EFFECTS OF EMPTY FRUIT BUNCHES (EFB) ON BACTERIAL COMMUNITY DYNAMICS AND DIVERSITY IN TOPSOIL RHIZOSPHERES OF OIL PALM (*Elaeis guineensis* .L).

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CERTIFICATION

This is to certify that this Project work was carried out by **OMOROWA** karen with Matriculation Number AGR2004422 of the Department of Soil Science, Faculty of Agriculture, University of Benin city, Nigeria.

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ABSTRACT

The oil palm industry generates large quantities of solid waste, particularly Empty Fruit Bunches (EFB), which are often discarded despite their potential value as organic soil material. This study examined the effects of EFB on bacterial communities in oil palm rhizosphere soils collected from the Nigerian Institute for Oil Palm Research (NIFOR) in Edo State, Nigeria. Laboratory analyses were conducted to determine how different EFB application rates influenced soil properties and bacterial population structures. The results showed that moderate EFB application at 50kg to 100kg improved key soil parameters such as pH, organic carbon, and nutrient content. Although bacterial counts slightly decreased with EFB addition, beneficial species such as *Bacillus subtilis* and *Enterobacter aerogenes* were more prominent, exhibiting plant growth-promoting traits like nitrogen fixation and phosphate solubilization. However, the presence of Escherichia coli and Staphylococcus aureus indicated potential biosafety concerns. In conclusion, EFB enhances soil fertility and supports beneficial microbes, when applied in moderate rates (50kg- 100kg)

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND STUDY

Oil Palm (Elaeis guineensis) is the major sources of natural food and valuable economic plants. The economic value of oil palm exceeds plants due to its usefulness for food, soap, cream and construction materials. However, harvesting and processing of oil palm fruits results in the generation of a lot of waste such as empty fruit bunches, mesocarp fibre, sludge and effluent.

EFB, a solid residue which accounts for 20% of the fresh fruit weight, is one of the palm biomass produced in abundance (4.42 t ha^{-1} y^{-1}) after oil extraction at palm oil mills. In the past, EFB was burned to produce steam at the mills and the ash produced was used as fertilizer or soil conditioner. However, incineration of EFB was soon discouraged because of environmental pollution and health hazards. However, EFB can be very useful as a substrate for mushroom cultivation or left to rot and returned to the fields as supplementary fertilizer (Chang, 2014).

Recent research has focused on utilizing EFB as a soil amendment to make soil richer and keep it productive longer. Shafiquzzaman *et al.,* (2016) demonstrated the effectiveness of composting EFB using Trichoderma strains, resulting in nutrient-enriched biocompost that improves soil health and plant growth. Similarly, (Pupathy and Radziah 2016) also explored the effects of nitrogen-fixing bacteria derived from EFB compost on soil microbial populations, showing the benefits of EFB compost in promoting beneficial microbial communities.

The rhizosphere is the narrow soil area close to the plant roots and the hotspot of various microbes. The plant influences the nearby soil through the release of certain chemicals known as rhizodeposits, which mainly consist of carbohydrates, secondary metabolites organic acids, and amino acids (canarini *et al.,* 2019). Applying EFB as a soil amendment can influence the diversity and dynamics of bacterial communities in the rhizosphere, thereby affecting soil fertility, soil health and sustainability. By enhancing beneficial microbial populations, EFB amendments can make nutrients easier to get and reduce harmful soil germs, and promote plant growth, offering a sustainable solution for managing agricultural waste and improving soil health.

Despite the increasing interest in utilizing empty fruit bunches (EFB) as a soil amendment in oil palm cultivation, there remains a significant knowledge gap regarding its exact impacts on soil microbes particularly the bacterial populations in the rhizosphere. While EFB is known to increase soil nutrients and organic content, its influence on the diversity, abundance, and function of rhizosphere bacteria has not been clearly established which necessitate this experiment.

Therefore, it is essential to study how application of EFB effects bacteria composition in oil palm rhizosphere and soil quality.

1.2 Objectives of the Study

To determine the:

1. effects of EFB application rates on bacteria population and composition.

2. pathogenity of the possible introduced bacteria to the cultivation and growth of oil palm.

CHAPTER TWO

LITERATURE REVIEW

2.1 OIL PALM CULTIVATION AND ORGANIC WASTE GENERATION

Oil palm *Eleais guineesis*, which was derived from the Greek word elaion (oil) and guineesis, suggesting its origin (Equatorial Guinea). *Eleais guineesis*, is a monocotyledon, monoecious (both male and female flowers on the same plant) and tropical perennial plant belonging to the family Arecaceae (Palmea). Eleais guineesis is the most versatile species of palm which is used in large scale plantations (Embrandiri *et al*, 2015). In the last few decades global demand for edible oils and fats has risen with the increased population and has resulted in a remarkable expansion in the area of oil crop cultivation mainly soybean and oil palm. With the increase in demand for palm oil in global markets, production rates have increased resulting in huge amounts of waste generated from the palm oil industry (Quaik et al., 2015).

The by-products of palm milling process include palm pressed fiber (mesocarp), palm kernel shell (endocarp), and empty fruit bunch (EFB), whilst palm trunk and frond are produced during the harvesting and pruning season (Samiran et al., 2015; Awalludin et al., 2015). The current practice of dumping in the most unscientific manner has led to excess nutrients in the soil. This is harmful to both the flora and fauna that live there. Despite the several applications of palm oil in recent times, the oil palm industry has drawn much negative attention among the scientific community because of many issues associated with oil palm cultivation like deforestation, biodiversity loss, peat land destruction and social conflicts. Likewise, palm oil industry also contributes to soil deterioration, water pollution and greenhouse gas (GHGs) emissions, posing threat to the environment. Thus, various strategies and policies are needed to be implemented to achieve the goal of sustainable management of palm oil industry. (Embrandiri et al., 2015)

2.2 Empty Fruit Bunches (EFB): Composition and Characteristics

The palm oil industry generates substantial quantities of solid waste materials, among which Empty Fruit Bunches (EFB) constitute a significant portion, accounting for approximately 20% of the weight of fresh fruit bunches (FFB) processed. This highlights the considerable volume of EFB produced during palm oil extraction processes (Tahir et al., 2019). These fibrous residues are the by-products obtained after fresh fruit bunches undergo sterilization and threshing at palm oil mills. Approximately 23% of the total weight of fresh fruit bunches (FFB) is converted into empty fruit bunches (EFB), while other by-products include 21% crude palm oil (CPO), 14–15% palm pressed fiber (PPF), and 6–7% palm kernel shell (PKS) (Corley and Tinker, 2016).

Empty Fruit Bunches (EFBs) primarily consist of lignocellulosic materials, including cellulose (ranging approximately from 22% to 65%), hemicellulose (about 19.5% to 39%), and lignin (between 10% and 34%) (Hazirah et al., 2017; Kolajo et al., 2024). Their elemental composition includes notable amounts of carbon (around 41% to 68%), hydrogen (2.9% to 7.3%), oxygen (26% to 52%), nitrogen (less than 0.1% up to 2.2%), and sulfur (0.04% to 0.9%), although these values vary depending on sample origin, environmental factors such as soil fertility and climate, and analytical methods used (Hazirah et al., 2017; Asoka et al., 2021). Additionally, the pH of EFB generally ranges from 5.8 to 7.8, indicating a neutral to slightly alkaline characteristic that can be beneficial for neutralizing acidic soils (Asoka et al., 2021; Kolajo et al., 2024).

Due to its high carbon content and lignocellulosic composition, Empty Fruit Bunches (EFB) are considered ideal raw materials for organic soil amendments. The lignocellulosic biomass of EFB consists mainly of cellulose, hemicellulose, and lignin, which are essential components for soil organic matter and contribute to improved soil structure and fertility (Tahir et al., 2019). However, the inherent structural complexity makes it resistant to rapid decomposition, thereby necessitating pre-treatment or microbial inoculation for effective agricultural use (Wahyudi et al., 2021).

2.2.2 Agricultural Uses of EFB as Organic Amendment

The rapid increase in organic waste generation from households, industry, and agriculture driven by global population growth poses both environmental challenges and opportunities for sustainable resource management (Hoornweg *et al.,* 2013). Transforming these organic wastes into nutrient-rich amendments such as compost, manure, crop residues, and empty fruit bunches (EFB) has become a promising strategy to improve soil health and fertility through enhanced microbial activity (Bernstad *et al.,* 2016; Calabi-Floody *et al.,* 2017).

Numerous studies have documented the potential uses of Empty Fruit Bunches (EFB) in agriculture, either in raw form as mulch or after being processed into compost or biochar (Anyaoha *et al.,* 2018). As a mulching agent, EFB helps improve soil moisture retention, suppress weed growth, and reduce surface evaporation. When composted or pyrolyzed, EFB enhances soil nutrient availability and moisture content (Ahmad, 2018). These improvements are likely attributed to the reduction of carbon losses during composting or pyrolysis (Zhang *et al.,* 2022).

Despite its usefulness, the direct application of untreated EFB or other agricultural residues to soil can be problematic. Such wastes may harbor pathogens, weed seeds, heavy metals, or produce unpleasant odors (Vakili *et al.,* 2015). Composting provides a viable alternative to ensure safe usage (Zakri and Adam, 2021). The composting process transforms raw biomass into a more stable, nutrient-rich product that can safely be applied to agricultural soils (Supriatna *et al.,* 2023). This compost improves soil structure, increases microbial activity (Zakri and Adam, 2021), and enhances nutrient exchange through improved cation exchange capacity (CEC) (Padhan *et al.,* 2022).

Currently, Malaysia is utilizing palm oil mill waste such as EFB and palm oil mill effluent (POME) for compost production (Hoe *et al.,* 2016). Composting EFB can reduce its volume by up to 50–75%, and the product can be commercialized for agricultural use (Then *et al.,* 2016). However, composting EFB takes time due to its complex lignocellulosic structure. With the use of POME-derived microbial inoculants, the process can be completed in about 40 days instead of the typical 60–90 (Huzairi *et al.,* 2013). Still, the resulting compost is low in macronutrients, and nutrient enhancement with NPK fertilizers is often required to boost its effectiveness (Hau *et al.,* 2020).

While microbial inoculation benefits plant growth in the short term, it may also create unfavorable soil environments over time, potentially affecting long-term microbial balance.

2.2.3 Effects of EFB Application on Soil Properties and Rhizosphere Microbial Communities

The application of Empty Fruit Bunches (EFB) to agricultural soils demonstrates significant improvements across multiple soil parameters. Research indicates EFB mulching enhances three key physical properties: soil pH, aggregate stability, and reduced bulk density, collectively improving soil structure and nutrient availability (Carron *et al.,* 2018). These improvements extend to hydrological characteristics, with EFB amendments increasing soil permeability and water retention capacity while further reinforcing aggregate stability, thereby providing effective erosion control (Caliman *et al.,* 2017). Importantly, long-term EFB application contributes substantially to carbon sequestration efforts, as demonstrated by elevated permanganate-oxidizable carbon (POX-C) levels in treated soils relative to control groups (Noirot *et al.,* 2022).

The chemical benefits of EFB amendments are equally noteworthy. They effectively regulate pH balance, optimize moisture content, and enhance the bioavailability of critical nutrients - particularly potassium and calcium - creating ideal growing conditions for oil palm cultivation (Hsiao-Hang *et al.,* 2018). Beyond physicochemical improvements, EFB exerts profound influences on soil biological communities. As a nutrient-dense organic substrate, EFB stimulates microbial diversity and metabolic activity, with particular enhancement of bacterial populations involved in essential nutrient cycling processes (Situmorang *et al.,* 2014). The decomposition process further activates soil fauna and amplifies microbial functions, notably accelerating nitrogen and phosphorus cycling (Tao *et al.,* 2016).

Recent innovations in EFB utilization show particular promise when combined with Plant Growth-Promoting Rhizobacteria (PGPR). This enriched compost formulation significantly boosts beneficial microbial populations, simultaneously improving soil fertility and crop productivity (Hindersah *et al.,* 2021). Such microbial-based biofertilizers represent a sustainable alternative to chemical inputs, with PGPR strains performing multiple functions including nitrogen fixation, phosphate and potassium solubilization, and induction of plant pathogen resistance (Ajeng *et al.,* 2020; Rodriguez *et al.,* 2019).

The most extensively studied PGPR genera include *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholderia, Rhizobium, Enterobacter,* and *Flavobacterium* (Compant *et al.,* 2019). These microorganisms play pivotal roles in plant nutrition, stress tolerance, and soil fertility enhancement across diverse crops including rice, wheat, grapevine, soybean, and sugar beet (Edwards *et al.,* 2015; Víctor *et al.,* 2019). The microbial resilience theory (Allison and Martiny, 2008) provides a framework for understanding these interactions, proposing that microbial communities dynamically adapt to environmental changes in ways that influence ecosystem functioning. In EFB-amended soils, this manifests as oil palm seedlings selectively recruiting beneficial microbial consortia to their rhizosphere, optimizing growth across varying soil management regimes.

2.3 Effects of Organic Amendments on Soil Microbial Community dynamics

Soil microbial communities represent complex assemblages of microscopic organisms that perform indispensable ecosystem services. These bacterial populations drive nutrient cycling, organic matter decomposition, soil structural development, and plant growth support (Li *et al.,* 2023; Zhang *et al.,* 2024). Their composition varies dramatically in response to environmental factors including pH, organic carbon content, moisture levels, temperature, and nutrient availability (particularly phosphorus, nitrogen, sulfur, and various cations).

Organic amendments introduce a diverse array of organic compounds, essential nutrients, and, in the case of manure, active microbial populations into the soil. This influx profoundly influences the composition, abundance, and activity of soil bacterial communities, particularly within the topsoil rhizosphere where plant-microbe interactions are critical. Studies have shown that amendments like compost and manure increase overall microbial biomass and bacterial diversity, enriching the soil ecosystem (Paz-Ferreiro *et al.,* 2015; Pascale *et al.,* 2020;). Similarly, EFB a lignocellulosic byproduct of the palm oil industry serves as a carbon-rich substrate that supports microbial colonization and proliferation, though its slower decomposition rate reflects its complex structure. (Pascale *et al.,* 2020; Singh *et al.,* 2023).

The response of soil microbial communities to organic amendments depends on several factors, including the type of amendment, its chemical composition (such as carbon-to-nitrogen ratio and lignin content), and the frequency and quantity of application. Manure, rich in readily available nutrients and an endogenous microbiome, often triggers a rapid increase in bacterial diversity and activity (Li *et al.,* 2012; Zhao *et al.,* 2016). Compost, depending on its maturity and feedstock composition, can also stimulate diverse bacterial populations, especially when derived from mixed plant and animal wastes (Morales *et al.,* 2016; Wang *et al.,* 2017).

Organic amendments reshape microbial communities by stimulating microbial biomass and enzymatic activity, thereby increasing microbial functional diversity and enhancing nutrient cycling processes essential for plant growth (Paz-Ferreiro *et al.,* 2015; Pascale *et al.,* 2020). For example, compost and manure applications have been shown to alter microbial networks by increasing microbial abundance and shifting community composition. EFB, rich in lignocellulosic materials, provides a sustained carbon source that supports diverse microbial populations over longer periods. However, the structure of the organic matter, particularly its C:N ratio and lignin content, plays a crucial role in determining microbial colonization rates and succession dynamics (Thiele-Bruhn *et al.,* 2012).

2.3.1 Comparative Impacts of Organic Materials on Microbial Diversity and Activity in the Topsoil Rhizosphere

Several studies have compared the effects of various organic amendments on soil microbial diversity and activity. For instance, compost application has been consistently linked to improvements in soil structure, increased water-holding capacity, and reduced erosion, all of which create favorable conditions for diverse microbial communities (Diacono and Montemurro, 2010; Bernstad *et al.,* 2016). The biological degradation processes involved in composting not only stabilize organic matter but also enrich the final product with microbial metabolites and humic substances that further stimulate soil microbiota upon application (Bernstad *et al.,* 2016; Calabi-Floody *et al.,* 2017).

Manure amendments introduce both nutrients and living microorganisms, which can outcompete or complement native soil microbes, leading to shifts in community structure and function. The process of bioturbation, facilitated by soil fauna such as earthworms, further integrates manure-derived microbes into the soil matrix, enhancing microbial heterogeneity and functional potential (Groffman *et al.,* 2015; Esperschütz *et al.,* 2007).

EFB-based amendments, especially when composted or combined with palm oil mill effluent (POME), promote bacterial communities that break down cellulose and hemicellulose, while also supporting nitrogen fixation and phosphorus solubilization (Moreno *et al.,* 2017; Edwards *et al.,* 2015). Although EFB decomposes more slowly, it provides a steady source of carbon and energy for soil microbes. Its application has been shown to gradually increase microbial biomass and diversity, particularly in soils that start with low organic matter. Due to its resistant nature, EFB's impact on microbial communities becomes more evident over time, encouraging the growth of specialized decomposers such as lignocellulolytic fungi and actinobacteria (Thiele-Bruhn *et al.,* 2012).

The magnitude and persistence of these microbial changes depend on several factors, including amendment type, application rate, soil properties, and environmental conditions (Nicholson *et al.,* 2018; Zhang *et al.,* 2022). Some research indicates that while organic amendments generally enhance microbial diversity, the specific outcomes can vary, with some studies reporting limited or no long-term effects, and others observing significant increases in both genetic and functional diversity (Bastida *et al.,* 2016).

However, the long-term impact remains dependent on site-specific factors including soil texture, climate, and prior land management. Some studies report no substantial change in microbial diversity over time, while others highlight marked increases in both genetic and functional microbial diversity (Bastida *et al.,* 2013).

2.3.2 Functional Mechanisms of Organic Amendments

Carbon and Nutrient Provision

Amendments supply both labile and recalcitrant carbon alongside essential nutrients (N, P, K), directly fueling microbial metabolism (Paz-Ferreiro *et al.,* 2015; Moreno *et al.,* 2017). EFB's slow decomposition provides sustained energy, while manure offers immediate nutrient pulses.

Physical Property Modification

Compost and EFB improve soil structure by enhancing aggregation, porosity, and water retention creating favorable microbial habitats (Diacono and Montemurro, 2010; Morales *et al.,* 2016). EFB specifically reduces bulk density and improves infiltration (Caliman *et al.,* 2017).

Enzymatic Activation

Amendment decomposition stimulates extracellular enzyme production, accelerating nutrient mineralization (Pulleman *et al.,* 2012; Moreno *et al.,* 2017). This favors copiotrophic over oligotrophic microbial groups.

Chemical Environment Alteration

Many amendments improve pH and CEC, creating more stable conditions for microbial growth (Schefe, 2019; Vaia, 2024). They also enhance mycorrhizal associations, improving phosphorus uptake (Churchland and Grayston, 2014).

The long-term effects are particularly notable with EFB, which supports specialized decomposers like lignocellulolytic fungi and actinobacteria (Thiele-Bruhn *et al.,* 2012). When combined with POME, EFB fosters bacterial communities capable of cellulose degradation, nitrogen fixation, and phosphorus solubilization (Moreno *et al.,* 2017; Edwards *et al.,* 2015).

Rhizosphere dynamics further amplify these effects through rhizodeposition-mediated microbial recruitment (Ni and Su, 2024). The resulting microbial assemblages enhance plant health and carbon stabilization (Peixoto *et al.,* 2020). Manure's introduction of exogenous microbes increases community diversity and functional redundancy (Li *et al.,* 2012; Zhao *et al.,* 2016), while all amendments collectively boost soil organic matter - the foundation of long-term soil health (Thiele-Bruhn *et al.,* 2012; Lal, 2020).

Critically, these effects are highly context-dependent, varying with amendment quality, application methods, soil type, and management history (Jurburg *et al.,* 2017; Kidd *et al.,* 2015). This underscores the need for site-specific amendment strategies to optimize microbial community outcomes.

2.4 Nutrient Cycling and Soil Fertility Implications of EFB Application

Empty Fruit Bunches (EFB), a significant by-product of oil palm plantations, are essential for nutrient cycling in tropical soils through their decomposition and the release of nutrients. When applied as an organic amendment, EFB significantly affects the dynamics of key nutrients carbon (C), nitrogen (N), and phosphorus (P) which are crucial for maintaining soil fertility and supporting sustainable agricultural productivity in tropical environments (Rosenani *et al.,* 2016; Zakri *et al.,* 2020; Caliman *et al.,* 2018).

The use of EFB enhances soil carbon dynamics. Once incorporated, EFB decomposes, resulting in considerable mineralization of organic carbon. Research indicates that approximately 50% of the initial dry matter of EFB decomposes within the first 90 days, with the carbon-to-nitrogen (C:N) ratio decreasing from around 80 to about 20 within six months. This change reflects increased microbial activity and the transformation of organic matter into more stable soil organic compounds (Lim *et al.,* 1987). The addition of organic carbon from EFB amendments boosts soil carbon stocks, enhancing soil fertility and helping to mitigate carbon loss in tropical soils vulnerable to degradation (Carron *et al.,* 2015; Tarigan, 2018).

Nitrogen dynamics in soils amended with EFB reveal a slower yet sustained release pattern. Unlike carbon, nitrogen mineralization occurs gradually, with a significant portion remaining bound in the organic material even after 11 months (Abu Bakar *et al.,* 2011; Anyaoha *et al.,* 2018). This slow-release characteristic provides a consistent supply of nitrogen, minimizing leaching risks and enhancing nitrogen use efficiency (Rahman *et al.,* 2019). Additionally, the application of chemical fertilizers can accelerate EFB decomposition, further increasing nitrogen availability (Gisong, 2022). EFB application has been shown to double soil nitrogen content compared to untreated soils, thereby improving nutrient status and productivity (Kour *et al.,* 2020).

Phosphorus, which is often limited in tropical soils, also benefits from EFB application. Although EFB contains lower levels of phosphorus compared to nitrogen or potassium, its decomposition gradually releases plant-available phosphorus, facilitated by microbial activity (Ismail, 2017; Hastuti and Rohmiyati, 2020). Furthermore, improvements in soil structure and organic matter content from EFB enhance phosphorus retention and reduce losses (Lim *et al.,* 2015).

2.4.1 EFB Decomposition Enhances Soil Fertility

The decomposition of empty fruit bunches (EFB) significantly enhances soil fertility by increasing organic matter, releasing nutrients, and improving soil structure. EFB is a rich source of organic carbon; as it decomposes, it contributes to the buildup of soil organic matter, which enhances aggregation, porosity, and moisture retention essential factors for root growth and microbial activity (Denang *et al.,* 2020; Teh Boon Sung *et al.,* 2010).

As EFB breaks down, it releases vital nutrients that replenish soil fertility. EFB contains considerable amounts of potassium, nitrogen, phosphorus, calcium, and magnesium, which become available to plants through the mineralization process during decomposition (Denang *et al.,* 2020; Rosenani *et al.,* 2016). This gradual nutrient release supports sustained plant nutrition and minimizes nutrient losses.

Additionally, EFB application improves soil structure by reducing compaction and increasing porosity, which enhances water infiltration and retention. This improvement supports better root development and boosts soil microbial biomass and activity, further accelerating organic matter decomposition and nutrient cycling (Rosenani *et al.,* 2017). Field studies indicate that mulching with EFB enhances soil chemical properties, including pH, organic carbon levels, cation exchange capacity (CEC), and available phosphorus, collectively improving soil fertility and crop yields (Denang *et al.,* 2020).

2.4.2 Interaction Between Nutrient Cycling and Bacterial Community Function in EFB-Amended Soils

Nutrient cycling and bacterial community function are closely interconnected in soils enriched with empty fruit bunches (EFB) under oil palm plantations. The lignocellulosic organic matter in EFB serves as both a substrate and habitat for diverse microbial communities. High-throughput sequencing indicates that bacterial communities in decaying EFB are primarily composed of phyla such as Planctomycetes, Proteobacteria, and Actinobacteria, which are adept at degrading lignin, cellulose, and other resistant compounds (PMC6594733; PMC8881682).

These bacteria produce enzymes that decompose EFB polymers, gradually releasing carbon (C), nitrogen (N), phosphorus (P), and other nutrients into the soil. Additionally, nitrogen-fixing and phosphate-solubilizing bacteria associated with EFB compost further enhance nutrient availability (Sitanggang *et al.,* 2021). The microbial succession that occurs during EFB decomposition fosters effective decomposers and nutrient cyclers, such as Bacillus, Streptococcus, and Micrococcus, thereby boosting soil enzymatic activities and nutrient pools (ScienceDirect article).

Biofertilizer formulations that combine EFB compost with beneficial bacteria have shown to increase root colonization and nutrient uptake in oil palm seedlings, thereby reducing reliance on chemical fertilizers and promoting sustainable soil health (E3S Web of Conferences, 2020).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Experimental Site

The experiment was conducted at Field 14 of the Nigerian Institute for Oil Palm Research (NIFOR), located in Edo State, Nigeria. This site falls within the country's tropical rainforest zone, characterized by high humidity, consistent rainfall, and relatively stable temperatures throughout the year, which are conducive to oil palm cultivation. The precise geographical coordinates of the field range from latitude 6°32'59.6904" to 6°33'3.97348" N and longitude 5°37'11.1306" to 5°37'12.7848" E, covering an area of approximately 2.5 hectares. Field 14 was established in 2015 with the East-West-South (EWS) hybrid Tenera oil palm variety, a high-yielding cultivar widely adopted in commercial plantations due to its superior oil content and adaptability to tropical climates.

3.2 Sampling Technique

Soil samples were collected from the experimental field after applying the treatments. Using an auger, samples were taken from each treatment replicate plot at depths of 0-15 cm and 0-30 cm.

For the first treatment plot, which received 100 kg of EFB amendment, samples were collected from 3 replicates, each containing 4 sub-replicates. Samples from the sub-replicates were combined to create a composite sample for each replicate, and this process was repeated across all 3 replicates. The same procedure was followed for the second treatment plot (150 kg EFB amendment), the third treatment plot (50 kg EFB amendment), and the control plot, which had only 1 replicate with 4 sub-replicates. Composite samples from each depth were then bagged, labeled, and prepared for analysis. In total, 20 samples were obtained from the sampling exercise.

3.3 Soil analysis

3.3.1 Physical and Chemical Analysis of Soil

Analysis of the soil samples focused on physicochemical properties which includes pH, organic matter content, and nutrient concentrations like nitrogen, phosphorus, and potassium. The samples were air-dried at ambient temperature and sieved through a 2 mm sieve, following the standard laboratory techniques.

3.3.1.1 Soil pH determination

15 grams of the fine soil sample was weighed and placed into a clean extraction cup (two replicates are recommended). 30 ml of deionized or distilled water was added to the soil to create a slurry, maintaining a soil-to-water ratio of 1:2. The mixture is thoroughly stirred intermittently for 30 minutes then allowed to rest for a few additional minutes. The pH is measured and recorded using a calibrated pH meter (KBS LTER 2023)

3.3.1.2: Soil Particle Size Analysis

- A 51 g sample of air-dried soil was measured into an extraction cup, to which 50 ml of sodium hexametaphosphate solution was added to facilitate particle dispersion
- Then, 100 ml of distilled water was poured into the cup, which was capped and stirred vigorously at medium speed for about 10 minutes
- The mixture was transferred to a 1000 ml sedimentation cylinder and topped up to the 1000 ml mark with distilled water
- After thorough mixing, hydrometer and temperature readings were taken at 40 seconds
- The cylinder was allowed to settle undisturbed for an additional 2 hours, after which hydrometer and temperature measurements were repeated (Lesikar *et al.,* 2005)

- The particle size distribution was then determined using these data

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

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

$$\%\,silt\ = (\%\,silt\ +clay) - \%clay$$

$$\%\,sand = 100 - \%(silt + clay)$$

Where:

HR = hydrometer reading

T = temperature

For every degree the temperature falls below 20°C, a subtraction was made, and for every degree it rises above 20°C, an addition was applied

3.3.1.3: Determination of Soil Organic Matter

Organic matter content was determined by first measuring organic carbon using the Walkley-Black method (1934), as outlined 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 of organic carbon was then calculated from the titration volumes obtained:

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

Where;

B = blank titre volume

T = sample titre volume

F = correction factor (1.33)

N = normality of ferrous sulphate

A conversion factor of 1.724 is commonly used to convert organic carbon to organic matter, based on the assumption that organic matter contains approximately 58% organic carbon. However, this factor applies primarily to certain soils or components of soil organic matter and can vary depending on factors such as vegetation cover, soil depth, and organic matter composition. To express the results in g kg^{-1}, the percentage organic matter values are multiplied by 10 (Eurofins, 2021; Minasny *et al.,* 2017)

3.3.1.4 Soil Nitrogen Determination

Nitrogen content was determined using the Kjeldahl method. 1 gram of air-dried soil sample was weighed and mixed with one Kjeldahl tablet and twenty milliliters 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 ten milliliters 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 three milliliters 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; The percentage of nitrogen was calculated from the titration volumes obtained:

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

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 in Soil Determination

Phosphorus was determined using the Bray-1 method. 5 grams of air-dried soil were weighed into an extraction cup, and 30 ml of Bray-1 extracting solution was added. The mixture was shaken mechanically for approximately 5 minutes, then filtered into an extraction bottle. 1 milliliter of the filtrate was transferred into a 50 ml volumetric flask, followed by the addition of 6 ml distilled water and 2 ml of color-developing reagent, which were mixed thoroughly. Next, 1 ml of ascorbic acid solution was added, and the mixture was left to stand for about 10 minutes to allow color development. The absorbance was measured at 650 nm using a visible spectrophotometer. A standard curve plotting absorbance against phosphorus concentration (ppm) was constructed, and the intercept was used to determine the phosphorus concentration (Cho *et al.,* 2017)

3.3.1.6 Exchangeable Base Cations Analysis (K, Ca, Mg, Na) in Soil

The ammonium saturation method was used to determine the exchangeable bases. 10 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).

3.3.1.6 Exchangeable Acidity (H and Al) Determination in Soil

Exchangeable acidity was determined by titration. 5 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.

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

3.3.1.8: Total Elemental Composition Analysis of Soil

One gram of air-dried soil was weighed into a digestion tube, and 10 ml of nitric acid (HNO_3) was added. The mixture was heated in a block digester for approximately one hour and then allowed to cool. Five milliliters of 2M hydrochloric acid (HCl) was added, followed by dilution with 10 ml of distilled water. The solution was filtered through filter paper into a 100 ml volumetric flask and brought up to the mark with distilled water. The resulting extract was stored in a plastic reagent bottle for subsequent instrumental analysis (Ahmed, *et al*,. 2022)

3.3.2: Microbial Analysis

3.3.2.1: Procedures for Sterilization of Soil Testing Materials and Equipment

Materials such as Petri dishes, pipettes, and glassware including conical flasks and round-bottom flasks were thoroughly drained and dried. They were then wrapped in aluminum foil and sterilized in a hot-air oven at 160°C for one hour. After sterilization, the items were allowed to cool before use. An aseptic working environment was maintained by using a Bunsen burner flame and disinfecting work surfaces with alcohol

All culture media were obtained from Oxoid and prepared according to the manufacturer's instructions. The media used in this study included Plate Count Agar, Bacillus cereus Agar (BCA), Eosin Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA), Pseudomonas Cetrimide Agar (PCA), Triple Sugar Iron Agar (TSI), Simmons Citrate Agar (SCA), and Mueller Hinton Agar (MHA) (Oxoid, 2022; Willey *et al.,* 2008)

3.3.2.2 Enumeration and Isolation of Bacterial Isolates from Samples

The samples were serially diluted tenfold by mixing 25 g of sample with 225 ml of sterile saline water (SSW). From this mixture, 1 ml aliquots were transferred sequentially into test tubes containing 9 ml of SSW to achieve successive dilutions. Subsequently, 1 ml from the fourth dilution tube was inoculated onto sterile Petri dishes containing nutrient agar supplemented with 1% fluconazole, while another set of plates contained potato dextrose agar supplemented with 1% chloramphenicol. Replicates were prepared for bacterial cultures using the pour plate method. The dilution factor calculation is provided below in equation 1

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

Where:

Final volume = aliquot volume (sample volume) + diluent volume

(Igbinosa, et al., 2021)

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

(Equation 2)

$$\frac{cfu}{g} = \frac{nmber\ of\ colonies\ x\ diltion\ factor}{volume\ of\ innoculation}$$

Following the pour plate culture of bacterial isolates from the samples, single colonies were subcultured onto tryptone soya agar and incubated for 24 hours at ambient temperature (28 ± 2°C). The colonies were Gram-stained and identified using standardized cultural and biochemical methods as outlined by Bridson (2006) in the Oxoid manual. Differential media from Oxoid, including Chromogenic Bacillus cereus agar with selective supplement, Sorbitol MacConkey agar with Cefixime-Tellurite supplement, Eosin Methylene Blue agar, Pseudomonas cetrimide agar (supplemented with glycerol), Salmonella Shigella agar, Mannitol Salt agar, and Triple Sugar Iron agar slants, were employed for the successful isolation and cultivation of bacterial isolates. Further bacterial identification was confirmed through biochemical tests such as citrate utilization (Simmon's citrate agar, Micromaster), indole production, oxidase, urease, sugar fermentation, catalase, 3% KOH test, gas production, and hydrogen sulfide (H_2S) formation.

3.3.2.3 Membrane Filtration Method for Soil Microbial Analysis

For each collected water sample, duplicate aliquots of 50 mL were filtered through 0.2 µm pore-sized cellulose nitrate membrane filters (Whatman Laboratory Division, Maidstone, England) using a Sartorius 16824 water pump. The membranes were aseptically transferred onto plates containing appropriately prepared growth media, taking care to avoid trapping air bubbles. The filters were incubated on nutrient agar, and after 24 hours, colonies were counted using colony counters before subculturing. Colony counts were recorded for the duplicate samples

$$Total\ colonies/100ml(cfu/ml) = \frac{\text{colonies counted } x \text{ 100}}{\text{mL sample filtered}} \qquad \text{(Equation 3)}$$

3.3.2.4 Phenotypic Identification of Bacteria from Samples

Pure bacterial cultures were obtained by subculturing single colonies isolated through the pour plate technique. These isolates were characterized based on cultural, morphological, and biochemical properties. Various tests were conducted, including Gram staining, catalase, urease, indole, oxidase, citrate utilization, as well as their reactions on triple sugar iron agar

3.3.2.5 Gram Staining of Soil Bacterial Isolate

A Gram staining procedure was performed to differentiate Gram-positive from Gram-negative isolates. Sterile, grease-free microscope slides were labeled and smeared with the bacterial sample using a sterilized loop. The smear was air-dried and heat-fixed by passing it over a Bunsen burner flame. Crystal violet stain was applied to the fixed smear for one minute, then rinsed off with distilled water. Lugol's iodine, acting as a mordant, was added for one minute and washed off. The smear was then decolorized with 95% ethanol for 30 seconds, followed by a distilled water rinse. Safranin was applied as a counterstain for one minute and rinsed off with distilled water. After air drying, immersion oil was added to the slide for examination under an oil immersion objective lens. Organisms were identified based on their color, shape, and arrangement: Gram-positive bacteria retained the purple color of crystal violet, while Gram-negative bacteria appeared pink from the safranin stain

3.3.2.6 Potassium Hydroxide (KOH) Test for Soil Bacteria

The potassium hydroxide (KOH) test was employed to rapidly distinguish Gram-negative bacteria from Gram-positive ones, serving as a complementary method to Gram staining. KOH dissolves the thin peptidoglycan layer of Gram-negative bacterial cell walls but does not affect the thicker walls of Gram-positive bacteria. This disruption causes lysis of Gram-negative cells, releasing their contents, including genetic material. A drop of 3% KOH solution was placed on a labeled, clean microscope slide and mixed with a loopful of pure bacterial culture. The mixture was gently stirred and observed for up to 60 seconds. The formation of a viscous, slimy, or mucoid string indicated a positive result, confirming the presence of Gram-negative bacteria, whereas the absence of this viscous string indicated a negative result

3.4 Statistical Analyses

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 soil physical and chemical properties

The effects of empty fruit bunches (EFB) (0, 50, 100, and 150 kg) on some of the physical and chemical properties of the topsoil (0–15 cm) and subsoil (15–30 cm) are presented in Table 1.The soil pH values ranged from 5.4 and 6.0, which falls within the typical pH range of tropical soils (Isong *et al.,* 2022). The highest pH (6.0) was recorded in the 50 kg treatment in the topsoil, implying that moderate application of EFB temporarily buffered soil acidity through the release of basic cations during decomposition. This observation agrees with Zhang *et al.,* (2023), who reported that organic amendments help reduce soil acidity during their early decomposition stages. At higher application rates (100–150 kg), a slight drop in pH was observed, likely due to the accumulation of organic acids, which can counteract the alkalinizing effect of cations. Similar trends have been reported by Adeleke *et al.,* (2017), where organic amendments sometimes led to acidification in tropical soils. Electrical conductivity (EC) increased progressively with higher EFB additions, peaking at around 440 µS/cm with the 100 kg treatment in the topsoil. This rise indicates increased soluble ions in the soil solution as decomposition released nutrients, thereby improving short-term nutrient mobility (Mahmud and Chong, 2021). Organic carbon (OC) and organic matter (OM) also increased significantly, rising from 11.87 g/kg and 20.42 g/kg in the control to 17.30 g/kg and 29.80 g/kg respectively in the 100 kg treatment. However, the aplication of 100kg EFB treatment significantly increased the OC and OM at the top and sub soil respectively. These results highlight the role of EFB as a rich carbon source, consistent with the findings of Noirot *et al.,* (2022) that EFB incorporation enhances soil carbon stocks.

Table 1: Physical and Chemical Properties of EFB Amended Top soils and Sub 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 – 30cm 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	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 (TN) and available phosphorus (Av. P) reached their highest levels under the 100 g treatment, with measurements of 1.60 g/kg TN and 1.18 mg/kg Av. P in the topsoil. This suggests that the decomposition of empty fruit bunches (EFB) releases macronutrients that can beneficial to the soil. The slight decrease in some nutrients at the 150 kg treatment may reflect nutrient immobilization or leaching caused by excessive amendment, potentially reducing nutrient availability. However, this contradicts the report from the research by Boafo *et al* (2019) that highlighted that EFB contains ample amount of nutrients that enriches the soil and also improves soil quality.

Also, the application of moderate amounts of empty fruit bunches (EFB) significantly 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 capacity although, this contradicts the research by Adeleke *et al,* (2017) that decomposition of organic amendments can acidify the soil. The base saturation also varied with the application rate, with the highest at the 50 kg application, which is 49.52. It increased from 33.21 in the control treatment, reached its peak at 50 kg and declined from 100 kg application. This is in line with the research that highlighted that application of organic amendments tends to increase exchangeable bases thereby improving base saturation (Wang *et al,* 2023).

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 soil. This suggests that the soil has a low water holding capacity, high infiltration and susceptibility to nutrient leaching (Brady and Weil, 2016).

4.2 Bacterial population in Colony Forming Units (cfu g kg^{-1})

The bacterial population observed in Figure 1 revealed that the subsoil control (15–30 cm) harbored more cultivable bacteria than the topsoil control (0–15 cm). This is contrary to the general expectation that topsoil typically supports higher microbial abundance due to greater organic matter and root biomassas observed by Sun *et al*, (2021). The higher counts in the subsoil may reflect localized conditions in the oil palm rhizosphere, which includes greater moisture retention, reduced fluctuations in temperature, and deeper root penetration that releases exudates into lower soil layers. Studies have shown that root systems of perennial crops like oil palm extend deeply into the soil, potentially enriching subsoil microbial populations through the continuous release of carbohydrates and organic acids (Intara *et al,*2018). In contrast, the topsoil, though rich in organic matter, may be affected by leaching and fluctuations in aeration, while the higher lignin content of organic residues further limits the stability and turnover of bacterial communities. In all the treatments, both topsoil and subsoil amended with empty fruit bunches (EFB) had lower bacterial populations than their respective controls. This suggests that EFB did not stimulate immediate proliferation of bacterial, instead, its high lignocellulose composition and slow decomposition rate likely caused nutrient immobilization, thereby reducing available substrates for a wide range of bacterial groups (Tahir *et al,*2019).

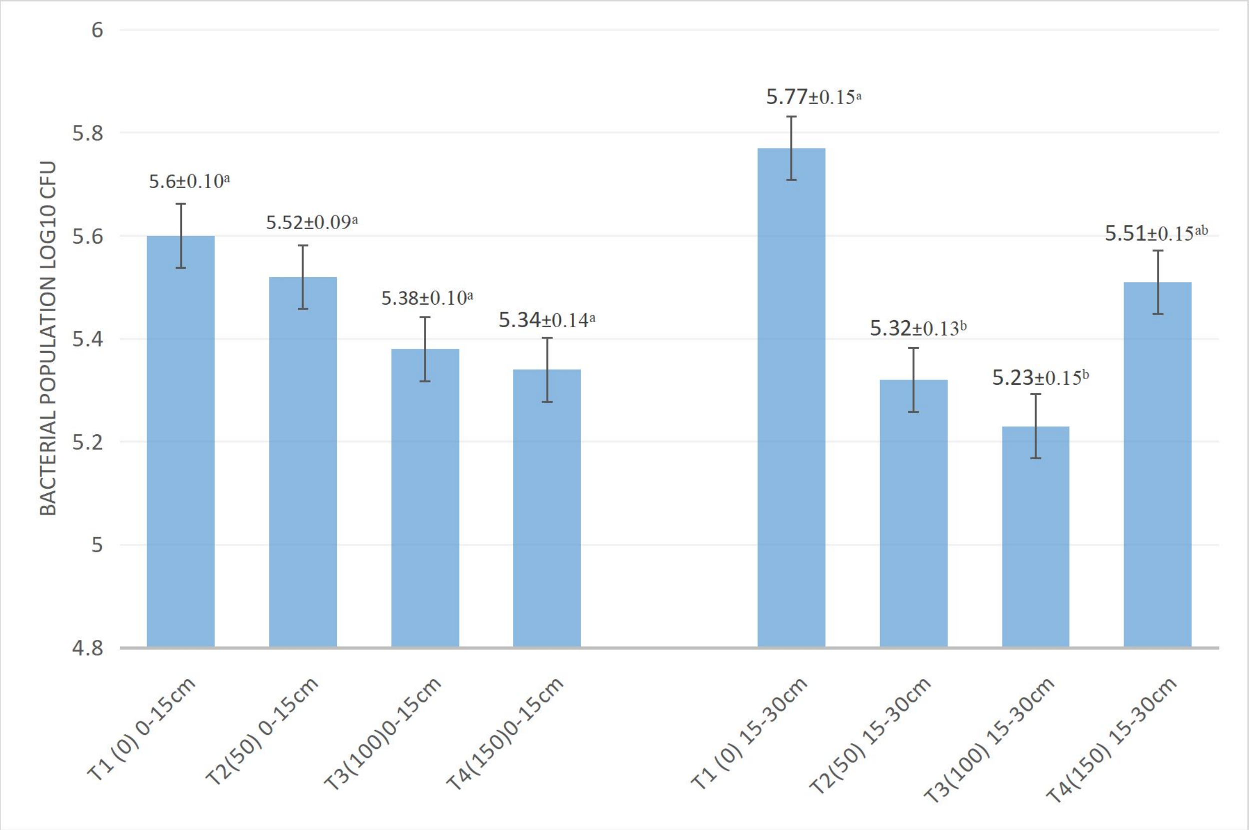

Figure 1: Bacterial population in Colony Forming Units (cfu)

4.3 Identification of Isolates

The cultural, morphological, microscopic, and biochemical characteristics of bacterial isolates obtained from the soil samples are presented in Table 1. The table provides a comprehensive characterization of the bacterial isolates recovered from the treatment samples. These isolates were subjected to a series of cultural, morphological, and microscopic examinations, alongside standard biochemical tests. Colony pigmentation, shape, margin, elevation, and growth patterns were among the primary features that distinguished these bacteria during culture. The results demonstrate a substantial level of microbial diversity, with clear variation in structural and physiological traits.Based on the cumulative cultural, microscopic, and biochemical tests, the isolates were identified as: *Bacillus subtilis, Enterobacter aerogenes, Serratia marcescens, Klebsiella oxytoca, Micrococcus luteus, Escherichia coli* and *Staphylococcus aureus*

These identifications are consistent with previous studies that have reported on the dominance of *Bacillus spp*. and *Enterobacter spp*. as common rhizosphere inhabitants with plant growth-promoting attributes (Hidayat *et al,*2024). At the same time, the detection of *E. coli* and *S. aureus* points to the presence of opportunistic and potentially pathogenic bacteria in the rhizosphere. This underscores the dual role of rhizosphere microbial communities in providing beneficial plant–microbe interactions while also presenting possible biosafety risks.

Table 2. Cultural, morphological and microscopic characteristics of bacterial isolates obtained from samples

Morphological							
Elevation	Raised	Flat	Flat	Raised	Raised	Flat	Flat
Margin	smooth	Undulate	Entire	Entire	Undulate	Undulate	Undulate
Color	Cream	Cream	Cream	Cream	yellow	Cream	Cream
Shape	Irregular	Irregular	Circular	Circular	Irregular	Irregular	Irregular
Size	Small	large	Small	Medium	Small	Large	Large
Gr. diff. agar	MSA	BCA	EMB	EMB	MSA	EMB	EMB
Colour	Yellow	Straw	Pink	opaque	Pink	green	pink
Staining							
Gram stain	+	+	-	-	+	-	-
cell type	Cocci	Rod	Rod	rod	Cocci	Rod	Rod
Arrangement	clusters	disperse	disperse	disperse	tetrads	disperse	disperse
Color	purple	purple	pink	pink	purple	pink	pink
Spore staining	-	+	-	-	-	-	-
Biochemical							
KOH String Test	-	-	+	+	-	+	+
Catalase	+	+	+	+	+	+	+
Indole	-	-	-	-	-	+	-
Citrate	+	+	+	+	+	-	+
Oxidase	-	-	-	-	+	-	-
Motility	-	+	-	+	-	+	+
Urease	+	-	+	-	+	-	-
Glucose	+	+	+	+	-	+	+
Sucrose	+	+	+	+	-	-	+
Lactose	+	+	+	+	-	+	+
Mannitol	-	+	-	+	-	-	-
Gas formation	-	-	+	-	-	+	-
H_2S formation	-	-	-	-	-	-	-
TSI (Slant/Butt) reaction	A/A*	A/A	A/AG	K/A (*A/A)	K/K*	A/AG	A/A(K*) G*
Esculin Hydrolysis	-	-	+	-	-	-	+
Identity	*Staphylococcus aureus*	*Bacillus subtilis*	*Klebsiella oxytoca*	*Serratia marcescens*	*Micrococcus luteus*	*Escherichia coli*	*Enterobacter aerogenes*

4.4 Percentage bacteria occurrence

The percentage occurrence of these bacterial isolates (Figure 2) further highlights the ecological distribution of the community. *Bacillus subtilis* (21.82%) and *Escherichia coli* (20%) were the most abundant species, followed by *Enterobacter aerogenes* (14.55%) and *Serratia marcescens* (14.55%). Less frequent isolates included *Micrococcus luteus* (10.91%), *Klebsiella oxytoca* (9.09%), and *Staphylococcus aureus* (9.09%). This distribution shows that the rhizosphere supports both beneficial and potentially harmful microorganisms.

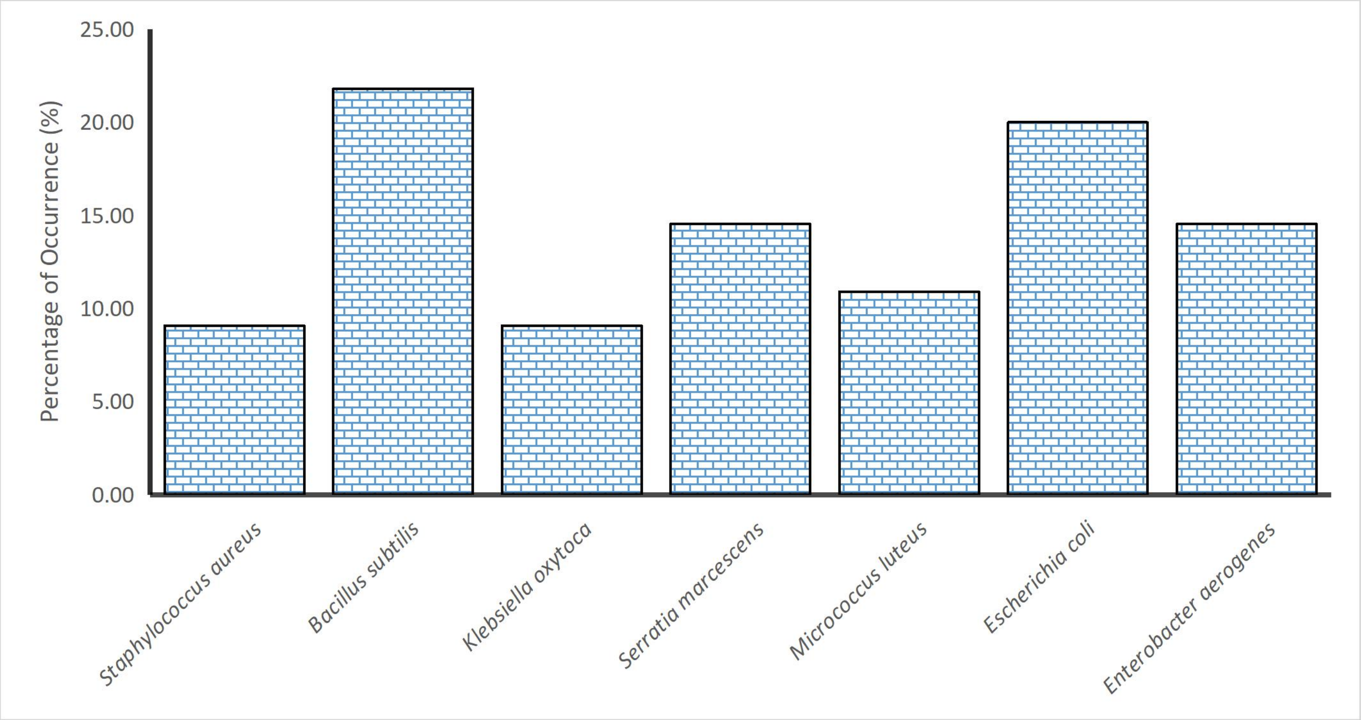

Fig 2: Bacterial Percentage of Occurrence

4.5 Plant growth- promoting properties

Functionally, several of the dominant isolates exhibited important plant growth-promoting (PGP) properties (Table 3). *Bacillus subtilis* demonstrated the ability to produce indole-3-acetic acid (IAA), solubilize phosphate, and fix nitrogen, making them crucial contributors to nutrient cycling and root development. *Enterobacter aerogenes* and *Micrococcus luteus* displayed PGP functions such as phosphate solubilization and IAA production, while *Serratia marcescens* had a more limited role but contributed to rhizosphere stability. These findings suggest that, despite lower overall bacterial counts in EFB-amended soils , the bacterial community shifted toward functionally valuable groups that could support oil palm growth.

Table 3. Plant growth-promoting properties of bacterial isolates

Isolates	Nitrogen Fixation	Ammonia Production	IAA production	Phosphate Solubilization	PSI
Staphylococcus aureus	-	-	-	-	0
Bacillus subtilis	+	+	+	+	7
Klebsiella oxytoca	-	-	-	-	0
Serratia marcescens	+	+	-	-	0
Micrococcus luteus	+	+	+	+	5
Escherichia coli	-	-	-	-	0
Enterobacter aerogenes	+	+	-	+	5

Key: + = Positive, - = negative

However, the pathogenicity test highlighted the biosafety risks within the bacterial community. The presence of Escherichia coli and Staphylococcus aureus both opportunistic pathogens raise concerns, since their occurrence in notable proportions suggests that organic amendments such as empty fruit bunches (EFB) may inadvertently introduce or encourage the persistence of undesirable microbes. While their ecological role in nutrient cycling may be limited, their presence emphasizes the dual nature of microbial enrichment in agricultural soils: the simultaneous increase of beneficial PGPRs and potentially harmful organisms.

Isolates	Hemolysin Production	Gelatinase	Urease	Protease	Lipase
Escherichia coli	-	-	-	-	-
Serratia marcescens	-	+	-	-	+
Bacillus subtilis	+	+	+	+	+
Pseudomonas aeruginosa	+	-	+	+	-
Enterobacter cloacae	-	+	+	-	-
Enterobacter aerogenes	-	-	-	+	-
Micrococcus luteus	-	-	+	-	+

Key: + = Positive, - = negative

Hemolysis = beta for positive and negative for gamma and alpha

Table 4: Pathogenicity Testing

CHAPTER FIVE

5.0 Conclusion

The study demonstrated that the application of empty fruit bunches (EFB) significantly influenced both the physicochemical and microbial properties of oil palm rhizosphere soils. Moderate application rates (50–100 kg) improved key soil fertility indicators such as pH, organic carbon, total nitrogen, available phosphorus, and exchangeable bases, showing that EFB is an effective organic amendment for enhancing soil quality in sandy tropical soils. Although EFB-amended soils showed slightly lower bacterial populations compared to the control, the bacterial community composition shifted towards functionally beneficial groups. Dominant isolates such as *Bacillus subtilis* and *Enterobacter aerogenes* exhibited plant growth-promoting traits including indole-3-acetic acid production, nitrogen fixation, and phosphate solubilization, indicating their potential role in nutrient cycling and root development. However, the detection of opportunistic pathogens such as *Escherichia coli* and *Staphylococcus aureus* highlights the need for caution, as EFB may also favor the persistence of undesirable microorganisms. EFB has proven to be a valuable soil amendment capable of improving soil fertility and supporting beneficial microbial activity, but its application must be managed properly to maximize its agronomic benefits while minimizing biosafety risks.

5.1 Recommendations

1. Apply EFB at moderate rates (50–100 kg) to achieve optimal soil improvement without causing nutrient imbalances or acidification.
2. Encourage pre-treatment or composting of EFB before field application to enhance decomposition, improve nutrient release, and reduce the presence of potential pathogens.

3. Increase farmer awareness through training and extension services to promote the safe and effective use of EFB as a soil fertility enhancer.
4. Conduct further long-term studies using molecular techniques to better understand microbial community dynamics and ensure the safe integration of EFB into sustainable oil palm production systems.

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APPENDIX


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


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