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Bacterial and Fungal Assessment of Top Soil Cultivated with Oil Palm Seedlings.

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Research Article

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ABSTRACT

The fungal and bacterial populations in soil waste dumping site (WDS) and oil palm ecosystem (OPE) cultivated with oil palm seedlings have been studied. The month of April had the highest occurrence with 20.1 x 10^5 cfu/g from WDS. This was followed by May with 12.8 x 10^5 cfu/g (WDS), July 10.1 x 10^5 cfu/g (OPE) and August showed the lowest occurrence with 7.1 x10^5 cfu/g (OPE). The mean bacterial counts for the month of July recorded the highest occurrence with 8.42 x 10^3 from OPE. This was followed by May with 5.98 x 10^3 cfu/g (WDS), April 4.45 x 10^3 cfu/g (WDS) and August showed the lowest with 1.60 x 10^3 (OPE). The biochemical tests revealed the occurrence of eleven isolates. The *Bacillus subtilis* was the most occurred while *Flavobacteria devorans* was the least occurred. The frequency of occurrence of fungi isolated revealed that *Penicillium expansium* had the highest occurrence with 11.7%. The least occurrence was *Trichoderma polysporum* with 1.1%. The high counts of fungi and bacteria obtained in soil from WDS were an indication that the soil was influenced by the degrading matters at the sites. The soil from waste dumping sites best supported high fungal and bacterial populations while soil from oil palm ecosystem less supported the populations.

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) industry provides direct employment to about four million Nigerian people in about twenty oil palm growing states in Nigeria, and indirectly to other numerous people involved in processing and marketing [1]. It is a perennial crop that originated from the tropical rain forest of West Africa [2]. It is endemic to the humid tropical rain forest regions of Eastern Nigeria and South Eastern Cameroon. It can however be said to be a native of Africa covering a total area of about 2 million hectares in Nigeria and endemic to the south eastern states of Nigeria and later spread to South America in the 16th century and to Asia in the 19th century [3]. Despite the enormous potential of the oil palm, there is need to access the fungal and bacterial populations of top soil cultivated with oil palm seedlings.

Plant roots are favourable media for the growth of microorganisms, numerous and different populations of which being found on, as well as around them. The interactions between microorganisms from the soil and the roots of the plant fulfill important nutritive needs both for the plant and for the associated microorganisms. This is demonstrated by the large number of microorganisms found in the rhizoplane and in the rhizosphere [4].

Soil microorganisms are vital for the continuing cycling of nutrient and for driving above ground ecosystems. It is important to study microbial diversity not only for basic scientific research, but also to understand the link between diversity and community structure and function. Soil bacteria and fungi play pivotal roles in various biogeochemical cycles (BGC) Wall and Virginia [5]. Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition [6], plant health [7], soil structure [8] and soil fertility [9].

The degradation of organic matter in soil largely results from the interaction between macro- and microorganisms, although microorganisms have a greater participation in this process because of their higher biomass values [10]. *Bacillus* species are widely distributed and well known plant growth promoting bacteria, commonly present in the rhizosphere. They increase plant growth by vanishing plant pathogens by their unique antimicrobial activities, including production of antibiotics [11] and toxins [12] to compete with pathogenic organisms.

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Some strains are also known to induce systemic resistance in plants against variety of pathogens. Application of *Bacillus* species are ubiquitous in nature found around the diverse habitats and have ability to grow and multiply on different types of organic substrates including plant and animal materials. *Bacillus* species can multiply on a variety of substrates, including cheap agricultural byproducts [13]. Solid state fermentation is helpful for production of bacterial metabolites at low cost on large scale [14].

Gram staining (GS) is the principal staining technique used for microscopic examination of bacteria. Unknown bacteria can be classi-fied into Gram-positive or Gram- negative. Some gram positive bacteria decolorize more rapidly, and incorrectly identified as gram-negative. Adding to this, factors like composition of growth medium, age of culture1 and antibiotic treatments may allow crystal violet to wash out, and the sample may appear gram-variable, with some cells staining pink and others staining purple [15].

MATERIALS AND METHODS

Collection of samples

Samples were collected from different top soil locations within Benin city. These Collected samples were brought to the laboratory in plastic bags and kept at about 4^{o}C in the refrigerator till used for bacterial isolation.

Isolation of spore-forming bacteria

Travers *et al*., [16] method was used to isolate spore forming bacteria from the collected samples. The colonies from these plates were sub-cultured on the nutrient agar medium for purification and then kept in slants and saved in the refrigerator for further study

Microbial counts

A serial decimal dilution was performed by adding 10 g soil, wet weight, to 95 ml of a 0.1% (w/v) sodium pyrophosphate solution. Aliquots of these suspensions were later transferred to Petri dishes containing specific media for counts of microbial groups. Total bacteria were counted using the medium of Nutrient agar.

Determination of colony forming units

The colonies were counted after seven days of incubation at 28^{0}C. The best dilution is the one that allows the development of a number of 50-300 colonies [17]. The formular for calculating the number of colony forming units (cfu) in a gram sample [18] is:

$$a \times 10n / v;$$

Where: a-number of colonies, 10n- dilution in which calculation was carried out, v-inoculum volume.

Fungal percentage frequencies were recorded. During the above period a total of 293 pathogenic aerobic bacteria were isolated from various clinical samples. All the strains were subjected to gram staining, KOH string test and biochemical tests.

Gram staining

Smears were flooded with crystal violet for one minute and then washed gently in tap water. In the second step, smears were exposed to Gram's iodine for one minute, and then washed with tap water. In the third step, slides were exposed to acetone for de-colorization and washed immediately with tap water. Finally, dilute Carbol Fuchsin was added as the counter stain and washed after 60 seconds.

Microscopic examination

A smear of 24-48 hour-old culture of each isolate was prepared on a clean microscopic slide and stained with gram stain. The smear was then examined microscopically under magnification (X100) to show the shape of the cell, the presence of the spores, their position and the dimensions of the cell in addition to the gram reaction.

KOH string test

A loopful of growth from a bacterial colony was emulsified on the surface of a glass slide in a suspension of 3% KOH. The suspension was stirred continuously for 60 seconds after which the loop was gently pulled from the suspension. The test was considered positive if string occurred within the first 30 seconds after mixing the bacteria in KOH solution [19].

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Biochemical tests

Different tests were carried out to confirm the identity of the isolate gram negative or positive. These tests were catalase, oxidase coagulase, citrate utilization, Urease and indole production. Also was sugar fermentation test carried out according to Cheesebrough [20].

Statistical analysis

The data are presented as the means of four samples. Results were evaluated with students two-tailed test. The level of significance was set at 5%

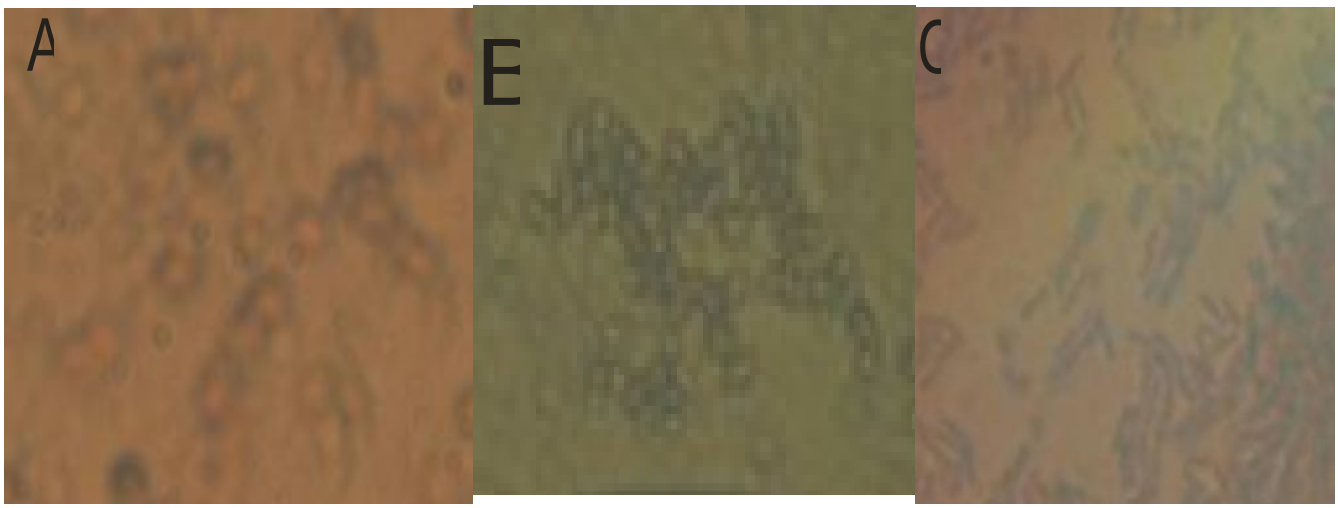
RESULTS AND DISCUSSION

The present study showed that mean fungal counts for the month of April had the highest occurrence with 20.1 x 10^5 cfu/g (WDS). This was followed by May with 12.8 x 10^5 cfu/g (WDS), July 10.1 x 10^5 cfu/g (OPE) and August showed the lowest with 7.1 x10^5 cfu/g. from OPE. The mean bacterial counts for the month of July recorded the highest occurrence with 8.42 x 10^3.(OPE) This was followed by May with 5.98 x 10^3 cfu/g (WDS). April 4.45 x 10^3 cfu/g (WDS) and August showed the lowest with 1.60 x 10^3 from OPE (table 1).

Table 1: Total viable fungal and bacterial counts from top soil cultivated with oil palm seedlings

Sample No	Point of collection	Mean Fungal counts (cfu/g)	Mean Bacterial counts (cfu/g)
March top soil	Oil palm field 1	8.0 x 10^5	1.87 x 10^3
April top soil	Oil Palm Ecosystem (OPE) Section 29	20.1 x 10^5	4.45 x 10^3
May top	Waste dumping site (WDS) Section 19	12.8 x 10^5	5.98 x 10^3
June	Waste dumping site (WDS) Oil palm field 2	10.0 x 10^5	4.20 x 10^3
July	Oil Palm Ecosystem (OPE) Excavated soil	10.1 x 10^5	8.42 x 10^3
August	Oil Palm Ecosystem (OPE) Excavated soil 1 Oil Palm Ecosystem (OPE)	7.1 x 10^5	1.60 x 10^3

Figure 1: Light microscope photomicrographs of sporulated spores



A) *Bacillus subtilis* with creamy color and irregular shape, B) *Pseudomonas putida,* with short rod and circular shape C) *Flavobacterium devorans* (x100 magnification)

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All the eleven bacterial isolates were selected for characterization including cultural and morphology (table 2). Circular and irregular shape, cocci and rod were observed in all isolates after staining and examining the cells by light microscopy (figure 1). The different biochemical tests of catalase, indole, and sugar fermentation were used to isolate and characterized the eleven bacteria solates (table 2). The occurrence of the eleven isolates from March till August are shown in table 3. The *Bacillus subtilis* was the most occurred while *Flavobacteria devorans* was the least occurred.

Table 2. Characteristics of bacteria isolates from top soil at section 29 WDS cultivated with oil palm seedlings

Isolates	*Steptoco-ccus* sp.	*Escheri-chia coli*	*Acinetob-acter calcoa-Ceticus*	*Bacillus subtilis*	*Klebsiella pneumo-Niae*	*Miccro-coccus varians*	*Enterob-acter* sp.	*Alcalig-enes* sp.	*Pseud-omonas putita*	*Flavoba-cterium devorans*	*Coryne b-acteriu m* sp.
Cultural											
Elevation	Convex	Low convex	Low convex	Flat	Convex	Convex	Low convex	Convex	Low convex	Low convex	Convex
Margin	Smooth	Entire	Entire	Entire	Smooth	Entire	Entire	Entire	Entire	Entire	Entire
Colour	Cream	Ceam	Cream	Cream	Cream	Yellow	Cream	Cream	Cream	Yellow	Cream
Shape	Circular	Circular	Circular	Irregu-lar	Circular	Circul-ar	Circular	Circular	Circul-ar	Circular	Circula r
Morph-ological											
Gram staining	+	-	-	+	-	+	-	-	-	-	+
Cell type	Cocci	Rod	Rod	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Rod
Cell arrangem-ent	Chains	Single	Single	Chains	Single	Single	Single	Single	Single	Single	Single
Biochem-Cal											
Catalase	-	+	+	+	+	+	+	+	+	+	+
Oxidase	+	-	-	-	-	-	-	+	+	-	-
Coagulase	-	-	-	-	-	-	-	-	-	-	-
Urease	+	-	+	+	+	+	-	+	-	+	+
Indole	-	+	-	-	-	-	-	-	-	-	-
Citrate	+	-	+	+	+	+	+	+	+	+	+
Glucose	+	+	-	+	+	+	+	+	+	+	+
Lactose	-	+	-	-	+	-	+	-	-	-	-

Table 3: Total occurrence of bacterial isolates from top soil cultivated with oil palm seedlings

Genera – species	Gram stain	March	April	May	June	July	August
Steptococcus sp.	+	+	+	-	+	-	+
Escherichia coli	-	-	-	-	-	+	-
Acinetobacter calcoaceticus	-	-	+	-	-	+	-
Bacillus subtilis	+	+	+	+	+	+	+
Klebsiella pneumoniae	-	-	+	+	+	-	-
Miccrococcus varians	+	-	+	-	+	+	+
Enterobacter sp.	-	-	-	+	-	-	-
Alcaligenes sp.	-	-	-	-	-	-	-
Pseudomonas putita	-	+	-	-	-	-	-
Flavobacterium devorans	-	-	-	-	-	-	+
Corynebacterium sp.	+	-	-	-	+	-	-

The frequency of occurrence of fungi isolated revealed that *Penicillium expansium* had the highest occurrence with 11.7%. These were followed by *Aspergillus flavus* 7.9%, *Xyleria* sp. 7.9% and *Aspergillus niger* 7.0%. The least occurrence was *Trichoderma polysporum* with 1.1% (table 4).

The high counts of fungi obtained in soil from section 29 WDS in the month of April was an indication that it was influenced by the degrading matters at the site. For bacterial counts, the month of July had the highest counts from excavated soil in oil palm field of OPE. It was also influenced by pruned and decayed palm fronts, bunches and logs. All these are organic matters that decayed and created avenues for mass breeding of fungi and bacterial. This agrees with Holt et al. [21] and Ogunwonyi et al. [22], they reported that when soil is polluted by waste it causes a pressure on the sensitive of microorganisms and so changes the diversity of soil microflora.

The lowest in fungal and bacterial counts from excavated soil from oil palm field 1 of OPE in the month of August may be due to insufficient availability of decaying matters / nutrients. The frequency of fungal species recorded may not be surprising as these pathogens are indigenous to soil environment. This was in agreement with a similar result by Atlas and Bartha [23], who reported that such pathogens are known to persist in such environment.

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Table 4: Mean total colony frequency of fungal species in top soil from section 29 WDS cultivated with oil palm seedlings

Fungal species	March	April	May	June	July	August	% frequency
Aspergillus niger	1	3	1	2	4	1	7.0
Aspergillus flavus	2	4	2	4	3	2	7.9
Aspergillus fumigatus	-	2	-	1	2	-	3.0
Aspergillus sp.	1	3	1	2	2	1	5.8
Aspergillus sp. 1	-	2	-	1	2	1	3.5
Aspergillus sp. 2	1	-	2	1	1	2	4.1
Ceratocystis paradoxa	-	3	-	1	2	2	4.7
Curvularia sp.	2	1	2	3	-	2	5.8
Fusarium oxysporium	1	4	2	-	2	1	5.8
Fusarium oxysporium sp	-	2	-	-	1	-	1.8
Fusarium oxysporium sp. 1	1	-	-	1	2	1	3.0
Fusarium oxysporium sp. 2	-	2	-	1	2	-	3.0
Penicillium expansium	3	5	3	4	3	2	11.7
Penicillium sp.	-	2	-	1	2	1	3.5
Penicillium sp. 1	-	-	1	1	-	2	2.3
Rhizopus oryzae	2	-	2	-	1	2	4.7
Trichoderma hamatum	1	3	1	3	2	1	6.4
Trichoderma polysporum	-	1	-	-	-	-	1.1
Trichoderma sp.	2	2	3	1	1	2	6.4
Xyleria sp.	3	3	2	1	2	2	7.6

CONCLUSION

The soil from waste dumping sites best supported high fungal and bacterial populations while soil from oil palm ecosystem less supported the populations. The abundance of these soil borne microbes may be strongly influenced by some abiotic and biotic factors.

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