

Research Article

Fungal Diversity in Selected Cashew Plantation Soils in South East Nigeria

C.A. Alum¹, F.O. Tasie², L.N. Agbo³, V.I. Onoh⁴

^{1, 2, 4} Department of Applied Microbiology and Brewing, ESUT, P M B 01660 Enugu, Nigeria

³ Technology Incubation Centre Enugu, National Board for Technology Incubation, Abuja, Nigeria

ABSTRACT: The soils collected from three locations in three cashew plantations in South East Nigeria (Okigwe, Akamaoghe and Ugwu-onyeama) were studied using standard microbiological techniques. The soil collection was carried out in the rainy season and nine soil samples were taken from 0-15 cm soil depth. Each soil sample was analyzed for soil physical and chemical properties and studied for soil fungi using the soil dilution plate method. The emerging fungal colonies were enumerated and identified to the species level based on morphologic and microscopic characteristics. Twelve (12) species of both sporulating yeast and mesophilous fungi were isolated from soil samples collected from the different locations of cashew plantations studied. Of these, 9 species (*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium solani*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Penicillium*, *Trichoderma* and *Rhodotorula* spp.) belong to Class Deuteromycetes, 2 species (*Mucor indicus* and *Rhizomucor pusillus*) belong to Zygomycetes and 1 specie (*Microsporum canis*) belong to Eurotiomycetes. The plantation site which had the highest Shannon-Wiener's diversity index of soil fungi was Akamaoghe2 and Ugwuonyeama2. The highest similarity of fungal community composition between two locations calculated by Sorensen's index of similarity occurred between Okigwe2 and Ugwuonyeama1. The analysis of variance of mean values of soil properties among revealed that the soils were not significantly different from each other except in sodium and phosphate. The relationships of soil fungi with all the soil factors analyzed by Kruskal-Wallis test showed that soil fungi related positively with the Sodium, Phosphate, Calcium and Magnesium contents in soils. It is recommended that the Cashew plantations should be properly managed, so that there are no abrupt changes in their soil environmental conditions, in order to keep the existing diversity of soil fungi.

Keywords: Cashew Plantations, Fungal Diversity, identification, isolation, Soil fungi.

1. INTRODUCTION

Defining the number of fungi on earth has always been a point of discussion and several studies have focused on enumerating the world's fungal diversity [1]. From the late 1940s there has been a growing interest in soil mycology and soil borne fungal diseases of plants and this too has motivated the studies on soil fungi and their ecology. There are over 1.5 million fungal species distributed widely throughout the globe [2], and about 75,000 species of soil fungi in the world [3]. Many studies of soil fungi in the past emphasized species diversity in soil samples collected from various agricultural areas and forest types [4][5]. However, relatively few studies have tried to compare quantitatively the fungal diversity among different habitats. There have been also very few studies reported on the relationships of soil fungal diversity with environmental factors. Cashew plantation has assumed increasing importance and acceptance in tropical countries like Nigeria where they are grown in large commercial scale. Cashew (*Anacardium occidentale* L.) is a tree of forestry as well as horticultural significance grown in different parts of the world [6]. The plantations established vary in structure and composition and these variations may alter soil microbial communities. As a result of the very limited knowledge of the abundance and diversity of soil fungi in cashew plantation soils, this study is aimed at isolating and identifying fungi in selected cashew plantation soils in south east Nigeria.

1.1 Fungi in Soil

The kingdom Fungi is one of the most diverse groups of organisms on Earth, and they are integral ecosystem agents that govern soil carbon cycling, plant nutrition, and pathology. Fungi are widely distributed in all terrestrial ecosystems [7]. They are abundantly found in a host of environments, and live an independent, dependent or mutually beneficial existence. Fungi are vital in maintaining the proper functioning of the ecosystem by decaying dead matter and releasing vital nutrients in the soil and atmosphere. Common fungi found in soil are *Aspergillus*, *Cladosporium*, *Penicillium*, *Alternaria*, *Trichoderma*, and *Mucor*.

II. MATERIALS AND METHODS

2.1 Materials

The major raw material used in this work is soil which was obtained from selected cashew plantation soils in Okigwe, Akamaoghe and Ugwuonyeama and instruments for analysis were obtained from Department of Applied Microbiology and Brewing, ESUT, Enugu.

2.1.1 Media

Sabouraud dextrose agar (Sda)

2.1.2 Reagents

Lactophenol cotton blue stain, Normal saline, Acetone, chloramphenicol

2.2 Methodology

Soil dilution plate method was used to obtain fungal colonies. Soil pH, temperature and moisture content were determined using a soil pH meter and soil thermometer. Microwave digestion procedure was used in analyzing chemical properties in soil using Absorption Spectrophotometer 20 machine.

2.2.2 Sterilization of Glass Wares

All glass wares were thoroughly washed with detergent and rinsed with clean water, allowed to air dry, wrapped in aluminium foil paper and were sterilized in hot air oven, which was preheated at 160°C. The glass wares were sterilized for 1 hour. Then, the oven was switched off and allowed to cool. The glass wares were brought out and stored under sterile aseptic condition (Air tight container for subsequent use).

2.2.3 Collection of Samples

Samples were collected randomly from three different locations each in three different cashew plantations of Ajalli cashew plantation in AkamaOghe (6° 44 N and 7° 32 E), Ugwuonyeama (6° 43 N and 7° 41 E) and Okigwe (5° 82 N and 7° 35 E). The soil type is predominantly aerosols and the vegetation of the area are dominated by forest species, located within the humid tropical rainforest zone Nigeria. Soil auger was used to collect the soil sample at 0-15cm depth and put in sterile Ziploc bags that were well labelled. The pH, soil type, color, temperature and texture were observed and thereafter taken to the laboratory for isolation to get pure cultures and identification of isolates using both morphologic and microscopic examination.

2.2.4 Determination of Soil Characteristics

Soil pH was determined by inserting the pH meter into the soil up to a depth of 15cm and allowed to stay for some minutes after which the pH reading was obtained and recorded. Soil temperature was determined by inserting the soil thermometer into the soil to a depth of 15cm and allowed to stay for some minutes after which the temperature reading was obtained and recorded.

2.2.5 Determination of Soil Moisture Content

Total 30g of soil from each sample was weighed and put in a container. The soil samples were dried in hot air oven at 110°C for 24hrs. The weight was measured every 2hrs until a constant weight was gotten. The dry soil was weighed and recorded. The percentage moisture content of soil sample was determined by the equation:

$$W\% = (\text{weight of dry soil} / \text{weight of wet soil}) \times 100$$

2.2.6 Chemical Analysis of Soil Sample

Each of the soil samples were divided into two and one half was taken to PRODA Enugu for chemical analysis. The soil was firstly digested using the closed microwave digestion procedure by weighing 0.2g of each of the samples into a beaker and adding 4ml of Aqua regia (1part nitric acid and 3parts hydrochloric acid), it was then put in a microwave for 20mins at 100-120°C after which it was allowed to cool down and transferred quantitatively to a volumetric flask and filled to 50ml with doubled deionized water. The samples were then taken to the Atomic Absorption Spectrophotometer 20 machine for the quantitative determination of the chemical elements using the absorption of optical radiation (light) by free atom in the gaseous state.

2.2.7 Preparation of Sabouraud Dextrose Agar

Total of 16.25g of SDA was measured with a weighing balance and was dissolved with 250ml of distilled water in a 500ml conical flask. It was mixed thoroughly to form a solution, the mixture was boiled over a Bunsen burner to dissolve the agar completely. The conical flask was plugged with cotton wool covered with aluminum foil paper and the mixture was autoclaved at 121°C for 15minutes to sterilize the medium. Thereafter allowed to cool to 50°C. Then 0.25mls of sterile chloramphenicol was added to the media and mixed properly then was poured into sterile petri dish 15-20ml each aseptically and then allowed to set and stored at 4°C until needed.

2.2.8 Sample Inoculation

The soil samples where firstly cultured raw to check if they will yield growth and they did, then one gram (1g) of individual samples of soil was weighed and suspended in 9ml of normal saline. The stock was sieved using a 1mm spore size sieve. From the above suspension, 1ml was then serially diluted to 10^{-1} - 10^{-9} . Then, 1ml of the diluted samples (10^{-5}) and (10^{-7}) was then inoculated using the pour plate and spread plate methods onto the sterile Sabouraud Dextrose Agar (SDA) and incubated at room temperature (about 28°C) for 14days with continuous observation for growth. At the end of the 14days, some mixed colonies of fungi in varied colors, shape, size and texture that merged were noted and recorded. These mixed colonies of fungi were separated by sub-culturing (i.e. into fresh culture media) for pure cultures.

2.2.9 Identification

Fungal growth observed were identified using pictorial atlas of soil and seed fungi and reported in colony forming units per gram of soil (cfu/g of soil). Macroscopically, physical appearance which include the color, size, texture and shape were noted. Microscopy was carried out after staining with Lactophenol cotton blue stain and identification was done.

2.2.10 Microscopic Examination

Two drops of Lactophenol cotton blue stain was placed on a clean grease free glass slide. A sterile needle was used to pick the isolated organisms from the growth medium. The cut portion was dropped on top of the drop of lactophenol cotton blue stain in the glass slide. Flamed sterile needle was used to tease out the hyphae to ease microscopic examination. The preparation was covered with a cover slip, then excess stain was removed by use of blotten paper as well as to remove air bubbles and also to help spread the organism evenly throughout the preparation. The preparations were left to air dry before they were examined under the microscope using X10 and X40 objectives [8].

2.3 Data Analysis

1. The number of colonies of a soil fungus/1g dry weight soil/plantation site was converted to the number of colony forming units (CFU)/g dry weight soil/ plantation site of a soil fungus. By summing up all the individual abundance records of a soil fungus in a plantation site, the total CFU/g dry weight soil for a plantation site was obtained.

2. The Shannon-Wiener's index (H') was chosen to measure the fungal species diversity in a plantation using the equation:
 $H' = \sum p_i \ln p_i$

Where: \sum is summation, p_i is the number of individual species/total number of samples.

3. The Sorensen's coefficient (CC) was used to compare fungal community composition between two plantation sites, using the Equation:

$$CC = [2C / (S1+S2)]$$

Where: C is the number of species the two plantation sites have in common, S1 is the total number of species found in plantation site 1, and S2 is the total number of species found in plantation site 2.

4. Soil environmental factors, measured as physical properties (pH and temperature) and soil chemical properties (Sodium, phosphorus, potassium, calcium and magnesium) in the plantation sites where analyzed statistically to identify any differences among the means by analysis of variance (ANOVA).

III. RESULTS

A total number of 12 fungal species were isolated from the soil samples. The number of fungal species identified from the soils in Okigwe1 (O1), Okigwe 2 (O2), Okigwe 3 (O3), Akamaoghe 1 (A1), Akamaoghe 2 (A2), Akamaoghe 3 (A3), Ugwuonyeama 1 (U1), Ugwuonyeama 2 (U2) and Ugwuonyeama 3 (U3) were 4, 3, 3, 8, 6, 4, 5, 4 and 2 respectively. The results are recorded and presented in nine (9) separate tables. Table 1 enumerates the soil characteristics. The cashew plantation soils were acidic with U2 having the highest pH and A1 having the highest temperature and moisture content. All of the soil sample colors are brown with the exception of U2 and U3 which is red. Table 2 shows the chemical components of soil samples. The chemical analyzed where NA, CA, MG, K and P. O1, A1, U2 and A2 had the highest composition in NA, CA, MG and P respectively while there was no trace of K. TABLE 3 and 4, highlights the result of the study expressed in colony forming units per gram soil sample (cfu/g). Total colony count for dilutions 10^{-5} and 10^{-7} was 2.16×10^7 cfu/g and 2×10^9 cfu/g respectively and the average count was 2.4×10^6 cfu/g and 2.22×10^8 cfu/g. Table 5, shows the physical and microscopical characteristics of the isolates. Table 6, highlights the specific isolates recovered from the plantation sites with A1 having the highest number of isolates. Table 7, shows the frequency or occurrence of each fungal specie isolated among the nine sites expressed in percentage. From Table 8, A2 and U2 had the highest diversity and evenness of 0.69 and 1.0 respectively based on Shannon Wiener's Diversity Index and Evenness of fungi species in the cashew plantations. The similarities in fungal plantation composition between two cashew plantation sites calculated by Sorenson's Coefficient of Similarity (CC) are shown in Table 9. The results revealed that the greatest similarity in soil fungi occurred between O2 and U3 (80%), followed by O2 and U1 (75%) and A3 and U2 (75%). The similarity of soil fungi between these three (3) pairs of plantations were considered high (>50%). The pairs between which the similarity of soil was considered low were A2 and U1 (18%), O2 and A1 (20%) and A1 and U3 (20%).

Table 1: Soil Characteristics

SAMPLE SITE	pH	TEMPERATURE (°C)	MOISTURECONTENT (%)	SOIL COLOR
O1	4.5	28	47.60	Brown
O2	6.0	28	48.13	Brown
O3	6.0	30	50.83	Brown
A1	5.0	31	54.00	Brown
A2	5.5	27	51.90	Brown
A3	6.0	29	51.73	Brown
U1	5.5	27	53.50	Brown
U2	6.5	25	51.00	Red
U3	5.5	28	49.10	Red

Key:

- I. O1-Okigwe1
- II. O2-Okigwe2
- III. O3-Okigwe3
- IV. A1-Akamaoghe1
- V. A2-Akamaoghe2
- VI. A3-Akamaoghe3
- VII. U1-Ugwonyeama1

- VIII. U2-Ugwuonyeama2
IX. U3-Ugwuonyeama3

Table 2: Chemical Components of Soil
VALUES (ppm)

PARAMETER	NA	CA	MG	K	P (%)
O1	61.30	26.00	1.11	NIL	0.23
O2	60.00	26.00	1.20	NIL	0.18
O3	58.00	26.30	1.18	NIL	0.31
A1	6.30	44.00	1.58	NIL	0.52
A2	34.00	1.76	1.21	NIL	0.69
A3	40.00	18.21	1.23	NIL	0.58
U1	59.00	22	1.25	NIL	0.56
U2	51.80	6.53	1.76	NIL	0.49
U3	56.40	20.00	1.62	NIL	0.52

Key:

- I. Na-Sodium
II. Ca-Calcium
III. Mg-Magnesium
IV. K-Potassium
V. P-Phosphate

Table 3: Average Fungal Concentrations by Specific Sample Sites (10^{-5})

SAMPLE SITES	AVERAGE NO. OF COLONIES FOR S AND P	DILUTION FACTOR 10^{-5}	CFU/g OF SOIL
O1	25	10^{-5}	2.5×10^6
O2	30	10^{-5}	3×10^6
O3	6	10^{-5}	6×10^5
A1	24	10^{-5}	2.4×10^6
A2	25	10^{-5}	2.5×10^6
A3	22	10^{-5}	2.2×10^6
U1	27	10^{-5}	2.7×10^6
U2	29	10^{-5}	2.9×10^6
U3	28	10^{-5}	2.8×10^6

Total colony count for dilution factor $10^{-5} = 2.16 \times 10^7$ cfu/g

Average count for dilution factor $10^{-5} = 2.4 \times 10^6$ cfu/g.

Key:

- I. S-spread plate
II. P-pour plate
III. O1-Okigwe1
IV. O2-Okigwe
V. O3-Okigwe
VI. A1-Akamaoghe1
VII. A2-Akamaoghe2

- VIII. A3-Akamaoghe3
IX. U1-Ugwonyeama1
X. U2-Ugwonyeama2
XI. U3-Ugwonyeama3.

Table 4: Average Fungal Concentrations by Specific Sample Sites (10^{-7})

SAMPLE SITES	AVERAGE NO. OF COLONIES FOR S AND P	DILUTION FACTOR 10^{-7}	CFU/g OF SOIL
O1	7	10^{-7}	7×10^7
O2	15	10^{-7}	1.5×10^8
O3	10	10^{-7}	1×10^7
A1	36	10^{-7}	3.6×10^8
A2	30	10^{-7}	3×10^8
A3	34	10^{-7}	3.4×10^8
U1	20	10^{-7}	2×10^8
U2	30	10^{-7}	3×10^8
U3	18	10^{-7}	1.8×10^8

Total colony count for dilution factor $10^{-7} = 2 \times 10^9$ cfu/g

Average count for dilution factor $10^{-7} = 2.22 \times 10^8$ cfu/g.

Key:

- I. S-spread plate
II. P-pour plate
III. O1-Okigwe1
IV. O2-Okigwe
V. O3-Okigwe
VI. A1-Akamaoghe1
VII. A2-Akamaoghe2
VIII. A3-Akamaoghe3
IX. U1-Ugwonyeama1
X. U2-Ugwonyeama2
XI. U3-Ugwonyeama3

Table 5: Physical and Microscopical Characteristics of Isolates

ISOLATES	MORPHOLOGY	MICROSCOPY
<i>Aspergillusniger</i>	Front colour is black and reverse is white to pale colour.	Hyaline hyphae. Long round radiate head with biseratephialide
<i>Aspergillusflavus</i>	Surface is greenish yellow to olive and may have a white border. Reverse is tan to yellowish	Septate hyphae with long conidiophores. Round radiate head with uniseratephialide
<i>Aspergillusfumigatus</i>	Surface is blue- green to grey-green with narrow white borders. Reverse is white to tan to pale yellow	Round columnar head with Uniseratephialide
<i>Fusariumoxysporium</i>	Reverse is pale to yellow	Septate hyphae are hyaline with short conidiophores

<i>Fusariumsolani</i>	Colour varies from white to yellow	Has monophialides
<i>Rhizomucorpusillus</i>	Colour is white and like cotton candy. Reverse is white to pale.	Has scarcely or non-septate broad hyphae. Has rhizoids and branched sporangiophore.
<i>Mucorindicus</i>	White wooly growth like cotton candy. Reverse is pale white.	Has broad hyphae which is scarcely or non septate. Phialides are flask shaped.
<i>Microsporiumcanis</i>	Wolly, fluffy and silky. Has light yellowish pigment at the edge. Reverse is pale tan to yellow.	Has septate hyphae. The macroconidia are spindle or fusoid in shape.
<i>Penicilliumchrysogenum</i>	Surface is velvety to powdery. Colour is grey-green with white edges. Reverse is tan to yellow.	Septate hyaline hyphae. Conidiospores are simple or branched.
<i>Penicilliumsp.</i>	Whitish colour and changes to grey-green with time	Produces septate, hyaline hyphae. Branching or simple conidiophores supporting phialides in brush-like clusters.
<i>Rhodotorulasp.</i>	Pink to coral or orange to red	Blastogenousconidium formation
<i>Trichodermasp.</i>	White with black or grey	Conidia are ovoid, hyaline, one celled and borne in small terminal clusters

Table 6: Isolates Recovered from Locations

LOCATION	TOTAL NO. OF ISOLATES	ORGANISMS
O1	4	<i>Aspergillusniger</i> , <i>Aspergillusfumigatus</i> , <i>Rhizomucorpusillus</i> , <i>Microsporiumcanis</i>
O2	3	<i>Aspergillusflavus</i> , <i>Aspergillusniger</i> , <i>Rhizomucorpusillus</i>
O3	3	<i>Mucorindicus</i> , <i>Aspergillusfumigatus</i> , <i>Rhizomucorpusillus</i>
A1	8	<i>Fusariumoxysporum</i> , <i>Fusariumsolani</i> , <i>Aspergillusfumigatus</i> , <i>Penicilliumchrysogenum</i> , <i>Mucorindicus</i> , <i>Penicilliumsp.</i> , <i>Aspergillusniger</i> , <i>Rhodotorulasp.</i>
A2	6	<i>Penicilliumchrysogenum</i> , <i>Trichodermasp.</i> , <i>Aspergillusfumigatus</i> , <i>Microsporiumcanis</i> , <i>Rhizomucorpusillus</i> , <i>Mucorindicus</i>
A3	4	<i>Penicilliumsp.</i> , <i>Rhodotorulasp.</i> , <i>Microsporiumcanis</i> , <i>Rhizomucorpusillus</i>
U1	5	<i>Aspergillusniger</i> , <i>Aspergillusflavus</i> , <i>Fusariumoxysporum</i> , <i>Rhizomucorpusillus</i> , <i>Rhodotorulasp.</i>
U2	4	<i>Rhizomucorpusillus</i> , <i>Microsporiumcanis</i> , <i>Mucorindicus</i> , <i>Penicilliumsp.</i>
U3	2	<i>Aspergillusniger</i> , <i>Rhizomucorpusillus</i>

Key:

- I. O1-Okigwe1
- II. O2-Okigwe
- III. O3-Okigwe
- IV. A1-Akamaoghe1
- V. A2-Akamaoghe2
- VI. A3-Akamaoghe3
- VII. U1-Ugwonyeama1

- VIII. U2-Ugwuonyeama2
IX. U3-Ugwuonyeama3

Table 7: Frequency Distribution of Fungal Isolates

Fungi specie isolated	Total no. of sample sites	No. of positive sites	PERCENTAGE FREQUENCY
<i>Aspergillusniger</i>	9	5	55.5
<i>Aspergillusflavus</i>	9	2	22.2
<i>Aspergillusfumigatus</i>	9	4	44.4
<i>Fusariumoxysporium</i>	9	2	22.2
<i>Fusariumsolani</i>	9	1	11.1
<i>Rhizomucorpusillus</i>	9	8	88.8
<i>Mucorindicus</i>	9	4	44.4
<i>Microsporiumcanis</i>	9	4	44.4
<i>Penicilliumchrysogenum</i>	9	2	22.2
<i>Penicilliumsp.</i>	9	3	33.3
<i>Rhodotorolasp.</i>	9	3	33.3
<i>Trichodermasp.</i>	9	1	11.1

Key:

- I. Sp-specie

Table 8: Shannon Wiener's Diversity Index of Soil Fungus in Cashew Plantation Sites.

Plantation sites	Dilution Factor 10^{-5}	Dilution Factor 10^{-7}	Shannon Wiener's Diversity Index
O1	25	7	0.53
O2	30	15	0.64
O3	6	10	0.66
A1	24	36	0.68
A2	25	30	0.69
A3	22	34	0.67
U1	27	20	0.68
U2	29	30	0.69
U3	28	18	0.67

Key:

- I. O1-Okigwe1
II. O2-Okigwe
III. O3-Okigwe
IV. A1-Akamaoghe1
V. A2-Akamaoghe2
VI. A3-Akamaoghe3
VII. U1-Ugwuonyeama1
VIII. U2-Ugwuonyeama2
IX. U3-Ugwuonyeama3

Table 9: Sorenson's Similarity Coefficient (CC) of Isolates between Different Locations (%)

	O1	O2	O3	A1	A2	A3	U1	U2	U3
O1	*	57	57	33	50	50	44	50	67
O2	*	*	30	20	33	29	75	43	80
O3	*	*	*	36	67	29	25	57	40
A1	*	*	*	*	43	33	46	33	20
A2	*	*	*	*	*	40	18	60	25
A3	*	*	*	*	*	*	44	75	33
U1	*	*	*	*	*	*	*	22	57
U2	*	*	*	*	*	*	*	*	33
U3	*	*	*	*	*	*	*	*	*

Key:

- I. * Not applicable
- II. O1-Okigwe1
- III. O2-Okigwe2
- IV. O3-Okigwe3
- V. A1-Akamaoghe1
- VI. A2-Akamaoghe2
- VII. A3-Akamaoghe3
- VIII. U1-Ugwonyeama1
- IX. U2-Ugwonyeama2
- X. U3-Ugwonyeama3

3.1 Statistical Analysis

The analysis of variance of the mean values of the soil properties among the three plantations shows that only the means of two soil chemical properties (sodium and phosphate) were significantly ($P < 0.05$) different. The means of soil chemical properties including, pH, temperature, moisture content, Calcium and Magnesium contents were not significant. Even though sodium and phosphate showed significant difference, organisms can still adapt. Organisms found in one is likely to be found in the other.

IV. DISCUSSION

All of the soil fungi in all nine locations, which belonged to the classes Deuteromycetes (*Aspergillusflavus*, *Aspergillusniger*, *Asergillusfumigatus*, *Fusariumsolani*, *Fusariumoxysporum*, *Penicilliumchrysogenum*, *Penicilliumsp.*, *Rhodotorula* and *Trichodermaspp.*), Zygomycetes (*Mucorindicus* and *Rhizomucopusillus*) and Eurotiomycetes (*Microsporumcanis*) are very common soil fungi that have been reported in various agricultural and forest soils in South East Nigeria. The soil dilution plate method used for the isolation of soil fungi in this study is a suitable method for isolating fungi in these three classes, as it supports releasing and mixing of numerous spores produced by the fungi into the diluted soil suspension. Most of the identified soil fungi have been reported to be organic matter decomposers, with the exception of *Fusariumoxysporum*, a phytopathogenic specie. *Penicillium* and *Trichodermaspp.* can be antagonists against other fungal species, especially phytopathogenic species [9]. The antagonistic fungi in this study may be isolates that have a high potential for use in the biological control of phytopathogenic species if screening test were carried out. The soils had high moisture content. An indicator of the high moisture content was the isolation of *Mucor* sp. at very high abundance levels from majority of the plantation sites. This fungi prefer high moisture content habitats [10]. Only two (2) of the species (*Aspergillusniger* and *Rhizomucopusillus*) were found at 50% or more of the locations so that their geographic distribution was somewhat higher

than the other species. This may indicate that these species are fit to survive and form spores under diverse conditions. Therefore they can be considered ecological generalists. Fungi are favored in soil that are slightly acidic (pH 5.0) and temperatures greater than 15-20°C. This suggests why the fungi isolates studied was within the above range which is in line with [11] which states that warmer climates favors the growth of these fungi. Na, P, Ca and Mg are trace elements necessary for fungal growth. They are required by fungi in very low concentrations, in the order of 1×10^{-3} M for P and Mg, and 1×10^{-4} M for Ca [12]. The fungi that had close positive relationships with P in the current study grew in the soils having P concentrations (0.18) much lower, and Ca (44) and Mg (1.76) concentrations much higher than reported by [12]. Land use changes have had direct and indirect impacts on the soil physical and chemical properties and more importantly, on soil microorganisms [10]. Most of the plantations where these fungi appeared (A1 and A2) have been completely undisturbed or not disturbed for a long time, so it is assumed that the soils were naturally balanced to support fungal growth. If there is no abrupt land use change, natural soil fungi will exist. When comparing A1 plantation site which had the highest number of fungal species with A2 plantation site which had the second highest number of soil fungal species, Table 3 shows there was not much variation in colonies of Akamaoghe, Okigwe and Ugwuonyeama. The colony count was used as a divisor in the Shannon wiener's index and the plantations had slightly different fungal diversity and evenness. The high similarity indices of soil fungi between O2 and U3, between O2 and U1, and between A3 and U2 should indicate the high similarity between the soil environmental factors in these plantation sites. The fungal community composition was more similar in soils having similar organic matter and pH. The analysis of soil environmental factors in the current study supported the high fungal similarity indices, perhaps due to their variation among the mean values of the soil environmental factors. Therefore, in future research of this kind, the number of soil samples per plantation should be increased.

VI. CONCLUSION

A total of 12 species of soil fungi were isolated from O1, O2, A1, A2, U1 and U2 cashew plantation soils. They belonged to the Class Zygomycetes, Deuteromycetes and Eurotiomycetes. Some soil fungi appeared in all plantation sites, but some appeared in only a few plantation sites or in as little as one. The plantation location that had the highest Shannon-Wiener's diversity index of soil fungi were A2 and U2. The highest similarity of fungal community composition between locations in plantations calculated by Sorensen's Coefficient of similarity occurred between O2 and U3, and O2 and U1, and A3 and U2, while the lowest similarity index of soil fungi was between A2 and U1. Among the plantation locations, the soil environmental and chemical properties revealed that the soils were similar to each other. The relationships of soil fungi with all the mentioned environmental and chemical factors showed that soil fungi related positively with the Na, P, Ca and Mg contents in the soil. Since the selected cashew plantation sites in South East Nigeria vary in the number and the species of soil fungi that contribute to the soil fungal diversity of the plantations, so, cashew plantations should all be managed using methods which will not allow any abrupt changes in the soil environmental conditions of the plantations in order to keep the existing diversity of soil fungi and since the isolates are capable of causing infections and deteriorating the cashews, it is recommended that the plantation workers are educated on how to protect themselves, and the consumers on how to wash the cashew with salt and water before consumption.

REFERENCES

- [1] Crous, P.W., Summerell, B.A., Mostert, I. and Groenewald, J.Z., How many species are there at the tip of Africa? *Studies in Mycology*, 55, 2006, 13-33.
- [2] Hawksworth, D.L., Fungal diversity and its implications for genetic resource collections. *Studies in Mycology*, 50, 2004, 19-22.
- [3] Finlay, R. D, The fungi in soil, in Van Elsas, J.D., Jansson, J.K. and Trevors J.T. (Eds.), *Modern Soil Microbiology*, (New York: Crc Press, 2007) 107-146.
- [4] Manoch, L., Microfungi from different forest types of HuayKhaKhang Wildlife Sanctuary: In the Proceedings of 37th Kasetsart University Annual Conference, Bangkok, 2000, 436-444.

- [5] Dethoup, T., Manoch, L., Visarathanonth, N., Chamswarn, C., Chawpongpan, S., Anunand, T. and Kijjoa, A., Diversity of Talaromyces from soils and their effects on plant pathogenic fungi *in vitro*, In the Proceedings of 45th Kasetsart University Annual Conference, Bangkok, 2007, 563-570.
- [6] FAO (Food and Agriculture Organization), Cashew Production in Africa, 2000s, 1961-2000. Production database.
<http://apps.fao.org/page/collections>.
- [7] Wardle D. A. and Björn, L. D., in disentangling global soil fungal diversity. *Science Magazine*, 346(6213), 2014, 1052-1053.
- [8] Tasie, F. O. and George-Okafor, U. O., Antimicrobial sensitivity of *Ocimum gratissimum* (scent leaves) on bacterial associated with *Otitis externa*. *Journal of Applied Science*, 9(2), 2006, 6499-6502.
- [9] Elad, S. and Freeman, S., Biological control of fungal plant pathogen, in Esser, K. and Bannett, J.W. (Eds.), *The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research, Agricultural Applications*. (New York: Springer, 2002) 93-110.
- [10] Grishkan, I., Tsatskin, A. and Nevo, E., Diversity of cultured microfungi communities in surface horizons of soils on different lithologies in upper Galilee, Israel. *European Journal of Soil Biology*, 44, 2005, 180-190.
- [11] Sylvia, D. M., Fuhrmann, J.F., Hartel, P.G. and Zuberer, D.A., *Mycorrhizal symbiosis, Principles and Applications of Soil Microbiology*, 2 (New Jersey: Pearson Education Incorporation, 2005) 263-282.
- [12] Griffin, D.H., Chemical requirements for growth, In: *Fungal Physiology*, 2 (USA: Wiley-Liss Inc., 1994) 458.