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Original Research Article

Assessment of Fungi and Bacteria Species in the *Moringa oleifera* Lam. and *Annona muricata* L. Rhizosphere of Arboretum, Rivers State University

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Abstract

The assessment of fungi and bacteria species in the Rhizosphere of Rivers State University Forest Arboretum. The experiment was laid in a completely randomized design (CRD). A composite soil sample of *Moringa oleifera* (Lam.) and *Annona muricata* (L.) of 0-35cm depth was collected using soil auger. The fungi and bacteria isolated and identified from the Rhizosphere of the Forest Arboretrum are *Rhodotorula sp*, (F.C. Harrison) *Aspergillus niger* (van Tieghem), *Microsporum audouinii* (Gruby), *Sporotrichum pruinosum* (J.C. Gilman and E.V. Abbot), *Aspergillus versicolor* (Budapest and Treaty). Though *Aspergillus versicolor* and *Microsporium audouinii* were absent in the soil of *Annona muricata.* However, the bacteria isolated from *Moringa oleifera* and *Annona muricata* are*; Bacillus sp* (Ehrenberg) *and Micrococcus sp* (Schroeter and Cohn). Generally, five (5) fungi and two (2) bacteria organisms were isolated, identified and classified into their different phylum, class and genera and cell morphology/microscopic characteristics. The result on the Simpson's diversity index of fungi and bacteria found in the Rhizosphere of *Moringa oleifera* and *Annona muricata* tree stand indicate that there was no diversity of Fungi found in both *Moringa oleifera* and *Annona muricata* tree stand (SDI = 1). Similarly the Simpson's diversity index value of bacteria population found in *Moringa oleifera* and *Annona muricata* tree stand (SDI=0.67) was more diverse when compared to the bacteria isolated from *Annona muricata* (SDI = 0.00). The soil microbial populations are indeed important factors for determining soil quality of the forest arboretum. Most micro-organisms isolated and identified are useful in nutrient recycling in this study. Therefore it is recommended that tree species of *Moringa oleifera* and *Annona muricata* promoted some good numbers of micro-organisms and the micro-organism found play an important role in soil food web in Rivers State University Arboretum. However, *Bacillus* based fertilizers could be applied to the soil to enhance the plant available for nutrients in the rhizosphere and help to control disease causing pathogenic microbes to induce pest defense systems.

Keywords: Rhizosphere, Forest Arboretum, soil augar, bacteria and fungi.

INTRODUCTION

Fungi are eukaryotic organisms with cell walls primarily made up of glucans and chitin and may reproduce both somatically and sexually, using spores (Cole, 1996; Bowman and Free 2006; Petersen, 2013). Fungi are microscopic cells that usually grow as long threads or strands called hyphae, which push their way between soil

particles, roots and rocks. Hyphae are usually only several thousandths of an inch in diameter. Fungi perform important services related to water dynamics, nutrient cycling and disease suppression. Fungi are important as decomposers in the soil food web. They convert hard to digest organic material into forms that other organisms can use. Fungi hyphae physically bind soil particles together, creating stable aggregate that helps increase water infiltration and soil holding capacity (Perterson, 2013).

Fungi are very successful inhabitants of soil, due to their high plasticity and their capacity to adopt various forms in response to adverse or unfavorable conditions (Sun *et al,* 2005). The diversity and activities of fungi are regulated by various biotic (plants and other organism) and abiotic (Soil pH, moisture, temperature and structure) factors (Lopez-Bucio *et al,* 2015; Rouphael *et al,* 2015).

The composition of soil fungi communities relates to tree growth. Fungi for long have been viewed as part of a black box of soil processes, in which the players are considered of minor importance (Allison and Martiny, 2018).

The high rate of tree growth which is characterized by high abundance of ectomycorrhizal fungi and agaricomycetes and low abundance of root-associated. Ascomycetes improve fungal communities. Agaricomycetes which include saprotrophs which have the capacity to decompose complex organic compounds (Floudas *et al,* 2012; Kohler *et al*, 2015) is of benefit to fungal communities. Fast decomposition results in mineralization of nutrients, which may indirectly favor tree growth (Van der Heijdan Bardgett, and Van Straalen 2008; Kyaschenko *et al,* 2017). It is also characterized by high soil pH, temperature and domination of spruce which improves tree growth. However, decreasing environmental stress favors the fungal communities because it enables the colonization of soil fungi with high capacity of nutrient mineralization, which is a benefit to tree growth.

Bacteria grow in many different microenvironments and specific niches in the soil. Bacteria populations expand rapidly and the bacteria are more competitive when easily digestible simple sugars are readily available around in the rhizosphere. Root exudates, dead plant debris, simple sugars, and complex polysaccharides are abundant is this region. About 10 to 30 percent of the soil microorganisms in the rhizosphere are actinomycetes, depending on environmental conditions (Sylvia *et al,* 2005).

Bacteria is important because as the soil is disturbed less and plant diversity increases, the soil food web becomes more balanced and diverse, making soil nutrients more available in an environment better suited to higher plants. Diverse microbial populations with fungus, protozoa and nematodes keep nutrients recycling and keep disease-causing organisms in check.

Many bacteria produce a layer of polysaccharides or glycoproteins that coats the surface of soil particles. These substances play an important role in cementing sand, silt and clay soil particles into stable micro-aggregates that improve soil structure. Bacteria live around the edges of soil mineral particles, especially clay and associated organic residues. Bacteria are important in producing polysaccharides that cement sand, silt and clay particles together to form micro-aggregates and improve soil structure (Hoorman, 2011). Bacteria do not move very far in the soil, so most movement is associated with water, growing roots or hitching a ride with other soil fauna like earthworms, ants, spiders, etc. (Lavelle and Spain, 2005).

Bacteria have the ability to adapt to many different soil microenvironments. They also have the ability to alter the soil environment to benefit certain plant communities as soil conditions change. Most soils are simply a graveyard for dead bacteria cells. Bacteria are so simple in structure that they have often been called a bag of enzymes and/or soluble bags of fertilizer (Dick *et al,* 2009). Since bacteria live under starvation conditions or soil water stress, they reproduce quickly when optimal water, food, and environmental conditions occur. Bacteria population may easily double in 15-30 minutes. Flourishing microbial populations increase soil productivity and crop yields over time. Forest ecosystems provide a broad range of habitats for bacteria, including soil and plant tissues and surfaces, streams and rocks, among others, but bacteria seem to be especially abundant on the forest floor, in soil and litter. Five phyla, *Acidobacteria, Actinobacteria, Proteobacteria, Bacteroidetes* and *Firmicutes,* appear to be abundant in most soils (Lauber *et al*, 2009). In addition to pH, which seems to be the bacteria community composition in the soils, organic matter content, nutrient availability, climate conditions and biotic interactions affect the composition of bacteria communities (Prescott *et al*, 2013). The high abundance of *Acidobacteria* and *Proteobacteria* across forest soil appears to indicate their functional importance. Bacteria perform many important ecosystem services in the soil including improved soil structure and soil aggregation, recycling of soil nutrients, and water recycling. Soil bacteria form micro-aggregates in the soil by binding soil particles together with their secretions. These micro-aggregates are like the building blocks for improving soil structure. Improved soil structure increases water infiltration and increases water holding capacity of the soil (Ingham, 2009). Bacteria perform important functions in the soil, decomposing organic residues from enzymes released into the soil. (Ingham, 2009) describes the four major soil bacteria functional groups as decomposers, mutualists, pathogens and lithotrophs. Each functional bacteria group plays a role in recycling soil nutrients.

Many soil bacteria processes nitrogen in organic substrates, but only nitrogen fixing bacteria can process the nitrogen in the atmosphere into a form (fixed nitrogen)

that plants can use. Nitrogen fixation occurs because these specific bacteria produce the nitrogenase enzyme. Nitrogen fixing bacteria are generally widely available in most soil types (both free living soil species and bacteria species dependent on a plant host). Free living species generally only comprise a very small percentage of the total microbial population and are often bacteria strains with low nitrogen fixing ability (Dick *et al.,* 2009).

The knowledge of some fungal and bacteria species found at the Rivers State University arboretum is not documented. Information on the composition of soil fungal and bacteria communities is of great importance in the rhizosphere. The microbial communities of the rhizosphere which include many genera of fungi and bacteria which is important to soil growth. The identification of fungi and bacteria species will increase our understanding in the interaction between fungi and bacteria, forest management, and ecosystem services. However, we are still at the beginning of understanding the mechanism of the ecosystem processes, in which fungi and bacteria takes part. Thus the importance of fungi and bacteria relative to other organisms in relation to the ecosystem and how such relationship may be useful for the development of forest management; hence this study is vital to researchers and scientist alike.

This research is aimed at assessing some species of fungi and bacteria in the rhizosphere of Forestry Arboretum Rivers State University, Nkpolu-Oroworukwo Port Harcourt.

Specific objectives of this research are to:

i isolate and identify the presence of fungi and bacteria found at the rhizosphere of the *Moringa oleifera* and *Annona muricata* tree stands in Forest Arboretum.

ii compare the abundance of fungi and bacteria in some selected tree rhizosphere in the Forestry Arboretum, using Simpson's Diversity Index (SDI)

MATERIALS AND METHODS

Study Area

The study was conducted in the Arboretum of Forestry and Environment, Rivers State University, Nkpolu-Oroworukwo situated in Latitude 4.5°N and Longitude 7.01°E at an altitude of 223 above sea level (Chukunda, 2014).

Methods of Sample Collection

In systematic random sampling design was employed since the trees are arranged in Plots in the Arboretum. The soil samples were collected in completely randomized design (CRD) due to the homogeneity of the arboretum soil. Then a soil sample of 0-35cm depth was obtained using soil auger borer from the experiment field, Forestry Arboretum and in accordance with the methods described by Chukunda and Alika (2018).

The soil samples were collected into sterile McCartney bottles. All the sample bottles will be preserved in an ice-cooled container and transported to the laboratory for physiochemical and microbiological analysis. All microbiological analysis was carried out under asceptic conditions.

Isolation and Enumeration of Soil Microbial Population

Isolation and enumeration of bacteria and fungi from the soil samples through serial dilution. Serial dilution of samples will be done up to three dilutions Aliquots (0.1ml) of appropriate dilution was spread and plated using a sterile bent glass rods onto the surfaces of fresh sterile dried nutrients agar plates for bacteria and potato dextrose agar plates (PDA) for fungi. (Harrigan and MacCane, 1990; Obire and Wemedo 1996; Ofunne 1999). The innoculated plates were incubated at 37°C for 24 hours for bacteria and 2-3 days for fungi. After incubation, plates that had significant growth was to be counted and the population of bacteria was recorded in colony forming units per gram (cfu/g) while population of fungi was recorded in spore forming units per gram (cfu/g) soil. Bacteria colonies were purified by sub culturing into fresh sterile nutrient agar plates which was incubated at 37^{o}C for 24hours and used as pure cultures for characterization of the isolates. Similarly discrete colonies of fungi was sub cultured into PDA plates which was incubated at 28^{o}C for 3-days and the pure cultures used for characterization of fungal isolates (Ofunne, 1999; Chukunda and Offor 2015).

Macroscopic and Microscopic Identification of Isolated Fungi and Bacteria

The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically by a compound microscope with a digital camera using a lactophenol cotton blue-stained slide mounted with a small portion of the mycelium (Gaddeyya *et al,* 2012; Reddy *et al,* 2014).

Abundance of Fungi and Bacteria in Selected Tree Rhizosphere in the Forest Arboretum

This method was carried out by assessing the similarities and difference between fungi bacteria in order to determine their abundance and diversity through selected trees in the arboretum.

The diversity of fungi and bacteria was calculated using Simpsons diversity index

Table 1a. Isolation of Fungi and Bacteria obtained from Rhizosphere of *Moringa oleifera* and *Annona muricata* tree stands in Rivers State University Arboretum

Micro-organism	*Moringa oleifera*	*Annona muricata*
Fungi		
1*) Rhodotorula spp*	+	+
2)*Aspergillus niger*	+	+
3*) Microsporium audouinil*	+	_
4*) Sporotrichumpruinosum*	+	+
5*) Aspergillus versicolor*	+	_
Bacteria		
1*) Bacillus spp*	+	_
2*) Micrococcus spp*	+	+

Key: + = Species of organisms present in the sample collected, - = species of organisms not present in the sample collected

Table 1b. Identification and Classification of fungi and Bacteria isolated into Phylum, class, genera and cell morphology/microscopy

Fungi/Bacteria Isolates	Phylum	Class	Genera	Cell morphology/microscopy
Fungi				
1) *Rhodotorula spp*	*Basidiomycota*	*Microbotyomycetes*	*Rhodotorula*	Oval and elongated pseudohyphae budding cells
2) Aspergillus niger	*Ascomycota*	*Euascomycetes*	*Aspergillus*	Septae hyphae with long smooth conidiophores and rough dark conidia
3)Microsporium audouinil	*Ascomycota*	*Euascomycetes*	*Microsporium*	Septae hyphae but no conidia
4)Sporotrichum pruinosum	*Basidiomycota*	*Hymenomycetes*	*Sporotrichum*	Broad septae hyphae with bridges known as clamp connection and branched with conidiophores
5)Aspergillus versicolor	*Ascomycota*	*Euascomycetes*	*Aspergillus*	Septae hyphae with smooth conidiophores
Bacteria				
1*) Bacillus sp*	*Firmicutes*	*Bacilli*	*Bacillus*	Irregular unstable spherical cells unable to form colonies
2) *Microccocus sp*	*Actinobacteria*	*Actinobacteria*	*Microccocus*	Gram positive cocci in pairs and tetrads

D=1-(£ n(n-1)/N(N-1)
n = the total number of organisms of a particular species
N = the total number of organisms of all species.
The value of D ranges between 0 and 1. With this index, 1 represents no diversity and 0, infinite diversity.

Abundance = $\frac{Total\ no\ of\ individual\ of\ the\ species}{No\ of quadrat\ per\ unit}\ X\ \frac{100}{1}$

Experimental Design and Statistical Analysis

The experiment was laid out in a Completely Randomized Design (CRD). The treatment was replicated three times. Data collection was analyzed using Simpson's diversity index.

RESULTS AND DISCUSSION

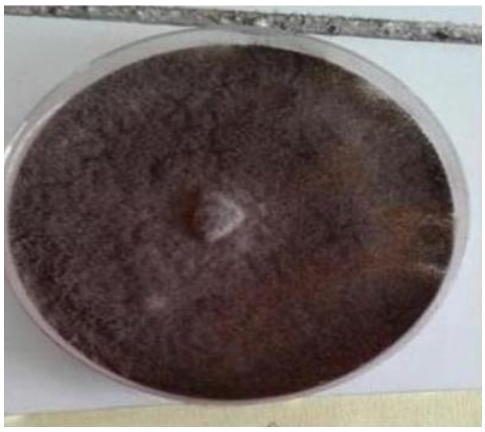
The results on the Isolation and Identification of Rhizosphere fungi and bacteria present in the *Moringa oleifera* and *Annona muricata* tree of the Forest Arboretum Rivers State University, Nkpolu-Oroworukwo, and Port Harcourt are presented in Tables 1a and 1b and Plates 1a,b,c. Results showed that the amount of fungi found in *rhizosphere of Moringa oleifera* and *Annona muricata* trees are *Rhodotorula sp; Aspergillus niger, Microsporum audouinil, Sporotrichum pruinosum* and *Aspergillus versicolor* though*, Microsporum audouinil* and *Aspergillus versicolor* were absent in *Annona muricata* tree stands at the Arboretum.

However, bacteria isolated from the Rhizosphere of both tree stands are *Bacilli sp* and *Micrococcus sp* though *Bacilli sp* was absent in *Annona muricata* tree sampled. Generally, five fungi and two bacteria were isolated, identified and classified into their different phyllum, class, genera and cell morphology/microscopic characteristics.

The results in the abundance of Fungi and Bacteria found in the Rhizosphere of *Moringa oleifera* and

Table 2. Abundance of Fungi and Bacteria found in both *Moringaoleifera* and *Annonamuricata* using Simpsons diversity index (SDI)

Fungi/bacteria class	Operational taxonomic (species)	Relative % of Fungi counts	
		Moringa	*Annona*
Microbotyomycetes	*Rhodotorula spp*	1	1
Euascomycetes	*Aspergillus niger*	1	1
Euascomycetes	*Microsporium audouinil*	1	1
Hymenomycetes	*Sporotrichum pruinosum*	1	1
Euascomycetes	*Aspergillus versicolor*	1	1
Bacteria class			
Bacilli	*Bacillus sp*	2	-
Actinobacteria	*Micrococcus sp*	1	2
Simpson's diversity index			
Bacteria diversity		0.67	0.00
Fungi diversity		1	1
Fungi		1	1
Bacteria		1.5	2



Aspergillus niger

Aspergillus vesicolor

Micrococcus spp

Plate 1. Organisms isolated from the soil rhizosphere

Annona muricata tree stands are shown in Table 2. The result indicated that there was no diversity of fungi isolated in both *Moringa* and *Annona* tree stands in the forest Arboretum. However, the Simpson's diversity index value of bacteria population found in the *Moringa* tree stand was (SDI =0.67) was more diverse when compared

to the bacteria isolated from *Annona muricata* (SDI=0.00).

Generally, there was a good number of microorganisms isolated and identified from the trees of *Moringa* and *Annona* found in the Arboretum of Rivers State University and hence *Aspergillus niger*, was a worldwide distributed member of *ascomycotina*, has been isolated from numerous habitats. *A. niger* is one of the fungi that has been labeled with the GRAS (generally recognized as safe) status from the US Food and Drug Administration. This dull or dark black looking fungus has several important products in fermentation industry. But due to cosmopolitan nature, human beings gets frequently exposed to spores and vegetative forms of *A.niger* present in air, on foodstuffs and others stored consumables products and suffers with allergic problems. *A.niger* may also produce certain mycotoxins which are *heptocarcinogenic*, *nephrogenic* immunological in nature. In addition, this fungus is also causative agent for many rot diseases in plants. So, the present review article is an important step to understand the diversity, pathogenicity and toxicology of this important spoilage organism.

Aspergillus niger (black mold), a filamentous *ascomycete* having ability of fast growth and pH tolerance is the most important cosmopolitan fungi associated with postharvest decay of different substrates. These results are in accordance with (Pitt and Hocking, 1997; Perfect *et al,* 2009; Perrone *et al,* 2007). This organism is a soil saprobe with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocelluloses. Because of their ability to produce extracellular organic acids some of them are commonly used in food industry. These features of *A. niger* enable them to cause decay of various organic substances including fruits, vegetables, nuts, beans, cereals, herbs, wood and herbal drugs. *A. niger* also plays a significant role in the global carbon cycle (Baker, 2006).

A. niger has been isolated from a variety of substrates but, these reports involve co-isolation with other perhaps more destructive microorganism or isolation from a stored product. The organism is considered as a strict saprophyte (Farr *et al,* 1989). There are reports of *A. niger* being as plant pathogen. This fungus can cause rotting of numerous fruits, vegetables and other food products, thus causing substantial economic loss. There are many examples of plant diseases caused by *A. niger*. Black rot of onions associated with *A. niger* is responsible for serious losses of onion bulbs in the field and storage (Narayana *et al,* 2007). Other plant pathogenic reports of *A. niger* are, spoilage of mangos (Prakash and Raoof, 1989), grapes (Sharma and Vir, 1986), Tomatos (Sinha and Saxena, 1987), stem rot of Dracaena (Abbasi and Aliabadi, 2008); root stalk rot of Sansevieria; and boll rot of Cotton; spoilage of cashew kernels, dates, figs, vanilla pods and dried prune (Bobbarala *et al,* 2009). *A. niger*can induce a crown rot of peanuts due to *A. niger*-infected seed under specific hot, humid growth conditions (Anderegg *et al,* 1976). Kharwar *et al.* (2008) isolated *A. niger* from *Catharanthesrosea* as an endophytic fungi which can alter its metabolite production.

Bacillus spp. are gram positive, ubiquitous in nature and recovered from all niches in the environment. These species have also been used to prepare medicinal, industrial and agricultural products (Lyngwi and Joshi, 2014). Bio-fertilizers can be used as alternatives to chemical fertilizers and pesticides and can provide new insights into enhancing plant growth and yield in the face of diseases (Choudhary, 2011). The plant-beneficial Bacillus spp. associate with roots or rhizospheres and develop biofilms to increase plant growth (Beauregard *et al,* 2013). The application of Bacillus-based fertilizers to soil can enhance the plant-available forms of nutrients in rhizospheres, control disease-causing pathogenic microbial growth and induce pest defense systems (Garcia-Fraile *et al,* 2015; Kang *et al,* 2015b). This review is focused on the growth-promoting potential of *Bacillus* spp. in crop plants and the involvement of these bacteria in reprogramming plant physiological changes to achieve abiotic and biotic stress tolerance. This present findings agreed with report of previous researchers.

Bacillus spp. convert the complex form of essential nutrients, such as Phosphorus and Nitrogen, to a simple available form that is used during uptake by plant roots (Kang *et al,* 2015a; Kuan *et al,* 2016). Phosphate is involved in nucleic acid, phospholipid, and adenosine *triphosphate* (ATP) metabolism, among other metabolic pathways, in plant cells (Theodorou and Plaxton, 1993). The secretion of phosphatases and organic acids from *Bacillus* spp. acidifies the surrounding environment to facilitate the conversion of inorganic phosphate into free phosphate (Kang *et al,* 2014a, 2015a). Additionally, Nitrogen is an important component of proteins, nucleic acids and other organic compounds in plants, and the available form of N in soil is limited, which slows plant growth in natural habitats (Barker *et al,* 1974; De-Willigen, 1986). Some of the *Bacillus* spp. release ammonia from nitrogenous organic matter (Hayat *et al,* 2010). Ding *et al,* (2005) reported that some of the *Bacillus* spp. have the gene and produce nitrogenase (EC 1.18.6.1), which can fix atmospheric N_2 and provide it to plants to enhance plant growth and yield by delaying senescence (Kuan *et al,* 2016).

The iron-chelating properties of *Bacillus* spp. via siderophore production help to solubilize iron from minerals and organic compounds in rhizospheres (Nadeem *et al.,* 2012). Siderophores bind Fe^{3+} in complex substances and reduce the Fe^{3+} to Fe^{2+}, which then enters plants (Walker and Connolly, 2008). Therefore, lubricant natures of the test trees are indications that the presence of *Bacillus* spp may have been responsible for it.

However, the bacteria species differed significantly from each subplots conversely the Fungi species there was not significant.

Therefore, based on the results available to boast soil

quality and microbial activities of forest health status and to promote productivity of Arboretum of Rivers State University, these bacterial and fungi isolated should be replicated.

CONCLUSIONS

It was concluded that soil bacteria and fungi play an important role in soil food web which supports other soil organisms for a healthy soil. Some population of soil bacteria and fungi can suppress root diseases of plants. The decomposers consume the easy-to-digest carbon compounds and simple sugars and tie up soluble nutrients like nitrogen in their cell membranes. Bacteria dominate in tilled soils but they are only 20-30 percent efficient at recycling carbon (C). Many bacteria produce a layer of polysaccharides or glycoproteins that coats the surface of soil particles. These substances play an important role in cementing sand, silt and clay soil particles into stable micro aggregates that improve soil structure. Bacteria live around the edges of soil mineral particles, especially clay and associated organic residues. Fungi perform important services related to water dynamics, nutrient cycling, and disease suppression. Along with bacteria, fungi are important as decomposers in the soil food web. They convert hard-to-digest organic material into forms that other organisms can use. Fungal hyphae physically bind soil particles together, creating stable aggregates that help increase water infiltration and soil water holding capacity for efficient plant plants.

RECOMMENDATIONS

1. *Bacillus* based fertilizers should be applied to the soil to enhance the plant available forms of nutrients in the rhizosphere and help to control disease causing pathogenic microbial growth and induce pest defense systems.
2. Fungi species should be applied to contaminated soils in order to reduce the harmful effects of toxic metals such as copper, lead, mercury, by accumulating them in their fruiting bodies.
3. More research is required to form the best way to maintain fungi/bacteria biodiversity in soil, taking into consideration fungal/bacteria functions, including disease control, contamination detection and bioremediation.

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