization may disrupt balance, allowing fast growing fungal taxa like *Trichoderma, Aspergillus,* etc. to dominate, thereby reducing species evenness and potentially supressing rare or slower growing species (Gong *et al*., 2023).

EFB has a high C:N ratio and when decomposers break it down, they require their own nitrogen to do so, and build their own biomass and this leads to a temporary nitrogen immobilization and a shift in the population of fungi that are not adapted to low nitrogen situations (Neswati t al., 2022).

Some pathogenic fungi species like *fusarium oxysporium*, and *ganoderma boninense* may also increase in number in soils enriched by raw EFB due to increased moisture and organic content that can enhance their growth (Ren *et al.,* 2020, Pulingham *et al.,* 2022). Although, research explored how EFB can be used as a fungicide to control *fusarium oxysporum* (Akalazu and Duru., 2024). Ren *et al.,* (2020) carried out an experiment that indicated that *Ganoderma boninense* which causes the basal stem rot of oil palm grows well in media rich in the lignocellulosic materials of raw EFB.

Composting and pre-treating EFB alters its chemical and physical properties, making it less conducive to the growth and multiplication of pathogenic fungal species, and also promote the multiplication of competitive beneficial fungi (Zakri and Adam., 2021).

Heavy applications of EFB to the soil can alter oxygen penetration in the soil, thereby supressing aerobic fungi, and favouring the anaerobic fungi, shifting fungal dominance away from saprotrophic fungi (Siddiquee and Shafawati., 2016).

2.5 The Rhizosphere

The Rhizosphere is the zone of the soil that is directly affected by the plant roots. It is influenced by the secretions made from the roots, called the root exudate, and the corresponding microbial activity and these exudates play roles in nutrient mobilization, allelopathy, and plant-microbe signalling (Canarini *et al.,* 2019).

It is home to a diverse and ever-changing community of microorganisms. This community includes bacteria, archaea, fungi, protozoa, and viruses, all of which form intricate networks of interactions that play a vital role in processes such as nutrient cycling, promoting plant growth, and protecting against disease. (Xiong *et al.,* 2021).

This zone is greatly impacted by soil type, plant species, and management methods, all of which can affect the efficiency of nutrient cycling, as the root architecture is considered a factor influencing the structure of microbial communities inhabiting the rhizosphere (Ogbemudia and Ogboghodo 2020). One recent study found that using composted EFB in oil palm plantations can lead to increased soil microbial activity and improved nutrient cycling, resulting in higher crop yields (Ishak *et al.,* 2021). This highlights the importance of considering these factors in order to optimize nutrient cycling and promote sustainable farming practices.

It is also heavily influenced by fungal communities. These communities are especially important for crops like oil palm. By using organic amendments like empty fruit bunch (EFB) compost, the diversity and structure of fungal communities can be altered, potentially impacting the availability of nutrients and the overall health of plants (Sukri *et al.,* 2021).

The rhizosphere is a complex and dynamic environment, and is a crucial factor in the interactions between plants and soil. It is responsible for nutrient cycling, plant health, and soil function, making it a vital component for sustainable agriculture and environmental resilience. To harness its potential, further research should focus on utilizing the biology of the rhizosphere to enhance crop productivity, improve soil health, and mitigate environmental degradation. This offers a promising frontier for innovation in the field of agriculture.

Chapter Three

3.0 Materials and Methods

3.1 Experimental Site

The experiment was conducted at Field 14, Nigerian Institute for Oil-palm Research (NIFOR), Edo State, Nigeria. The area falls within the lowland humid tropical climate zone of southern Nigeria. The average rainfall is between 1500 mm – 3500 mm (Ugwa *et al.,* 2023). This field lies within latitude 6° 32 '59.6904” – 6° 33' 3.97348” and longitude 5° 37’ 11.1306” – 5°37’ 12.7848” E.

The vegetation is characterised by Oil palm, Raphia palm, Date palm, Coconut palm, Shea tree, and other ornamental crops.

NIFOR is also characterised by highly weathered acidic sandy soils, that are well drained, with low to moderate fertility. The soil series of soils in NIFOR are Ahiara, kulfo, Orlu, and Alagba (Ugwa *et al.,* 2023).

The evaluated land size was 2.5ha and the palm planted in this site was the EWS hybrid Tenera. It was planted in 2015, for the evaluation of conservation agriculture technique and use of oil palm wastes in oil palm cultivation.

3.2 Sampling Technique

Soil samples were collected from the experimental field after the application of the EFB treatments. Sampling was carried out using a soil auger at two depths: 0 –15 cm and 0 –30 cm.

For the first treatment plot (100 kg EFB amendment), soil was sampled from three replicates, each containing four sub-replicates. Samples from the four sub-replicates within each replicate were thoroughly mixed to obtain a composite sample, and this was repeated across the three replicates.

The same procedure was followed for the second treatment plot (150 kg EFB amendment) and the third treatment plot (50 kg EFB amendment).

For the control plot, which consisted of one replicate with four sub-replicates, samples from the four sub-replicates were composited for each depth.

In total, 20 composite soil samples were obtained across the treatments and depths. Each composite sample was bagged, properly labelled, and subsequently sent to the laboratory for analysis.

3.3 Soil analysis

3.3.1 Physical and chemical Soil Analyses

The soil samples were analysed for various physical and chemical properties, including pH, organic matter content, and nutrient concentrations such as nitrogen, phosphorus, and potassium. Standard laboratory procedures were employed. The soil samples were first air dried at room temperature and passed through a 2 mm sieve before analysis.

3.3.1.1 pH determination

The pH was determined by this method. 15 grams of the fine soil sample was weighed and transferred into a clean extraction cup, with two replicates recommended. 30 ml of distilled water was then added to the soil to form a slurry in a 1:2 soil-to-water ratio. The mixture was stirred intermittently over a period of thirty minutes. After thorough mixing, it was left to stand for a few more minutes, after which the pH was measured and recorded using a previously calibrated pH meter (KBS LTER, 2023).

3.3.1.2: Particle Size Analysis

To determine the particle size, fifty-one grams of air-dried soil was weighed into an extraction cup, after which 50 ml of sodium hexametaphosphate solution was added to disperse the soil particles. Subsequently, 100 ml of distilled water was added to

the mixture. The cup was capped and placed on a stirrer, where the solution was stirred vigorously for about ten minutes at medium speed. The resulting suspension was then transferred into a 1000 ml sedimentation cylinder and diluted to the 1000-ml mark with distilled water. After reaching the mark, the solution was agitated, and both hydrometer and temperature readings were taken at forty seconds. The cylinder was then left undisturbed for an additional two hours, after which the hydrometer and temperature readings were again recorded (Soil Survey Staff, 2014). The particle size distribution was then calculated accordingly;

$$\% \ (\boldsymbol{silt} \ + \boldsymbol{clay}) = \frac{\boldsymbol{40sec\ HR \pm 0.36\ (T-20)\ X\ 100}}{\boldsymbol{weight\ of\ soil}} \text{ -------------------- } \textbf{(3.1)}$$

$$\% \ \boldsymbol{clay} = \frac{\boldsymbol{2\ hours\ HR \pm 0.36\ (T-20)\ X\ 100}}{\boldsymbol{weight\ of\ soil}} \text{ ---------------------- } \textbf{(3.2)}$$

$$\% \ \boldsymbol{silt} \ = (\% \ \boldsymbol{silt} \ + \boldsymbol{clay}) - \%\boldsymbol{clay} \text{ ---------------------- } \textbf{(3.3)}$$

$$\% \ \boldsymbol{sand} = \boldsymbol{100} - \%(\boldsymbol{silt} + \boldsymbol{clay}) \text{ ------------------------ } \textbf{(3.4)}$$

Where;

HR= hydrometer reading

T= temperature

There was a subtraction for every drop in temperature below 20° while an addition for every rise in temperature above 20°.

3.3.1.3 Organic Matter Determination

The organic matter content was determined by first estimating the organic carbon using the Walkley–Black method of organic carbon determination, as described by FAO (2019). 1 gram of air-dried soil was weighed and placed in a 500-ml conical flask. 10 ml of 0.167 M potassium dichromate ($K_2Cr_2O_7$) solution was then added, and the flask was swirled gently to disperse the soil in the solution. With caution 20 ml of concentrated sulfuric acid (H_2SO_4) was rapidly added, directing the stream into the suspension. The flask was swirled gently until the soil and reagents were properly mixed, then more vigorously for a total of one minute. It was left to stand for thirty minutes to cool due to the heat generated from the reaction. After cooling, 100 ml of water was added to the flask and swirled again for proper mixing. Five drops of ferroin indicator were then added to the solution, which was subsequently titrated to a dirty brown endpoint. A blank determination was also carried out without the soil sample (FAO, 2019). The percentage organic carbon was then calculated from the titre volumes obtained.

$$\boldsymbol{OC}\% = \frac{\boldsymbol{(B-T)\ x\ N\ x\ 0.003Fx100}}{\boldsymbol{weight\ of\ soil}}$$

Where;

B = blank titre volume

T = sample titre volume

F = correction factor (1.33)

N = normality of ferrous sulphate

The percentage organic carbon was multiplied by 1.724 to convert to percentage organic matter. The final results were reported in gkg^{-1} by multiplying percentage organic matter values by 10 (Walkley; 1934, Brady and Weil; 2016).

3.3.1.4 Nitrogen Determination

The determination was carried out using the Kjeldahl method for nitrogen estimation. 1 gram of air-dried soil sample was weighed and mixed with one Kjeldahl tablet and 20 ml of concentrated sulfuric acid (H_2SO_4). The mixture was heated until it became clear, indicating complete digestion and decomposition of the organic matter, after which it was allowed to cool. Approximately 10 ml of distilled water was then added, and the contents were filtered into a 100-ml volumetric flask using Whatman No. 45 filter paper. The filtrate was made up to the mark with distilled water and swirled thoroughly for proper mixing.

10 ml of the aliquot was transferred into a 500-ml Kjeldahl flask, followed by the addition of 30 ml of water. 15 ml of sodium hydroxide (NaOH) solution was added to neutralize the mixture and release ammonia. About 20 ml of boric acid (H_3BO_3) solution was placed in a conical flask positioned under the tip of a condenser, ensuring that the tip touched the surface of the solution. The aliquot was then heated, and approximately 3 ml of ammonia distillate was collected in the boric acid solution. Five drops of indicator were added to the distillate, which was then titrated with 0.01 M standard hydrochloric acid (HCl) to a pink endpoint. A blank determination was run too, without soil samples.

The percent nitrogen was the calculated as follows from the obtained titre values;

$$\% \, \boldsymbol{nitrogen} = \frac{\boldsymbol{T\ x\ M\ x\ 14\ x\ V1\ x\ 100}}{\boldsymbol{1000\ x\ V2\ x\ weight\ of\ soil}}$$

Where;

T = sample titre volume

M = molarity of HCl

V1 = final volume of digest

V2 = volume of aliquot used for distillation

(FAO; 2021)

3.3.1.5 Available Phosphorus Determination

The Bray-1 method was employed for the determination of phosphorus. 5 grams of air-dried soil was weighed into an extraction cup, after which 30 ml of Bray-1 extracting solution was added. The mixture was agitated using a mechanical shaker for about five minutes and then filtered into an extraction bottle. From the filtrate, 1 ml was measured and transferred into a 50 ml volumetric flask. 6 ml of distilled water and 2 ml of colour-developing reagent were subsequently added and thoroughly mixed.

Thereafter, 1 ml of ascorbic acid solution was introduced into the mixture, which was allowed to stand for

approximately ten minutes to permit colour development. The absorbance of the resulting solution was measured at 650 nm using a visible-range spectrophotometer. A standard curve of absorbance against phosphorus concentration (in ppm) was then plotted, and the interception point on the curve was used to determine the phosphorus concentration (Soil Survey Staff, 2014).

3.3.1.6 Exchangeable Bases Determination (K, Ca, Mg, Na)

The determination was carried out using the ammonium saturation method. Ten grams of air-dried soil was weighed and placed in a 250-ml extraction bottle, after which 100 ml of 1N ammonium acetate solution was added. The bottle was capped and shaken on a mechanical shaker for about one hour. The resulting soil solution was then filtered into a 100-ml conical flask using Whatman No. 45 filter paper and made up to the mark with ammonium acetate solution. The concentrations of potassium (K) and sodium (Na) were measured using a flame photometer, while calcium (Ca) and magnesium (Mg) were determined with an atomic absorption spectrophotometer (Ibitoye, 2008).

3.3.1.7 Determination of Exchangeable Acidity (H, Al)

The exchangeable acidity was determined using the titration method. Five grams of air-dried soil was weighed into an extraction bottle, and 50 ml of 1M potassium chloride (KCl) solution was added. The mixture was shaken on a mechanical shaker for about one hour and then filtered into an extraction bottle using Whatman No. 42 filter paper. 23 ml of the filtrate was measured into a 250-ml conical flask, and five drops of phenolphthalein indicator were added. The solution was titrated with 0.05N sodium hydroxide (NaOH) to a permanent pink endpoint (Ibitoye, 2008). The exchangeable acidity was subsequently calculated from the obtained titration values.

$$\boldsymbol{EA = \frac{v\ x\ 0.005\ x\ 1000}{weight\ of\ soil}}$$

(Soil survey staff; 2014)

3.3.1.8 Total Elemental Analysis

The total elemental analysis was determined by this procedure. One gram of air-dried soil was weighed and placed in a digestion tube, after which 10 ml of nitric acid (HNO_3) was added. The mixture was heated in a block digester for about one hour and then allowed to cool. 5 ml of 2M hydrochloric acid (HCl) was added, followed by dilution with 10 ml of distilled water. The solution was filtered using filter paper into a 100-milliliter volumetric flask and made up to the mark with distilled water. The resulting extract was then stored in a plastic reagent bottle for subsequent instrumental determination (Ibitoye, 2008).

3.3.2 Microbial Analyses

3.3.2.1 Sterilization of Materials and Apparatus

The Petri dishes, pipettes, flasks, extraction cups and bottles, were first washed, and dried. They were then wrapped with aluminium foil and sterilized in a hot air oven for an hour at 160°C. Then they were allowed to cool after being sterilized before usage. A sterile working environment was achieved by using a Bunsen burner flame and disinfecting work surfaces with ethanol. All media used were obtained and prepared according to manufacturer’s instructions. The media used in this study include Potato dextrose agar (PDA)

3.3.2.2 Enumeration and Isolation of Fungal Species from samples.

This was done using the Waksman soil dilution method, as reported by Raja *et al.,* (2017). Samples were collected into sterile bottles. The samples were serially diluted with a factor of 10 where 25g of sample was mixed with 225 ml of sterile saline water (SSW), from where 1 ml of the aliquot was serially diluted, while transferring to the bottles containing 9 ml of SSW. 1 ml of the diluent was then transferred from the 4th bottle to sterile petri dishes containing PDA, supplemented with 1% chloramphenicol to suppress bacterial growth. Replicates of the samples were prepared for the fungal plates using the pour plate method, with the formula in equation 1 employed.

$$\boldsymbol{dilution\ factor = \frac{final\ volume}{aliquot\ volume}} \text{ - - - - - - - - - - - } \mathbf{(1)}$$

Where; $final\ volume = aliquot\ volume(sample\ volume) + diluent\ volume$

Enumeration of fungal isolates was then carried out using the formula in equation 2, as by Willey *et al.,* (2008)

$$\boldsymbol{\frac{cfu}{g} = \frac{number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ inoculum}} \text{ - - - - - - - - - - - - - - - } \mathbf{(2)}$$

Special fungal features such as the nature of spores, hyphae, conidia and other features were used during identification.

3.3.2.3 Identification of Fungal Isolates

After fungal isolates had been successfully enumerated and sub-cultured on PDA, the moulds and yeasts were morphologically characterized after being stained with Lactophenol cotton blue (LPCB). The results obtained were then compared with standard references for proper identification of the isolates.

A drop of lactophenol cotton blue stain was placed on a clean, grease-free, and sterilized glass slide. Using a sterile inoculating wire loop, a portion of mycelium was carefully transferred from the mould culture onto the slide. The mycelium was evenly spread on the slide and gently covered with a cover slip. The preparation was allowed to sit for a few seconds before being examined under a microscope at ×40 magnification. The colonial and morphological characteristics of each fungal isolate were recorded, including the appearance of special structures such as spores or ascospores, if present. Observations also included the growth pattern and colony appearance from the initial stage to full maturity, along with the presence or absence of septate hyphae.

3.4 Statistical Analysis

Data obtained were analysed using analysis of variance (ANOVA) with the GenStat software, 12th edition and the means were separated using the Duncan's Multiple Range Test (DMRT) at 5% level of probability.

Chapter Four

4.0 Results and Discussion

4.1 Effects of EFB amendments on some chemical and physical properties of soil

The effects of EFB application at the different rates (0, 50, 100, and 150 kg), in the topsoil (0-15 cm depth) and subsoil (0-30 cm depth) are presented in table 1.

The soil pH ranged from 5.4 to 6.0, which is typical for tropical soils (Afu *et al.,* 2022). The highest pH value of 6.0 was observed in the treatment with 50 kg of EFB in the topsoil. This suggests that moderate incorporation of EFB initially helped buffer acidity by releasing basic cations during the early stages of decomposition. This finding aligns with the study of Ayeni *et al.,* (2021) that highlighted that application of organic amendments especially in moderate rates help stabilize soil acidity in tropical soils, by releasing basic cations that help neutralise the soil acidity.

However, at higher rates of EFB (100–150 kg), the pH slightly declined, likely due to the production of organic acids during decomposition, which can outweigh the buffering effect of the released cations. This pattern is consistent with a previous study on organic amendments in tropical soils which highlighted that in some cases, organic acids generated during decomposition processes can lead to acidification that may exceed the buffering capacity of the basic cations released. (Adeleke *et al.,* 2017).

Furthermore, electrical conductivity (EC) increased with the rate of EFB, reaching approximately 440 µS/cm with the 100 kg treatment in the topsoil. This indicates an accumulation of soluble ions released during decomposition, thereby enhancing short-term nutrient mobility (Mahmud and Chong, 2021). Improvements in organic carbon (OC) and organic matter (OM) were pronounced. OC increased from 11.87 g/kg in the control treatment to 17.30 g/kg in the 100 kg EFB treatment, and OM from 20.42 g/kg to 29.80 g/kg. These increases reflect the role of EFB as a substantial carbon input and are consistent with studies reporting that EFB incorporation improves soil organic reserves (Noirot *et al.,* 2022).

Table 1: Some physical and chemical properties of EFB amended soils.

Treatments	pH (1:1)	EC NS/cm	0.C gkg^{1}	O.M gkg^{1}	T.N gkg^{1}	Av. P Mgkg^{1}	K Cmolkg^{1}	Ca Cmolkg^{1}	Mg Cmolkg^{1}	Na Cmolkg^{1}	EA Cmolkg^{1}	ECEC Cmolkg^{1}	B.S gkg^{1}	Sand gkg^{1}	Silt gkg^{1}	Clay gkg^{1}
0-15cm Soil Depth																
0	5.80^{b}	365.00^{d}	11.87^{d}	20.42^{d}	1.03^{c}	0.87^{d}	0.20^{c}	0.16^{d}	0.10^{d}	0.05^{c}	1.03^{a}	1.55^{c}	33.21^{b}	860.00^{a}	13.00^{a}	127.00^{a}
50	6.00^{a}	431.70^{d}	16.00^{b}	27.52^{b}	1.43^{b}	1.10^{b}	0.27^{b}	0.22^{b}	0.13^{b}	0.07^{b}	0.70^{b}	1.39^{d}	49.52^{a}	865.00^{a}	10.00^{b}	125.00^{a}
100	5.60^{c}	440.00^{a}	17.30^{a}	29.80^{a}	1.60^{a}	1.18^{a}	0.38^{a}	0.30^{a}	0.19^{a}	0.10^{a}	1.00^{a}	1.97^{a}	49.24^{a}	870.00^{a}	10.00^{b}	120.00^{a}
150	5.40^{d}	398.70^{c}	14.00^{c}	24.10^{c}	1.30^{b}	1.04^{c}	0.25^{b}	0.20^{c}	0.12^{c}	0.07^{b}	1.00^{a}	1.64^{b}	39.02^{b}	864.00^{a}	10.00^{b}	126.00^{a}
15-30 cm Soil Depth																
0	5.80^{a}	191.00^{c}	10.10^{a}	17.40^{a}	0.90^{a}	0.60^{b}	0.14^{c}	0.11^{c}	0.07^{c}	0.04^{a}	1.00^{b}	1.36^{c}	26.29^{c}	860.10^{b}	12.00^{a}	127.00^{a}
50	5.62^{b}	156.30^{d}	9.17^{b}	15.80^{b}	0.73^{b}	0.52^{c}	0.13^{c}	0.11^{c}	0.06^{c}	0.03^{a}	1.13^{a}	1.46^{b}	22.72^{d}	871.00^{a}	11.00^{a}	118.00^{a}
100	5.80^{a}	249.00^{a}	10.40^{a}	17.90^{a}	0.90^{a}	0.73^{a}	0.18^{b}	0.14^{b}	0.09^{b}	0.05^{a}	1.00^{b}	1.47^{b}	31.51^{b}	862.00^{b}	11.00^{a}	127.00^{a}
150 1	5.62^{b}	223.30^{b}	10.40^{a}	17.87^{a}	0.97^{a}	0.61^{b}	0.23^{a}	0.18^{a}	0.11^{a}	0.06^{a}	1.03^{b}	1.61^{a}	35.84^{a}	864.00^{b}	8.00^{b}	128.00^{a}

Total nitrogen (1.60g/kg) and available phosphorus (1.18mg/kg) attained a higher level under the 100 kg treatment, in the topsoil (0-15 cm). This suggests that the decomposition of empty fruit bunches (EFB) releases macronutrients that can be beneficial to the soil nutrient status. The slight decrease in some nutrients in the 150 kg treatment may indicate immobilization due to excessive amendment rates and this could alter nutrient availability. This could be due to the high C:N ratio of EFB. EFB has a high C:N ratio and when decomposers break it down, they require nitrogen to do so, and build their own biomass thus; leading to a temporary nitrogen immobilization (Neswati *et al.,* 2022).

Also, the application of moderate amounts of empty fruit bunches (EFB) significantly ($P \geq 0.05$) increased the levels of exchangeable bases, including potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na), and also effective cation exchange capacity (ECEC), with the greatest effect observed at 100 kg. This enhancement of effective cation exchange capacity (ECEC) further improves soil fertility and buffering. The base saturation also varied with the application rate, was higher (49.52) at the 50 kg application rate. It increased from 33.21 (control treatment), reached a peak at the 50 kg application rate (49.52) and then began to decline from 100 kg application rate (49.24). This is in line with the research that highlighted that application of organic amendments to the soil tends to increase exchangeable bases thereby improving base saturation (Diacono and Montemurro, 2011).

The particle size distribution across the treatments showed a predominance of sand (860 – 871 g/kg), very low silt (8-13 g/kg) and a modest clay content (120-128 g/kg). this classifies the soil as a sandy loam. This suggests that the soil has a moderate water holding capacity, high infiltration and susceptibility to nutrient leaching (Brady and Weil, 2016). Generally, the application of EFB amendments to the soil significantly influenced the chemical properties (i.e., pH. EC, OM, TN, Av P, available basic cations, ECEC, EA, BS) in the topsoil and subsoil, with no modification to the physical properties. Organic amendments do not affect physical properties of soil within a short period of time.

4.2 Effects of EFB Amendments on The Fungal Population in Oil Palm Rhizospheres

4.2.1 Cultural, Morphological and Microscopic Characteristics of Fungal Isolates Obtained from Soil Samples

The cultural, morphological and microscopic characteristics of fungal isolates obtained from samples are presented in table 2. The table illustrates a comprehensive characterisation of the fungal isolates obtained from the treatment samples. These isolates were subjected to a series of cultural, morphological, and microscopic tests. The colony pigmentation and reverse coloration were the primary features that distinguished these fungi during culture. The results demonstrate a substantial level of microbial diversity, with the range of morphological and physiological traits.

Based on cumulative morphological and microscopic tests, the isolates were identified as; *Aspergillus niger, Trichoderma* sp., *Penicillium* sp., *Rhizopus arrhizus*, and *Mucor mucedo*

These identifications tally with the findings of Kirkman *et al.,* (2022), who identified that the major class of fungi associated with oil palm plantations is the ascomycetes, with *Aspergillus, Trichoderma, Penicillium,* and other ascomycetes as the frequently occurring fungi.

4.2.2 Fungi Population (CFU/g of Soil)

The fugal population of EFB amended soils at the different depths, in $\log_{10}$ CFU/g is presented in Figure 1. Among the four treatments, T3 (100 kg EFB) had the highest fungal population with 3.99 ± 0.19 $\log_{10}$ cfu/g in the top soil, and the lowest population was in T1 (control treatment) with 3.81±0.14 log10 cfu/g. The fungal population increased as the application rate increased, until it reached a peak at the 100kg EFB application rate, and then the fungal community began to decline with an increase of the EFB rate to 150 kg. This may be due to the combination of slope-induced runoff, possible waterlogging in lower zones, and nutrient imbalance likely creating less favourable conditions, leading to a decline in fungal population (Wang *et al.,* 2019). The fungal population was generally higher in the subsoil, compared to the top soil and this may be due to less external disturbances such as temperature fluctuation, deeper root penetration, leaching, etc. compared to the top soil creating a more stable environment for fungi proliferation in the sub soil.

Table 2; Cultural, morphological and microscopic characteristics of fungal isolates obtained from sample.

Cultural Morphology					
Colour of mycelium on agar plate	**Dark coloured growth**	**Green mycelium**	**Army green and entire, non-luxuriant with concentric ring**	**Initially white, with age turning grey and developing black dots**	**grey to off-white or white**
Colour of plate culture reverse	Dark	Pale yellow	Orange	light grey	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 and Aspergillus but Trichoderma forms sticky clumps of conidia with a distinctive green pigment rather than in chains. Typical green spore clumps are identified as Trichoderma.	clear (not pigmented) hyphae with smooth-walled conidiophores, stipes are rather long and is biverticillate	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 vesicle.	Conidiophores hyaline and loosely branched at right angles. Phialides flask-shaped and inflated at the base, with very short collarettes	Conidiophore stipes smooth-walled; phialides mono- or biverticillate, flask-shaped. Phialides do not show long pointed extensions at the tips	Rhizoids occur at the junction of stolon and sporangiophore	sporangia are produced on the tips of sporangiophores. The 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*	*Mucor mucedo*

This may be the reason for the variation of fungi population at varying depths. (Sun *et al.,* 2018; Qi *et al.,* 2024).

4.2.3 Frequency of Fungal Occurrence

From the result, *Aspergillus niger* had the highest occurring fungi genera, with 28.57% occurrence while *Rhizopus arrhizus* had the least frequency of occurrence with 9.52 %. *Penicillium sp.,* and *Mucor mucedo* had the same occurrence of 19.05% while *Trichoderma sp.* had 23.81% occurrence. These results align with the findings of Kirkman *et al.,* (2022), that reported that *Trichoderma sp, Penicillium sp., Aspergillus sp.,* are the most abundant fungi members in the oil palm rhizospheres (Figure 2). It also aligns with the findings of Tahir *et al.,* (2019) that reported that *Trichoderma sp, Penicillium sp., Aspergillus sp.* are the major fungi often associated with EFB and other palm residues.

4.2.4 Plant Growth Promoting Properties of Fungal Isolates

The ability of these fungal isolates to contribute to plant growth was assessed. They were screened for their ability to enhance; nitrogen fixation and phosphate solubilization. *Trichoderma sp.* was the only isolate that exhibited nitrogen fixation and phosphate solubilization properties, with a PSI of 7, whereas, other isolates were not found to have phosphate solubilization abilities. This was in line with the work of Bononi *et al,* (2020) where they highlighted that *Trichoderma sp.* isolated from rainforest soils increased phosphorus uptake in soybean by 141% and improved plant growth by 2 – 41% under P – deficient conditions. It was also observed that *Trichoderma sp* and *Rhizopus arrhizus* exhibited nitrogen fixing abilities. However, since these isolates do not possess nitrogenase enzyme that enable them fix nitrogen directly, this ability is likely indirect. This effect may be as a result of decomposition of organic matter by the fungi that releases exudates or lower oxygen micro zones that favour the growth of free-living nitrogen fixing bacteria. Consequently, the positive nitrogen fixation signal observed may be due to these free-living nitrogen fixing bacteria present in the culture medium or co-isolated with the fungus (Poveda and Eugui, 2022).

Figure 1; fungal population in EFB amended soils, at different depths.

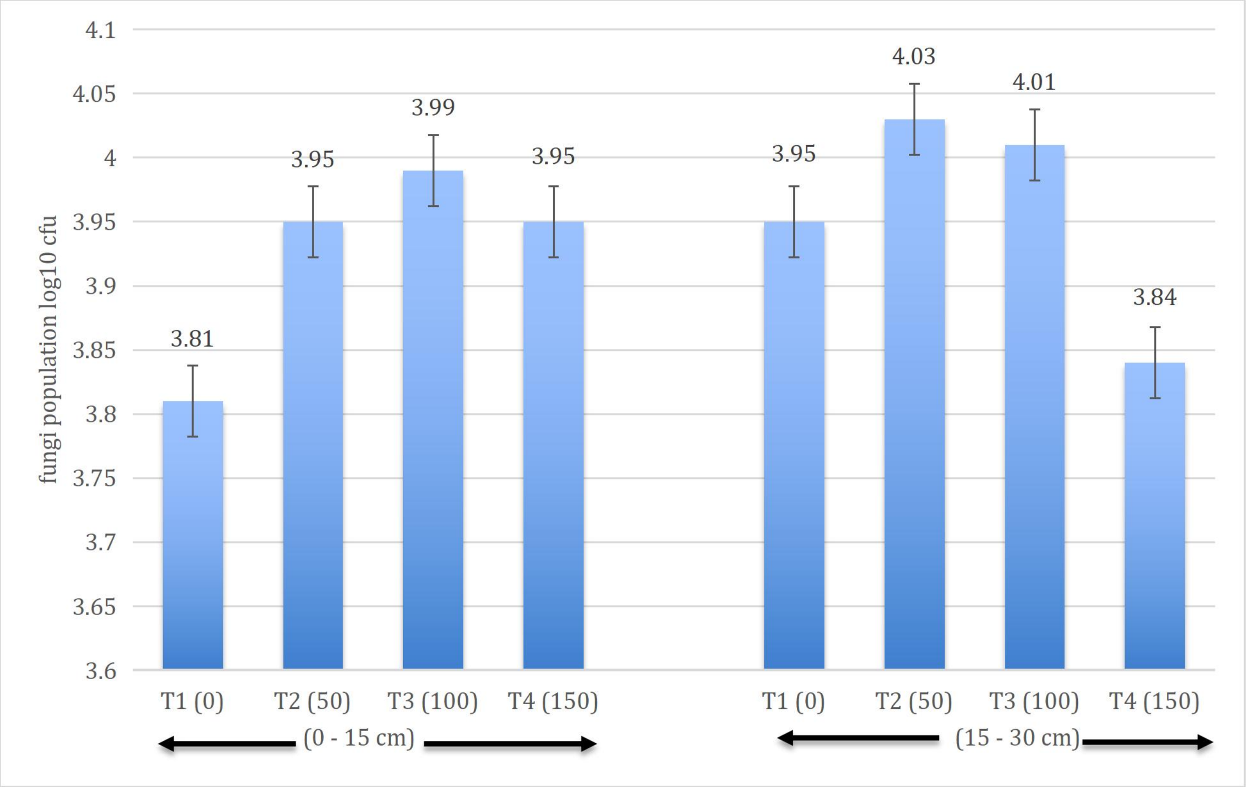

Figure 2. Frequency of fungi occurrence obtained from the different sample treatment.

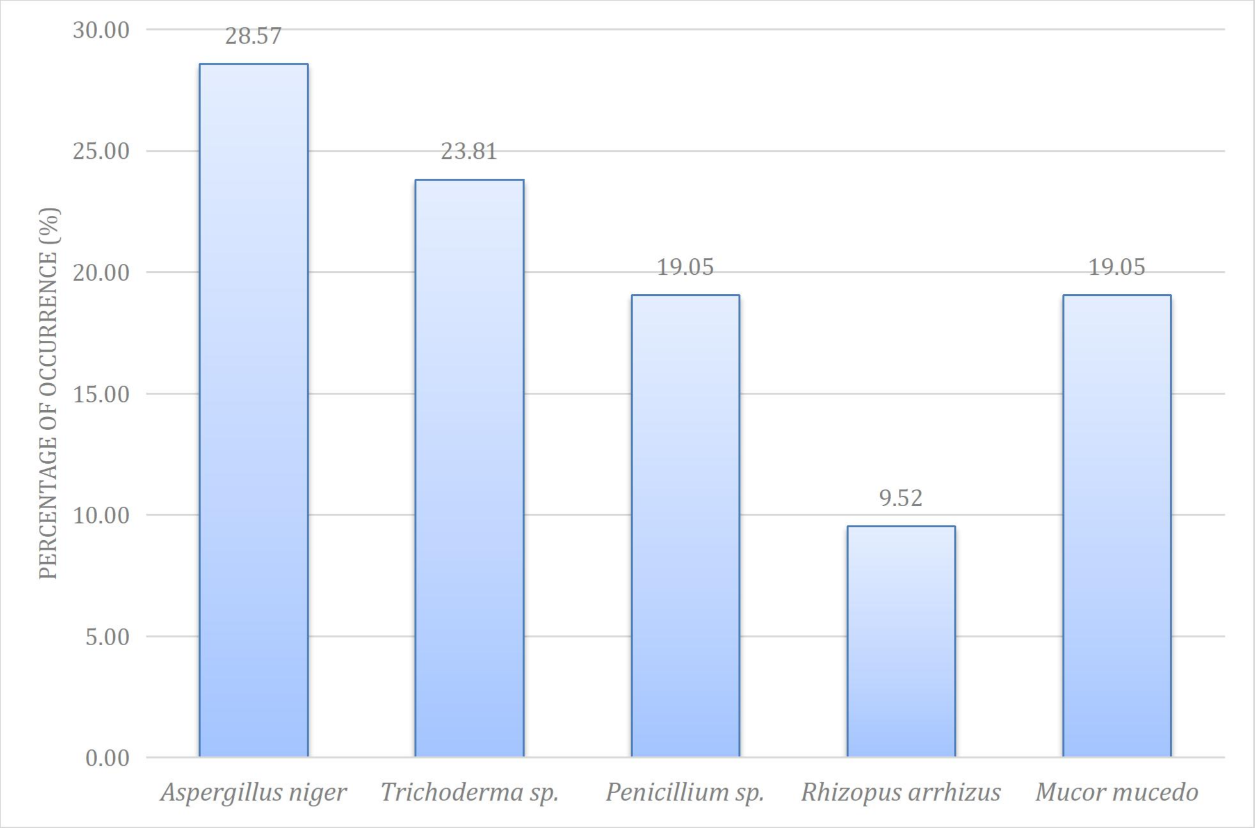

Table 3. Plant growth-promoting properties of fungal isolates

Isolates	Nitrogen Fixation	Phosphate Solubilization	PSI
Aspergillus niger	-	-	0
Trichoderma sp.	+	+	7
Penicillium sp.	-	-	0
Rhizopus arrhizus	+	-	0
Mucor mucedo	-	-	0

Key: + = possess property, - = does not possess property

Chapter Five

5.0 Conclusions and Recommendations

The study highlighted clearly that the application of EFB to the soil significantly modifies the soil environment and fungal community structure in oil palm rhizospheres. Moderate application rates, especially 100 kg, improved soil fertility indicators such as organic carbon, organic matter, total nitrogen, available phosphorus, exchangeable bases, and effective cation exchange capacity. The pH of the soil initially increased at moderate EFB application but declined at higher rates, suggesting that excessive amendments may lead to organic acid build-up and nutrient immobilisation. The fungal population responded positively to EFB application up to 100 kg but declined at 150 kg. This trend indicates that moderate EFB rates create favourable conditions for fungal proliferation and diversity, while excessive application can disrupt the balance of fungal communities. The subsoil showed higher fungal counts than the topsoil, reflecting its more stable micro-environment compared to the frequently disturbed topsoil. *Trichoderma sp.* displayed both phosphate solubilisation and nitrogen fixation traits, underlining its potential as a plant growth-promoting fungus.

From this study the moderate EFB incorporation can enhance soil fertility and stimulate beneficial fungal diversity, but excessive use may compromise fungal abundance, disrupt community structure and increase the risk of nutrient losses or pathogen build-up.

Based on the findings, these are the recommendations.

1) When applying fresh EFB to oil palm plantations, apply at the optimum rate of 100kg per palm stand to boost soil fertility and beneficial fungal diversity.
2) Avoid applying excess EFB to the palm stands.
3) Monitor soil properties and fungal communities after EFB application to maintain long term sustainability.

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APPENDIX I


FIELD: 14
YEAR PLANTED: 2015
PLANTING MATERIALS:
EWS Hybrid tenera.
SIZE OF THE FIELD: 2.5 Ha
PURPOSE:
Evaluation of Conservation Agriculture
Technique and use of Oil Palm waste
in Oil Palm Cultivation


NIFOR

APPENDIX II

SOIL NUTRIENT COMPARISM; PLANTATION WISE

Treatments	pH (1:1)	EC NS/cm	0.C gkg^{1}	O.M gkg^{1}	T.N gkg^{1}	Av. P Mgkg^{1}	K Cmolkg^{1}	Ca Cmolkg^{1}	Mg Cmolkg^{1}	Na Cmolkg^{1}	EA Cmolkg^{1}	ECEC Cmolkg^{1}	B.S gkg^{1}	Sand gkg^{1}	Silt gkg^{1}	Clay gkg^{1}
0	5.80^{a}	278.00^{a}	10.98^{a}	18.92^{a}	0.97^{a}	0.74^{a}	0.17^{b}	0.14^{b}	0.09^{b}	0.05^{b}	1.02^{a}	1.45^{bc}	29.75^{a}	860.50^{c}	12.50^{a}	12.70^{a}
50	5.81^{a}	294.00^{a}	12.58^{a}	12.55^{b}	1.08^{a}	0.81^{a}	0.20^{ab}	0.17^{ab}	0.09^{b}	0.05^{b}	0.92^{a}	1.43^{c}	36.12^{a}	868.00^{a}	10.50^{b}	12.15^{b}
100	5.70^{a}	344.50^{a}	13.85^{a}	23.85^{a}	1.25^{a}	0.96^{a}	0.28^{a}	0.22^{a}	0.14^{a}	0.08^{a}	1.00^{a}	1.72^{a}	40.38^{a}	866.00^{ab}	10.50^{b}	12.35^{b}
150	5.51^{b}	311.00^{a}	12.20^{a}	20.98^{a}	1.13^{a}	0.82^{a}	0.24^{ab}	0.19^{ab}	0.16^{ab}	0.07^{ab}	1.02^{a}	1.63^{ab}	37.43^{a}	864.00^{b}	9.00^{c}	12.70^{a}