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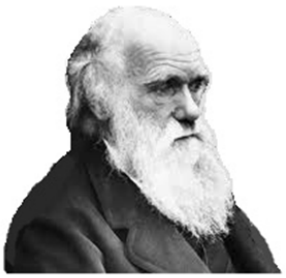

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Assessment of Microorganisms in the Rhizosphere Region of Plantain

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ABSTRACT

The populations of microorganisms that exist around the roots of plantain in the various farm locations across Toru Orua metropolis of Bayelsa, Nigeria are different due to difference in ecological locations. This paper assessed the different microorganisms in the Rhizosphere region of the sample plantain. Roots samples collected in University of Africa Toru-Orua (UAT) farm, Angalabiri farm and Koroye farm contain high population of bacteria compared to other Area in Toru-orua. In those locations where we have higher population of microorganisms like UAT farm, Angalabirir farm and Koroye farm, there was evidence of good nutrition in those locations. So, microorganisms in essence characteristically enhance the nutrition of plants Nitrobacter winogradkyi was found in roots samples collected in UAT, Angalabiri and Koroye farm due to the presence of cover crops around the plantain. These varieties of fungi were also isolated. The fungal population follows the same trend. They include aspergillus fumigatus, Aspergillus niger and Aspergillus flavus. These are the largest of all microorganisms in the soil. Others were Rhizobium, Nitrobacter, Winogradkyl, Azomona argillis and Pseudomonas aeruginosa. Practices that would enhance nutrition of the plants and the proliferation of bacteria and fungi around the roots of plantain are recommended such as organic matter accumulation in form of green manuring, zero tillage and non-use of chemicals and burning.

***Keywords*:** Farms, Microorganisms, Nutrition, Plantain, Roots

1. INTRODUCTION

Numerous microbial communities are housed by the rhizosphere, which is the thin soil layer that adheres to roots. Indicators of soil fertility and land use have been developed by

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several soil microbiologists using microbiological analyses of the soil. In general, microorganisms are in charge of the decomposition of organic matter, including hydrocarbons, the transformation of organic substances into various forms, and the generation of humus. These various kinds of creatures are classified into five different types: bacteria, actinomycetes, fungus, algae, and protozoa (Dita *et al*., 2018). Numerous animals and microbes live in the area around the root zone; however, it is generally accepted that microbes play a significant role in the release of nutrients, minerals, and carbon dioxide for plant development. It has been demonstrated that the endophytic and rhizospheric bacteria greatly enhance plant growth and health (Mendes *et al*., 2011). Researchers have looked into the possible advantages of helpful microorganisms in the rhizosphere/roots of Plantains (Dita *et al*., 2018).

One of the most significant staple food crops for millions of people in both developed and developing nations is the plantain (*Musa sapientum* var. *paradisiacal* Linn) (Oriola *et al*., 2017). Farmers find plantain farming appealing since it requires less labor to produce than cassava, maize, rice, and yam (Marriott and Lancaster, 1983). Therefore, it makes a substantial contribution to the food and financial security of those involved in its production and trading, especially in developing nations (Marriott and Lancester, 1983).

According to Solanke and Falade (2010), plantains are consumed across all socioeconomic strata and indigenous communities in Nigeria due to how simple they are to prepare and consume (Solanke and Falade, 2010). Nigeria yearly produces more than 2.11 million metric tons of plantains, which significantly improves the local subtropical population's diet (Akinsanmi, 2015). Despite the fact that many tons of plantains are produced each year, the price of plantains is always rising due to its high consumption rate. Lack of new and improved farming techniques is only one of several possible causes for this. In general, microorganisms are in charge of the decomposition of organic matter, including hydrocarbons, the transformation of organic substances into various forms, and the generation of humus. The distribution of rhizosphere microorganisms varies with textural changes and is higher in silty or silty clay horizons than in the sand or coarse horizons between them. According to several studies, microbes are crucial for the release of nutrients, minerals, and carbon dioxide for plant development. Microbial study of soil has been employed by several soil microbiologists as a gauge of soil fertility and land use. In reality, a lot of studies employ soil microbial analyses as a measure of the fertility of the soil and the usage of the land. However, there is little to no written material on the Isolation and Identification of microorganisms in the roots of plantain in Bayelsa state. It is on this basis this research was initiated to isolate and identify microorganisms in the roots of plantain in Toru-Orua metropolis.

2. LITERATURE REVIEW

2. 1. Plantain (*Musa sapientum* var. *paradisiaca* Linn)

All across the humid tropics and subtropics, plantains (*Musa sapientum* var. *paradisiaca* Linn) are grown for food. Plantains are a vital part of the diet and a major source of revenue for many small-scale farmers in West Africa. Millions of people in both industrialized and developing nations depend on it as one of their main sources of sustenance (Oriola *et al*., 2017).

Farmers find plantain farming appealing since it requires less labor to produce than cassava, maize, rice, and yam (Marriott and Lancaster, 2003). Therefore, it makes a substantial contribution to the food and financial security of those involved in its production and trading,

especially in developing nations. Due to its accessibility in Nigeria, it is consumed by both indigenous peoples and a wide range of socioeconomic strata.

Consuming plantains helps obese people lose weight and meets the caloric needs of many poor nations (Mohapatra *et al*., 2010). Plantain fruits have been used to make a variety of commercial items, including chips, beer, flour, and drinking drinks (Casimir and Jayaraman, 2001). Dodo Ikire, a common staple food made in several Western States of Nigeria, is named after the town of Ikire in Osun State. Fig. 1 shows the morphology of plantain (Rony and Rodomiro, 1997).

2. 1. 1. Scientific Classification (Taxonomic Position According to Cronquist (1981))

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Zingiberales
Family	Musaceae
Genus	*Musa*
Species	*Paradisiaca*

2. 1. 2. Morphology of plantain

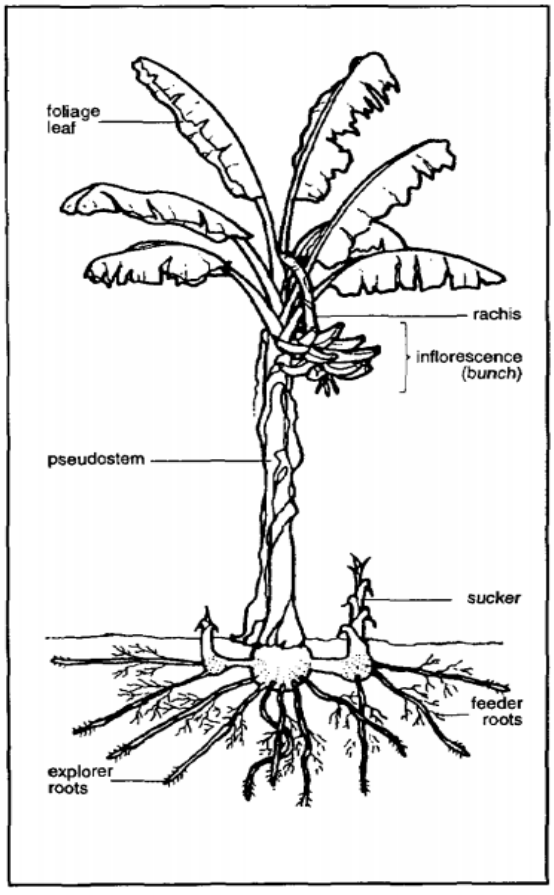

Fig. 1. The morphology of plantain (Rony and Rodomiro, 1997).

2. 1. 2. 1. Sucker

A corm produces 10-15 branches called suckers or daughters. Suckers are lateral shoots from the main plant. Suckers can be used for planting (Rony and Rodomiro, 1997). The whole unit of corm and suckers is called the mat or stool. There are four types of suckers:

- peepers are small suckers, appearing just above the ground
- sword suckers are large suckers with lanceolate leaves
- maiden suckers are large suckers with foliage leaves and large corms
- water suckers are suckers with small broad leaves and small corms (Rony and Rodomiro, 1997). Fig. 2 shows the plantain sucker (Rony and Rodomiro, 1997)

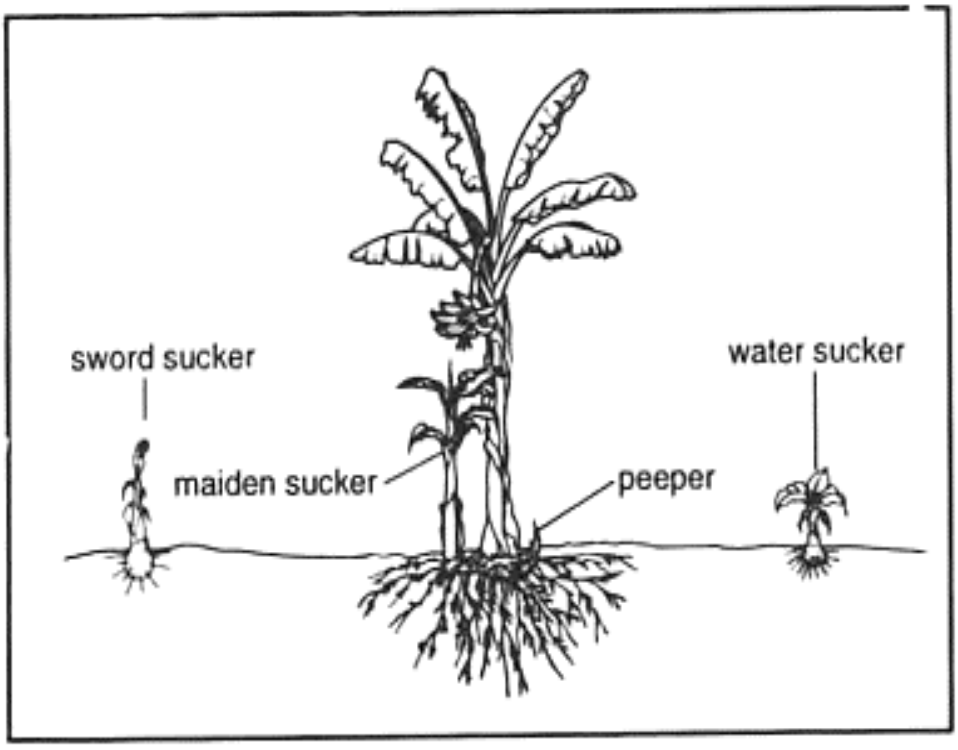

Fig. 2. Plantain sucker (Rony and Rodomiro, 1997)

2. 1. 2. 2. Pseudostem, Leaves

Plantain and banana are giant herbs, not trees. They do not produce wood. The cylindrical structure growing from the corm and carrying the foliage is a pseudo-stem, not a real stem, because the meristem remains near soil level. The pseudostem consists of overlapping leaf sheaths (Rony and Rodomiro, 1997).

Leaves are formed by the apical meristem and emerge from the middle of the pseudostem. There are two types of leaves:

- scale leaves
- foliage leaves

2. 1. 2. 3. Inflorescence (Bunch)

Upon flower initiation, the apical meristem converts into an inflorescence, which emerges through the center of the pseudostem, at the top of the plant. The inflorescence is supported by a rachis or fruit stalk, which is attached to the pseudostem for its support. The pseudostem and rachis provide a vascular connection between roots, leaves, and inflorescence (bunch) (Rony and Rodomiro, 1997).

2. 2. Medicinal and Nutritional Importance of Plantain

For the treatment of a wide range of human illnesses, plantain flowers, ripe fruit, unripe fruit, leaves, and stem extract and its active ingredients have been employed (Auta and Kumurya, 2015). Being a climacteric fruit, plantains experience a variety of physiochemical changes when harvested at the pre-climacteric matured green stage. These changes relate to changes in metabolic rates and biochemical responses including respiration, ripening, and senescence in the climacteric phase (Adeyemi and Oladiji, 2009).

Plantains are a good choice for blood pressure regulation since they provide a variety of minerals, are high in potassium, and are low in sodium (17 mg/100g) and fat (0.1 percent) (Mohapatra *et al.*, 2010). It is frequently advised for those who are intolerant to ingest. Therefore, this paper assessed the different bacteria and fungi microorganisms in the Rhizosphere region of plantain by isolation and identification methods.

2. 3. The Rhizosphere and Its Composition

Alexander (1977) defined the rhizosphere as the area of soil that is directly influenced by plant roots. It is the area of the soil through which all plant nutrients travel and through which any soil organism impacting the plant via the root must pass or transfer the influence. Here, there are a lot of soil interactions. The size of this zone varies on the soil's activity, although often the root's impact only reaches a few millimeters (Alexander, 1977). The soil environment particularly that closes to the roots has a significant impact on a plant's growth and development. It is a microenvironment that the plant provides, and the microorganisms play a significant role in it. The 'rhizosphere' is the name given to this area.

2. 3. 1. Structure of the Rhizosphere

The zone directly adjacent to the root surface is called the rhizoplane, and includes the epidermis and mucilage; the ectorhizosphere extends from the rhizoplane out to the soil. The term endorhizosphere was useful to identify and illustrate the importance of those microorganisms that exist in the internal portion of the root; however, if the rhizosphere is soil based, then the term endorhizosphere may be appropriate as it identifies the region of the root and not the rhizosphere. The ectorhizosphere is the actual portion of soil under the influence of plant roots. To avoid confusion between the endorhizosphere, rhizoplane, and ectorhizosphere, the terms root, rhizoplane, and rhizosphere, respectively, are used currently.

In plantain, the root system consists of primary, secondary and tertiary roots. Secondary roots are those that develop on primary roots, while tertiary roots develop on the secondary roots. Roots (all three types) have active growing tips (zones of elongation) which push the root through the soil. Just behind the growing tip of the primary root is a zone of 7-8 cm with root hairs. Behind the root hair zone is a bare zone, and then a long zone with secondary roots (Rony and Rodomiro, 1997).

Primary roots can be classified as:

- explorer roots
- feeder roots

Explorer roots are mainly for anchorage. Feeder roots, which usually grow from explorer roots, take up water and nutrients. Explorer roots are thicker than feeder roots (Rony and Rodomiro, 1997).

2. 3. 2. Interactions/changes in the Rhizosphere

2. 3. 2. 1. Physical Changes

The physical characteristics of the soil, such as its temperature, moisture content, and soil composition, have a big impact on how well plants absorb nutrients. The physical characteristics of the soil have a significant impact on the rhizosphere expansion because they influence root development and the transfer of ionic and molecular chemicals. Due to the substantial arbuscular mycorrhizal (AM) fungus population that promotes aggregate development in rhizosphere soils, plant roots have been demonstrated to boost the stability of surrounding aggregates in their rhizosphere region. It has a large capacity for retaining water, which enhances the soil's physical characteristics including bulk density, porosity, pore size distribution, etc.

2. 3. 2. 2. Chemical Changes

The rhizosphere, where plant roots and soil interact, undergoes a number of chemical changes. These interactions alter the soil's rhizodeposition, pH, nutrients, and redox potential, among other things, which has a substantial impact on the solubility of nutrients and the subsequent absorption of those nutrients. Plant roots not only sustain the plant and absorb water and nutrients, but they also release organic and inorganic substances into the rhizosphere. These compounds, which are secreted out from roots, have an impact on the availability of nutrients and rhizosphere microorganisms (Neumann and Romheld 2001). They protect the soil around the roots from pathogenic assault, mineralize nutrients, keep the soil wet, and stop the growth of rival plant species.

2. 3. 2. 3. Rhizosphere Biological Interactions

Due to nutrient-rich exudates, the rhizosphere is a hotspot of intensive biological activity. Specific plants and some soil microbes interact. These interactions may be advantageous or harmful. Mycorrhizae, legume nodulators, associative and free living dinitrogen fixers, nutrient solubilizers, and makers of antibacterial chemical are a few examples of the beneficial associations.

Vitamins, antibiotics, plant hormones, communication molecules, and other substances that promote plant development and reduce abiotic stress are also produced by rhizosphere bacteria. Another important location for endophyte entrance into plant roots is the rhizosphere. Important locations for endophyte entrance into the plant are the areas close to lateral root emergence and injured areas of roots. Later, this develops into a sophisticated plant-endophyte connection that helps the plants in a variety of ways, such as nutrition uptake, phytohormone synthesis, and induction (Rony and Rodomiro, 1997).

2. 3. 3. Microorganisms Related to Plant Rhizosphere

The type of root exudates the plant releases influences the microbial community in the rhizosphere either directly or indirectly. *Microflora* (bacteria, fungus, and algae) and micro- and meso-fauna make up populations in the root zone (nematodes, protozoa, mites, and insects). It is crucial to qualitatively and statistically examine the microbial population, their interactions with the root interface, and their participation in plant health since the rhizosphere soil is a zone of higher microbial activity.

2. 3. 3. 1. Soil microbial flora

a) Bacteria

Numerous bacterial species are the most prevalent microorganisms in farmed soil and participate in intricate rhizosphere activities (Ahemad and Khan 2012). They are thought to have between 106 and 108 cells per soil cubic centimeter. Per gram of soil, they are anything between a few million and a few billion bacteria cells. Up to 30 cm of cultivable soil contains the largest concentration of bacteria in the plough depth. Gradually fewer of them can be found in deeper strata.

A large proportion of motile bacteria, including amylolytic, proteolytic, ammonifying, denitrifying, and cellulose-decomposing bacteria, are found in the rhizosphere. Autochthonous and zymogenous bacteria are the two main categories of soil bacteria. Both zymogenous bacteria and autochthonous bacteria are part of the soil's natural microflora.

b) Fungi

Fungi are eukaryotic creatures that are absolute heterotrophs and mostly comprise aerobes or a group of organisms that ferment. The upper 20 to 30 cm stratum contains the majority of the fungus. In the top layers, the total mass of fungus is roughly similar to that of bacteria, and in soils from forests, it may even be higher. Between 0.001 to 1.0 billion fungi (about 1.5 tons/ha) are present on average. Both pathogenic and symbiotic fungi may be found in the rhizosphere, but whether one dominates in a given community depends on a variety of soil and plant-related variables. When choosing various rhizosphere fungus, root exudates have been identified as a key element (Buée et al. 2009) commonly occurring soil fungi.

2. 3. 4. Micro fauna

2. 3. 4. 1. *Protozoa*

Protozoan development is supported by moist soils. In a gram of dry soil, they may support populations of a few hundred to several million people. *Protozoa* make up 0.1–0.5 tons of soil mass each hectare. There are a few ciliates among them, but the majority of them are rhizopods (Amoebae) and flagellates. The *protozoans* rely heavily on bacteria as a source of food, but they are picky about which kinds of bacteria they consume. As a result, they have a selective impact on the makeup of the bacterial community. Due to their enormous biomass, quick turnover, and specific feeding strategies, Naked Amoebae are the most significant bacterial grazer group in soil.

They create a biofilm that sticks to the surface of the soil and the roots, giving them access to the majority of the bacteria present

2. 3. 4. 2. Nematodes

Nematode parasitism is frequently determined by environmental factors. Endoparasites (Heterodera) or ectoparasites make up the majority of plant parasitic forms (*Tylenchus* and *Dorylaminus*). Nematodes are prevalent in the top 30 cm of soil and are impacted by factors such as soil structure, texture, temperature, moisture, and others. The region of soil next to plant roots is known as the rhizosphere. This zone's metabolic activity is influenced by plant roots. To maximize the advantages of plant development and health from microbial sources, it is thus vital to understand the ecological interactions in the rhizosphere.

2. 3. 5. Effect of the Rhizosphere on Microbes

Numerous microorganisms are abundant in the rhizosphere, albeit their abundance tends to decline as the distance from the root rises. The ratio of the activity per unit volume of rhizosphere soil and the activity per unit weight of non-rhizosphere soil has traditionally been used to measure the rhizosphere effect (RS ratio) on a certain organism. A rough estimate of the degree to which a population is impacted by a rhizosphere may be made using this connection. An RS ratio of greater than one implies selective stimulation, one equal to one indicates no rhizosphere influence, and one less than one indicates rhizosphere activity suppression. Determining the rhizosphere competence of various plant microbe interactions can also be aided by the RS ratio.

2. 3. 6. Compounds Secreted in the Rhizosphere

The term "root exudates" refers to the organic substances that healthy roots secrete, which include more than 100,000 distinct low-molecular weight secondary metabolites (Jones and Darrah 1995; Brar *et al.*, 2006). In addition to a variety of amino acids, water-soluble sugars, organic acids, inorganic ions/gaseous molecules, and different vitamins and enzymes, root exudates are carbonaceous compounds (Uren 2001; Kumar *et al.*, 2006). Our understanding of root exudates as of this point is reliant on the use of methods from other disciplines. Chromatographic methods have made it possible for researchers to identify the wide range of carbonaceous compounds emitted from roots growing under axenic or sterile conditions, eventually revealing the complexity of root exudates.

2. 3. 7. Beneficial Role of Rhizosphere Microbes on Plants

Research on plant growth-promoting *rhizobacteria* (PGPR) has received a lot of funding in an effort to comprehend and make use of the microbial potential for plant performance. Making commercial goods based on microbes for growth promotion will be made easier with an understanding of their method of action. The microbial inoculant business has expanded to unprecedented heights in both the public and commercial sectors despite several obstacles. This demonstrates the enormous potential that microbes have to increase plant production.

The usage by plants of nutrients that are not readily available, such as iron, depends on microbes. According to predictions, the world's population will grow from its present level of seven billion people to 10 billion people in the next fifty years, necessitating a rise in agricultural output over the next few decades in order to feed everyone sufficiently. Some beneficial roles rhizosphere microbes perform include; Dinitrogen fixation, P-mobilization and solubilization, Nutrient uptake in deficient soils, Improvement of water uptake, Production of plant growth regulators, Promoting seed germination and early plant growth, Improvement in

soil structure, Competing with plant pathogens, Induced systemic resistance, Systemic-acquired resistance, Induced systemic tolerance, Overall biomass enhancement, Remediation of problematic soil etc.

2. 4. Application of Rhizosphere Microflora for Enhancing Crop Productivity: Bioformulations

An active ingredient is combined with an inactive or inert substance to create a bioformulation. A living germ, spore, or other latent form is the substance that is active in this situation. The phrase "biofertilizer" refers to formulations that contain microorganisms and/or a biological product that has the ability to fix atmospheric nitrogen, improve the solubility of soil nutrients, and/or potentially increase agricultural plant production. As a biocontrol agent, these microbes assist in creating a method to combat plant diseases that are environmentally friendly. A carrier-based biofertilizer should have 1 108 CFU/ml in liquid biofertilizers and 5 107 CFU/g in solid biofertilizers, according to the Bureau of Indian Standards (BIS) (Yadav 2009; Anandraj and Delapierre 2010).

Biofertilizers are used to apply bacteria that are helpful to plants. Biofertilizers are substances with advantageous microorganisms that promote plant development (Brahmaprakash and Sahu 2012). These helpful bacteria are transported to the field from their manufacturing location via an inoculant or formulation to promote plant development (Tittabutr *et al.,* 2007).

2. 4. 1. Types of Bioformulations

There are many variations available as biofertilizer formulations. The major types are:

- Solid carrier-based formulation
- Liquid formulation
- Polymer-entrapped formulation
- Fluidized bed dried formulation.

Granules, microgranules, wettable powders, and dust are examples of solid formulations (Guijarro *et al.,* 2007; Swapna *et al.,* 2016). Oil-in-water emulsions, mineral oil, organic oil, and culture broth are the bases for liquid inoculants. In a formulation with a polymer, the mass-multiplied inoculum is combined with the polymer before chemical solidification takes place (Jung *et al.,* 1982). This offers uniform beads that have living cells trapped inside of them. Typically, alginate is chosen while creating beads. It shields the imprisoned bacteria from adverse circumstances and gradually releases them into the surrounding environment. One of the major issues with the quality of biofertilizer is reducing contamination in the formulation.

Fluidized bed dried formulations of beneficial microorganisms are crucial in this regard since they have enhanced inoculant life and lowered contamination (Brahmaprakash and Sahu, 2012).

Granules are among the solid types of bioformulations and are defined as dry particles with an active ingredient, a binder, and a carrier that can be either coarse (100-1000 m) or micro (100-60 m) in size. Granules should be non-dusty, free-flowing, and should disintegrate in the soil to release the active ingredient. According to O'Callaghan and Gerard (2005), granules are safer and more focused on extending shelf life. Examples include wheat meal, corn meal bait, corn starch, cotton seed flour, alginate, and semolina wheat flour granules. Wettable powders,

the first type of bioformulation, are composed of 50–80% technical powder, 15–45% filler, 1–10% dispersant, and 3-5% surfactant (Brar *et al.*, 2006).

Aqueous suspensions of biomass suspensions in water, oils, or a mix of the two make up liquid formulations (Velineni and Brahmaprakash 2011). They are composed of 10–40% microorganisms, 1-3% suspender component, 1–5% dispersant, 3–8% surfactant, and 35–65% carrier liquid (oil/water) (Brar et al., 2006). The formulation's ability to support microbe viability is influenced by the type and amount of osmolytes utilized (Girisha *et al.*, 2006; Dayamani 2010; Dayamani and Brahmaprakash 2014). Liquid formulations are more effective since they keep more germs alive for a longer period of time (Bhaskara 2011). A stable suspension of an active substance in an oil or water-impermeable solvent is known as oil dispersion.

2. 4. 2. Mode of Application

There are three common ways of using biofertilizers:

1) Seed treatment
2) Root/seedling dipping
3) Soil application.

3. MATERIALS AND METHODS

3. 1. Collection of samples

The samples were taken from actively growing plantain roots and ball of the earth with the help of spade and cutlass, these samples were taken from three different farms in Toru-Orua metropolis (UAT farm, Anagabiri farm and Koroye farm). Samples were later put in the polythene bag, labeled properly and transferred to the laboratory immediately for laboratory analysis

3. 2. terilization and disinfection of materials

The plantain root samples were thoroughly washed in running tap water. They were then surface-sterilized using 70% ethanol for 2 mins and immersed in 150 ml of 1.5% sodium hypochlorite for 5 mins with shaking. The samples were then rinsed thoroughly in sterile distilled water and dried in sterile paper towels. Surface sterilized samples were macerated with a sterile mortar and pestle; they were later transferred to a petri-dish and kept in the refrigerator.

3. 3. Materials and Reagents

Nutrient Agar (NA), Mackconkey Agar (MA), sabouroaud Dextrose Agar (SDA), Bunsen burner, weighing balance, cotton wool, wire loop, incubator, conical flask, Beaker, pipettes, petri-dishes, Test tubes, Test tubes rack, autoclave, microscope, oil immersion, plantain root sample and volumetric flask.

3. 4. Serial Dilution and Inoculation

Serial dilution and inoculation was carried out for each sample, making normal saline diluted in distilled water according to the manufacture’s instruction. 9 ml of Normal saline was

introduced into 10-fold test tubes for serial dilution and sterilized before introducing the samples, 21 test tubes were used, using separate sterile pipettes, 1 gram of root sample was weighed out into the first test tube properly mixed. using a different sterile pipette, 1ml from the first test tube was pipette into the second test tube already containing 9 ml of diluted normal saline, this continued following the same procedure till the last dilution (the last test tube). Using the spread plate method for all media preparation and pour plate method for SDA, 1ml of each sample unit from the test tubes was pipetted into the sterile Petri dishes containing solidified Agars that were measured autoclaved and allowed to cool, media used was as Sabouraud Dextrose Agar [SDA], Nutrient Agar [NA], MacConkey Agar [MA].

After introducing the diluted samples from each test tube, Antibiotics was added to the SDA to inhibit the growth of Bactria on the SDA plate and the plate was incubated for 5 days, other plates were incubated at 37 °C for the 24hr. After incubation, there was growth on the MacConkey Agar plates. Growth were found on the NA and SDA plate, the representative colonies on the Nutrient Agar [NA] plates were subculture on fresh nutrients agar to run further tests and identify the growths on the plates using H_2O_2. Production of effervescence (bubbles) in 5-10 seconds is a positive test.

3. 5. Identification of Isolates

Identification of isolates was based on culture, morphological and biochemical characteristics following standard methods, biochemical test and characterization of isolate.

2. 5. 1. Gram Staining Technique

Colonies from different pure culture plates were emulsified into a drop of distilled water on a slide and a thin preparation was made. The smear was allowed to air dry, covered with crystal violet stain for 60 sec and was rapidly washed off with clean water. The smear was then decolorized with alcohol and washed off rapidly, counter stained with seferanine for 60 sec and washed off and examined microscopically under the ×100 objective lens.

2. 5 .2. Morphological Identification of Fungi

The plates were examined for the morphological characteristics of the fungal colonies. The macroscopic observation was aimed at determining the size, shape growth, and colour of the plate. This was done with a hand.

2. 5. 3. Microscopic examination of fungal isolates

The examination and microscopic examination of fungal isolates require the observation of microscopic features such as shape, size of hyphae, the shape of sporangia, conidia, conidiophores, and spores. Using a flamed inoculating needle, the edge of each colony is picked and slides of the different colonies are made, a drop of lacto phenol cotton blue stain is added to the slides and covered with cover slip and examine under the microscope using x100 and x400 magnification starting from third day of the culture. The microscopic characteristics observed were recorded accordingly.

2. 5. 4. Lacto phenol cotton blue staining technique

Lacto phenol cotton blue wet mount which is most widely used in the preparation of slides for microscopic examination of fungi. A drop of 70% ethanol was placed on a clean microscopic

glass slide. The test fungal isolate was then immersed in the drop of alcohol, drops of lacto phenol cotton blue were added and the wet preparation was covered with a glass cover slip. The wet preparation was examined using low power objective and thereafter, 40× objective.

2.6 Biochemical Test

a) Oxidase test

We add 0.2 ml of 1% α-naphthol, then add 0.3 ml of 1% p-aminodimethylaniline oxalate (Gaby and Hadley reagents). We shake vigorously to ensure mixing and thorough oxygenation of the culture. We used a cotton board, dipped into the solution and picked a part of the colony, We observed for color changes on the area with the picked organism, Microorganisms are oxidase positive when the color changes to deep blue within 15 to 30 seconds and Negative if there is no color change.

b) Catalase Test

Approximately 2-3 ml of freshly prepared 3% H_2O_2 hydrogen peroxide is taken in a test tube. A portion of the young bacterial colony is picked up using a sterile glass capillary, plastic or wooden stick and immersed into the tube contain.

c) Indole Test

Peptone water solution was measured and diluted following the manufacturer instruction, pure bacterial culture was grown in sterile tryptone broth over-night, 1.0ml of chloroform was added to the broth and shaken gently. After this, 5v drops of P-Dimenthylaminbenzaldehyde [KPVAC'S reagent] was added to the broth culture, Red color on the surface layer of the broth indicates positive while negative result appeared yellow on the surface of the broth.

4. RESULTS AND DISCUSSION

The populations of microorganisms that exist around the roots of plantain in the various locations across Toru-Orua metropolis of Bayelsa, Nigeria are different due to difference in ecological locations. Roots samples collected in UAT, Angalabiri and Koroye farm, contain high population of bacteria compared to other Area in Toru-Orua (Table 1). The fungal population follows the same trend. Five bacteria were isolated; they are micrococcus which are gram negative cocci in chain, oxidase negative, and indole negative (Table 5).

Table 1. Population of microorganisms found in plantain root across different Farm lands in Toru-Orua Metropolis

Location	Population count (cfu/g)	Fungi (cfu/g)
UAT farm	1×10^7, 3×10^7	2×10^4, 1×10^4, 1×10^4
Angalabiri farm	5×10^7, 9×10^7	
Koroye farm	4×10^7, 4×10^7, 5×10^7	

Characterization/identification of microbial isolates (morphological and biochemical characteristics), that is, morphological characteristics of isolates are shown in Tables 2 and 3.

Table 2. Morphological characteristics of microbial isolates (Mackconkey Agar Plate).

Mc (sample)	Size	Shape	Edges	Elevation	Colour	Texture	Opacity
Sample A 10^{-5} (1)	2 mm	Round	Entire	Raised	Creamy brown	Smooth and shiny	Opaque
Sample A 10^{-7}(2)	No growth	No growth	No growth	No growth	No growth	No growth	No growth
Sample C 10^{-7} (3)	1.5 cm	Irregular	Irregular	Flat	Yellowish brown	Rough	Transparent
Sample C 10^{-5}	2 mm	Round	Entire	Raised	Creamy brown	Smooth and shiny	Opaque
Sample B 10^{-5}	1.5 cm	Round	Entire	Raised	Red	Smooth and shiny	transparent
Sample B 10^{-7} (TNC)	1.5 cm	irregular	Entire	Raised	Brownish white	Rough	Opaque

Table 3. Morphological characteristics of microbial isolates (Nutrient Agar Plate)

Nutrient Agar (sample)	Size	Shape	Edges	Elevation	Colour	Texture	Opacity
Sample A 10^{-5} (1)	2 mm	irregular	lobate	umbonate	Yellowish white	Rough and shiny	Transparent
Sample A 10^{-7} (2)	2 mm	Irregular	lobate	Raised	Creamy brown	Smooth and shiny	Opaque
Sample C 10^{-7} (TNC)	1.5 cm	Round	lobate	umbonate	Yellowish white	Rough and shiny	Transparent
Sample C 10^{-5}	2 mm	irregular	Entire	Raised	Yellowish white	Rough	Opaque
Sample B 10^{-5}	2 mm	Round	Entire	Raised	Creamy brown	Smooth and shiny	Transparent
Sample B 10^{-7} (TNC)	2 mm	irregular	Rough	Raised	Yellowish white	Rough and smooth	Opaque

4. 1. Biochemical Tests

The results of the Biochemical tests carried out are shown in Fig. 3, Fig. 4 and Fig. 5, and Table 4. Five bacteria were isolated; they are micrococcus which are gram negative cocci in chain, oxidase negative, and indole negative.

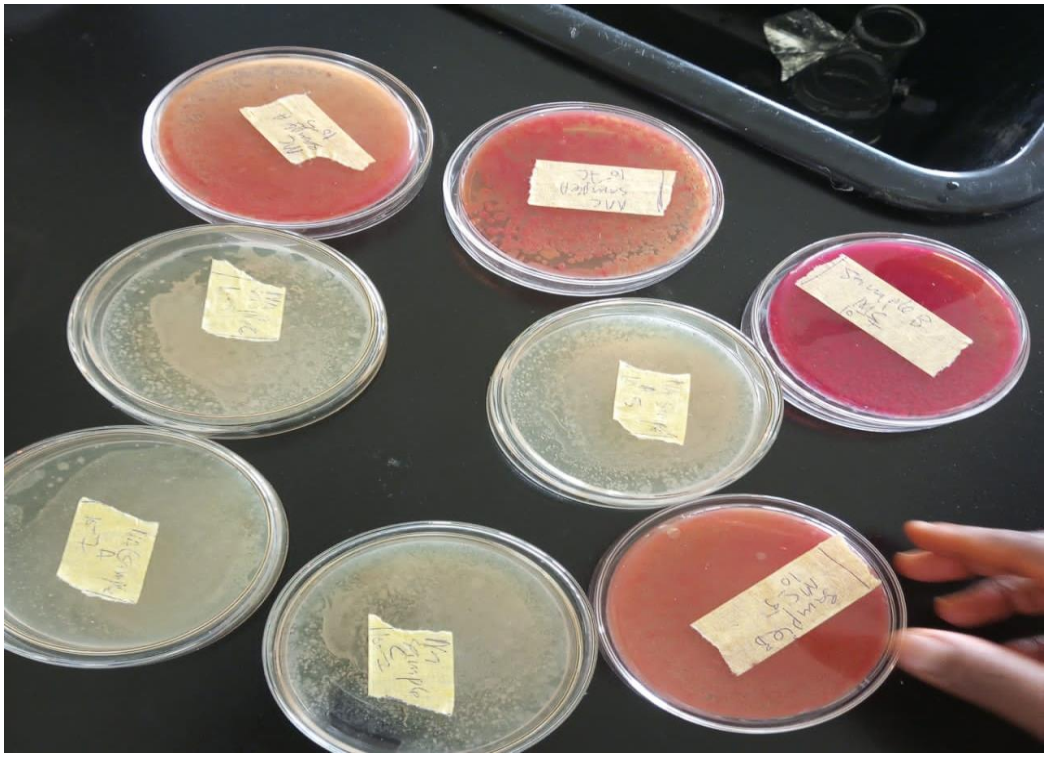

Fig. 3. Figure 4.1 Nutrient Agar Subculture plates

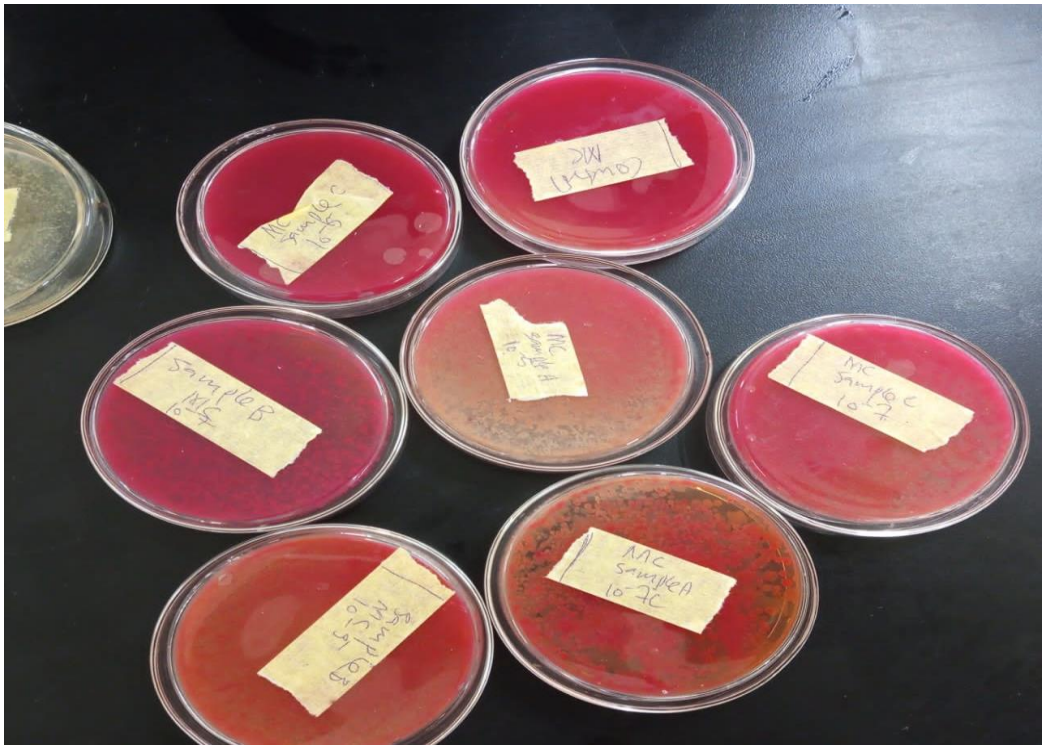

Fig. 4. MacConkey Agar Subculture plates

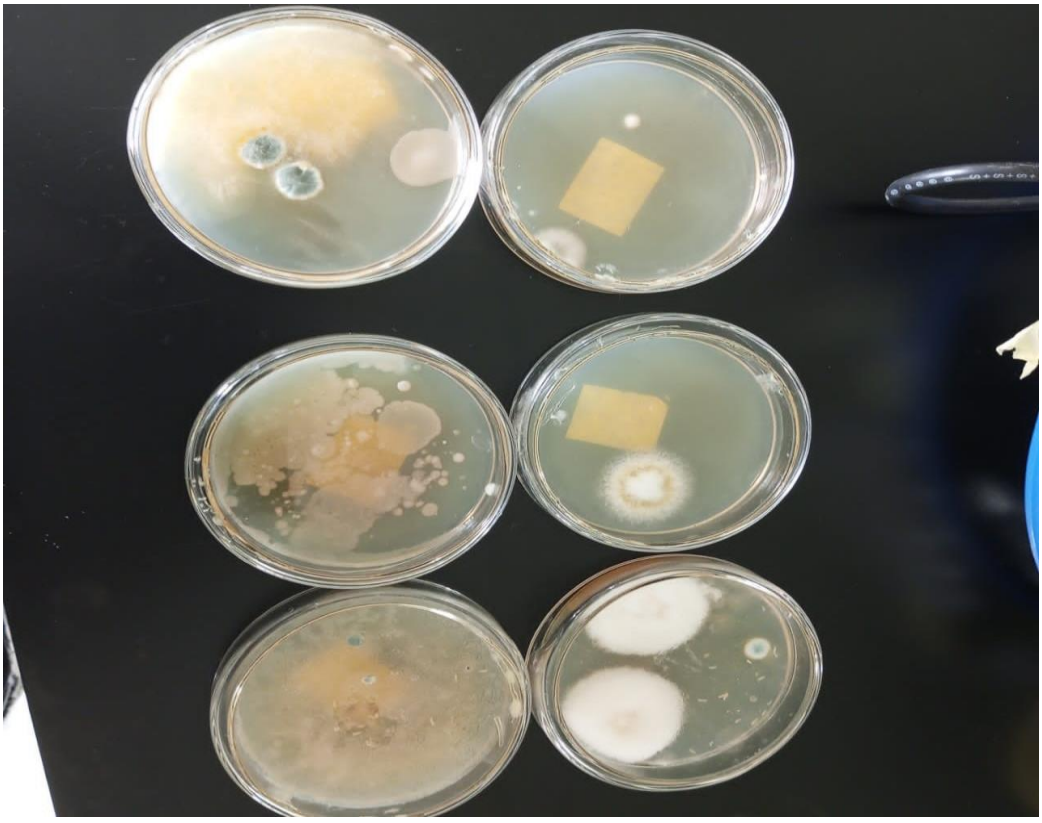

Fig. 5. Fungi plates

Table 4. Identification of fungal isolates

S/NO	Morphology	Microscopic examination with lactophenol	Fungi identified
1	Characterized by green echimulate conidia 2.5 to 3µm in diameter, produce in chains basipetally from greenish phialides, 6 to 8 by 2 to 3µm in size.	2.5-8µm wide, septate hyaline acute angle branching tree or fan like branching stipes may resemble hyphae of zygomycetes	*Aspergillus fumigates*
2	Black colouration in front and creamish in reverse view.	Aseptate hyphae with rough head of pigment	*Aspergillus flavus*
3	Colonies with loose white to yellow mycelium, rapidly turning dark brown and eventually black on the development of conidia.	Vesicle were light, yellow brown. Phialides growing radially along the periphery of vesicles. Primary phialides and secondary phialides are bogh brown	*Aspergillus niger*

Table 5. Index/Biochemical tests

Isolate	Gram reaction	Catalase test	Oxidase test	Indole	Most Probable organism
UAT farm	-ve (colli in chains)	-ve	-ve	-ve	*Micrococcus*
Koroye farm	-ve (Rod in chains)	-ve	-ve	-ve	*Nitrobacter*
Angalabiri farm	-ve (Stout rods in clusters)	-ve	-ve	-ve	*Rhizobium*
Isolate	**Gram reaction**	**Catalase test**	**Oxidase test**	**Indole**	**Most Probable organism**
UAT farm	-ve (Rod in chains)	-ve	-ve	-ve	*Azomonas agilis*
Koroye farm	-ve (Rod in chains)	-ve	-ve	-ve	*Pseudomonas aeruginosa*
Angalabiri farm	-ve (Rod in chains)	-ve	-ve	-ve	*Micrococcus*

5. DISCUSSION

In the Tables, the population of bacteria was higher than fungi in all the locations. The population of the organisms in school farm and axis between Toru-Orua and Angalabiri metropolis are different from other areas within Toru-Orua. The high population of bacteria in the soil corresponds to study of (Isirimah *et al*., 2006), an unpublished undergraduate project of 2004 which stated that the population of bacteria is higher than fungi in silty or silty clay soils. This indicates the populations of bacteria and fungi and the probable isolates present in plantain roots in different ecological zones of the state. In those locations where we have higher population of microorganisms (like school farm and axis between Toru-Orua and Angalabiri), there was evidence of good nutrition in those locations. So, microorganisms in essence characteristically enhance the nutrition of plants. Nitrobacter winogradkyi was found in roots samples collected in school farm and Toru-Orua and Angalabiri axis due to the presence of cover crops around the plantain. These varieties of fungi were also isolated. They include Aspergillus fumigatus, Aspergillus niger and Aspergillus flavus. These are the largest of all microorganisms in the soil. Others were Rhizobium, Nitrobacter, Winogradkyl, Azomona argillis and Psydormonads aeruginosa. Two different bacteria were found and isolated from samples collected at Koroye compound farm. This corresponds to (Isirimah *et al*., 2006) which state that different types of bacteria are found around the rhizosphere environment.

6. CONCLUSION

Isolation and identification methods of microorganisms in the roots of plantain were presented in this paper. It was revealed in the paper that the populations of bacteria and fungi

and the probable isolates present in plantain roots are differ in the different ecological zones of the case study state. In those locations, where we have higher population of microorganisms (like UAT farm and Angalabiri farm), there was evidence of good nutrition in those locations. So, microorganisms in essence characteristically enhance the nutrition of plants.

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