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ENDOPHYTIC MYCOFLORA OF *Garcinia kola* FROM AKAMKPA AND OBAN IN CROSS RIVER STATE, NIGERIA

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ABSTRACT
The Endophytic mycoflora of *Garcinia kola* (Heckl) growing at Akamkpa and Oban, Cross River State, Nigeria was studied. Barks and leaves of *G. kola* were collected in February - March and June - September, 2019. Pieces of leaves and barks of were cultured on Potato Dextrose Agar (PDA). A total of two hundred and forty four (244) isolates belonging to Hyphomycetes eight (8), Saccharomycetes one (1) and Zygomycetes one (1), species were recovered. *Acremonium* sp, with the highest colonization frequency of thirty two (32%), colonized both barks and leaves of *G. kola* in both locations and was significantly ($P< 0.05$) more, in the wet season in both locations. *Geotrichum candidum* significantly ($P<0.05$) colonized the barks at Akamkpa during the wet season. *Penicillium* sp was present in the barks from Oban during the wet season. *Rhizopus stolonifer* colonization was significant ($P< 0.05$) in the leaves and barks from both locations during the wet season but recorded only minimal growth in both plant materials especially during the dry season. *Verticillium* sp was significantly ($P<0.05$) present in the leaves from Akamkpa and Oban during both dry and wet seasons. The ecological indices suggest that *G.kola* from Akamkpa and Oban harbour diverse species of endophytic fungi.
Keywords: Akamkpa, Colonisation, Cross River State, Endophytic fungi, *Garcinia kola*, Microorganisms, Oban.

INTRODUCTION

The symbiotic relationship between plants and microorganisms is considered an important requirement for eukaryotic colonization of land (Heckman, *et al.,* 2001). "Endophytes" stands for any *in planta* microorganism (Singh, *et al.,* 2017). In a strict sense, fungal endophytes are "fungi that spend either full or a considerable part of their life inside living plant tissues without causing any visible harm" (Petrini, 1991). After decades of research on fungal endophytes, it is now clear that they are unexceptionally present in all taxonomic groups of the plant kingdom, vegetation types (alpine to tropical) and ecological types (hydrophytes to xerophytes) in great diversity (Persoh, 2013; Arnold, *et al.,* 2000; Rodriguez & Redman, 2000). The reports of high endophyte diversity in trees (Porras-Alfaro & Bayman, 2011; Arnold *et al.,* 2000) have led to an increase in research efforts in this direction especially on trees growing in tropical regions. Some tropical tree species have been reported to host a hyper diverse endophyte assemblage (Arnold *et al.,* 2000; Frohlich & Hyde, 1999). Endophytic fungi have been recovered from healthy tissues of plant species growing in different biomes such as tundra, deserts, and tropical rain- forests from the Arctic to Antarctica. Unlike mycorrhizal fungi, fungal endophytes reside entirely within plant tissue and may grow within roots, stems and/or leaves, emerging to sporulate at plant or host-tissue senescence (Stone, *et al.,* 2004).

Traditionally, fungal endophytes are divided into two major groups: Clavicipitaceous endophytes (C-endophytes) and Non-Clavicipitaceous endophytes (NC-endophytes). C-endophyte infections are limited to some cool-and warm-season grasses and produce a systemic intercellular infection. Their transmission is primarily vertical, passing from maternal plants to offspring through seeds (Suryanarayanan, 2013). Therefore, their community is characterized by having low diversity, in terms of number of species and genetic variability. Relationships of C-endophytes and their hosts have been studied extensively due to its mutualistic symbiosis, which typically increases their host fitness and consequently has applications in crop systems (Rodriguez *et al.,* 2009: Seiber, 2007: Stone *et al.,* 2004).

Endophytic fungi were first studied in temperate plants, but recently these studies have been extended to include tropical plants (Nwobodo, *et al.,* 2017; Pimentel, *et al.,* 2006). All plants maintain associations with fungal endophytes and epibionts. These associations between fungi and plants are generally a cryptic phenomenon in Nature. Fungal endophytes may invade tissues of roots, stems, branches, twigs, bark, leaves, petioles, flowers, fruits, and seeds, including xylem of all available plant organs. These fungi are alleged to affect the ecology of plants, by frequently enhancing the capacity of host plants to survive and resist environmental and biological stresses through ways that are only partially understood. It is also believed that endophytes have important roles in plant protection, acting against herbivores, insects and pathogens of the host and may also increase plant resistance to pathogens and biotic and abiotic stresses (Kogel, *et al.,* 2006; Ahlholm, *et al.,* 2002).

According to Hawksworth (2004), the extent of fungal diversity in tropical forests is unclear, and new species remain to be described. The greatest fungal diversity probably occurs in tropical forests, where a highly diverse population of angiosperms is present (Arnold *et al.,* 2000). In support of this proposal, a large number of endophytic fungal species have been described in association with plants in Asia, Australia, Africa, Central and South America, Mexico and some Pacific and Atlantic Islands. However, the diversity of endophytic fungi can vary across different biomes of a tropical forest.

Endophytic fungi have been accepted as sources for new secondary metabolites with useful biological activity. Interest in fungal endophytes is largely due to their chemical diversity. These represent a virtually untapped source of chemical reservoir that can be used in agriculture and therapeutics (Schulz *et al.,* 2002). Sampling and characterization of fungal endophyte diversity is an emerging challenge, which leads to the discovery of new species producing novel compounds and a better understanding of their role in ecosystems. Studies on the flora of endophytic fungi in tropical plants are relatively recent since it became evident that endophytes are rich sources of bioactive natural products, and many different agents have been isolated from these microorganisms with promising applications in development of natural drugs and other industrial products.

Garcinia kola (Heckel) belongs to the Kingdom; Plantae, Division; Tracheophyta, Class; Magnoliopsida, Order; Theales and Family; Clusiaceae (alternatively Guttiferae). Most of the trees remain in the wild and semi-domesticated form and has been rediscovered as so called neglected or underutilized nut (Stevens, 2019). This plant has been referred to as a "wonder plant" because every part of it has been found to be of medicinal impor-

tance (Dalziel, 1937). It is called Akilu/Akara by the Ibos and Efiat by the Ibibios of Southbeastern Nigeria (Meregini, 2005). It is a medium sized tree found in the humid forests of West-Central tropical Africa; Benin, Cameroun, Central African Republic, Cote d'Ivoire, Gabon, Liberia, Nigeria, Sierra Leone and Zaire), and south tropical Africa, Angola (Hutinson & Dalziel, 1956), where it plays an important role in African ethno medicine and traditional ceremonies (Iwu, 1982).

Garcia kola is listed as one of the priority species for conservation in the Sub-Saharan Forest Genetic Resources Programme (SAFORGEN). The tree is sometimes referred as a "wonder plant" because each of its parts can be used as medicine (Usunomena, 2012). The seeds which are among the most traded non-timber forest products (NTFPs) in West and Central Africa, are highly cherished for their therapeutic qualities where the plants are found. The seed commonly known as bitter kola is a masticatory agent used as a major kola substitute offered to guests at home and shared at social ceremonies. *Garcinia kola* seeds are also eaten as refreshing past time in West and Central Africa (Plowden, 1972). Investigation of the endophyte diversity of this species will add to the global fungal diversity estimates.

The specific objectives of this study were to determine diversity of endophytic fungi in leaves and barks of *Garcinia kola*, isolate, identify and enumerate and determine some ecological indices of the endophytic fungi in *G. kola* from the two study sites.

MATERIALS AND METHODS

Study Area:

Samples were collected from two locations Akamkpa Latitude 5º 25' 21″ North and Longitude 8º 31' 6.78″ East, and Oban Latitude 5º 19' 0″ North Longitude 8º 34' 0″ East; both in the Oban division of the Cross River National Park (CRNP). The park lies between Latitudes 5º 5' and 6º 29' North, and Longitudes 8º 15' and 9º30' East, towards the South-Eastern fringe of Nigeria. It covers two discontinuous sections, Oban, approximately 3000 km^2 in the South, established in 1989 and Okwangwo approximately 1000 km^2 established in 1991 (National Park Services, 2018). The park is only separated from the Karup National Park in the Cameroon by the International boundary between the two countries (National Park Services, 2018).

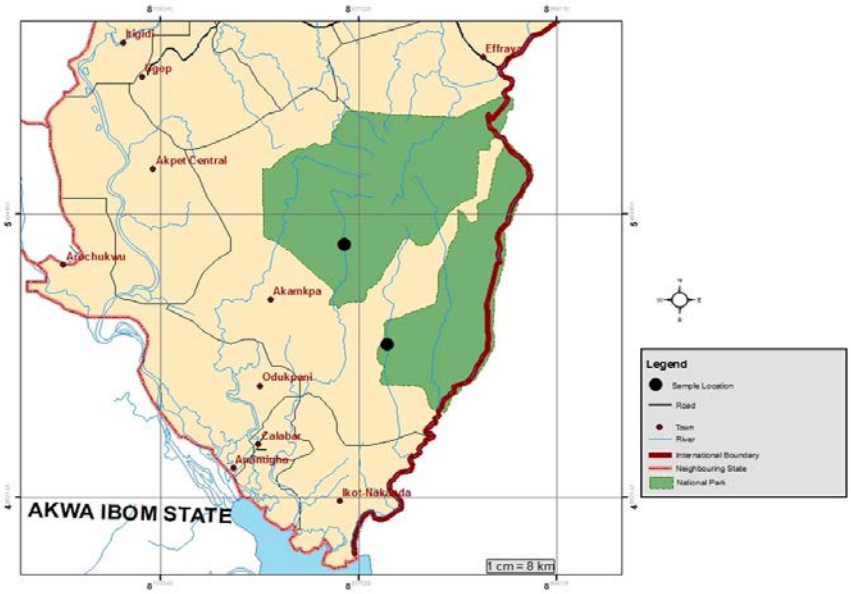

Fig1: Map of Oban Division of the Cross River National Park showing sample collection sites.
Source: Google maps

Sample Collection: Four (4) *G. kola* trees were randomly selected (Strobel & Daisy, 2003), each from Erokut Park in Akamkpa Latitude 5º 25' 21″ North and Longitude 8º 31' 6.78″ East, and Akin in Oban Latitude 5º 19' 0″ North and Longitude 8º 34' 0″ East. Barks and leaves were obtained by sampling these trees between the months of February-March and June-September, 2019 for the dry and wet seasons sampling respectively. Leaves and

barks were collected randomly from healthy looking *G. kola* trees at Erokut in Akamkpa and at Akin in Oban respectively. Using ethanol disinfected knives, bark tissues were collected about 1.5 metres above ground level from each of the selected mature trees and asymptomatic leaves of *G. kola* were also collected. The barks and leaves collected were put in labelled sterile polythene bags and taken in an ice box to the laboratory for isolation of endophytic fungi within forty eight hours.

Isolation of Endophytic Fungi:

The outer bark of the stem was removed and the inner portion containing the cortex dissected into bits approximately (1.0 cm^2. Using a cork borer, six pieces were randomly made from each of the ten leaves and barks collected respectively. The samples were washed in running tap water followed by distilled water to minimize the microbial load from sample surface (Verma *et al.*, 2007). To eliminate epiphytic microorganisms, all the samples were surface sterilized by dipping in ethanol (70%) for 3 minutes, followed by a solution of sodium hypochlorite (4% available chlorine) for 3 minutes and then rinsed in ethanol (70%) for 2-5 seconds, and a finally rinsed in sterilized distilled water. Samples were then allowed to surface dry under sterile conditions (Naik, *et al.*, 2008. The effectiveness of sterilization was confirmed by the leaf imprint method (Márquez, *et.*, 2007; Schulz, *et al*., 1998). The absence of fungal growth on the medium confirmed that the surface sterilization process was effective.

Six (6) $1cm^2$ pieces of barks and leaves each were randomly selected and placed on different 9 cm petri dishes containing the potato dextrose agar (PDA) augmented with chloroamphenicol 150 mg/l to inhibit bacterial growth. This was replicated four times, to yield a total of twenty four (24) pieces of bark and leaves per site respectively for each round of sampling. Sampling was done four times during the study; twice during the dry season (February – March) 2019 and twice during the wet season (June – September, 2019). This yielded a total of three hundred and eighty four (384) pieces of both barks and leaves of *G. kola.* The petri dishes were then incubated at 27±2ºC. The plates were screened on a routine basis and hyphal tips that grew from the tissues were cut and subsequently transferred onto fresh PDA plates for pure cultures. Each isolated fungus was assigned a number and stored in a refrigerator at 4^oC. For the characterization of the morphology of fungal isolates, slides prepared from cultures were stained with lactophenol in cotton blue and examined with a compound light microscope. The endophytic fungi were identified according to their macroscopic and microscopic characteristics such as the morphology of fruiting structures and spore, surface texture, margin character, aerial mycelium (Barnett and Hunter, 1998). Slide culture was carried out to help identify the endophytic fungi.

Statistical Analysis: The endophytic fungal isolates from *G. kola* plant tissue segment were analysed based on the percentage density of colonization (colonization frequency).

$CF = \frac{\text{Number of species isolated}}{\text{Number of segments screened}} \times \frac{100}{1}$ (Hata and Futai, 1995; Suryanarayanan *et al.,* 2000); relative percentage occurrence of different groups of fungi, RPO.

$RPO = \frac{\text{Density of colonisation of one group}}{\text{Total density of colonisation}} \times \frac{100}{1}$ (Suryanarayanan & Thennarasan, 2004) and percentage of endophytic infection rate (EIR),

$EIR\ (\%) = \frac{\text{Number of infected segments}}{\text{Number of segments screened}} \times 100$. The diversity indices; Simpson's Diversity index D, Shannon-Wiener Diversity index, H; Shatnnon's Equitability Index, $E_{H;}$ Species richness, *D* and Jaccard's Similarity Index were calculated. Shannon-Wiener Diversity Index, $H = -\sum (P_i \times \ln P_i)$, (Shannon & Weiner, 1963). Simpson's Diversity index = $1 - \frac{\sum n(n-1)}{N(N-1)}$, (Simpson, 1949); Shannon's Equitability Index, $E_H = \frac{H}{ln\ S}$ Species richness, $D = \frac{S}{\sqrt{N}}$ and Jaccard's Similarity Index = $Cj = \frac{j}{a+b+j}$ (Jaccard, 1901). Data generated was subjected to analysis of variance (ANOVA) and means of separated by Least Significant Difference.

RESULTS

A total of two hundred and forty four (244) isolates of endophytic fungi were recovered from three hundred and eighty four (384) plant parts (leaves and barks) of *G. kola* from Akamkpa and Oban as can be seen from Table 1. Ten (10) species of endophytic fungi were isolated; Hyphomycetes eight (8), Saccharomycetes one (1) and Zygomycetes one (1).

Table 1: Colonisation Frequency of Endophytic Fungi Isolated from *G. kola* growing in Akamkpa and Oban.

		Akamkpa				Oban			
		Leaves(96)		Bark (96)		Leaves (96)		Bark (96)	
S/No	Endophytic Fungi species	NOI*	+CF %	NOI*	CF+%	NOI*	CF+ %	NOI*	CF+%
1	*Acremonium* sp.	31	32.29	12	12.5	27	28.13	14	14.58
2	*Aspergillus niger*	0	0	1	1	0	0	0	0
3	*Aspergillus parasiticus*	9	9.38	6	6.25	2	2.08	0	0
4	*Candida* sp.	0	0	0	0	4	4.17	0	0
5	*Epicoccum* sp.	3	3.13	1	1	3	3.13	0	0
6	*Fusarium* sp.	0	0	2	2.08	0	0	0	0
7	*Geotrichum candium*	5	5.21	12	12.5	6	6.25	9	9.38
8	*Penicillium* sp.	3	3.13	4	4.17	3	3.13	4	4.17
9	*Rhizopus stolonifer*	9	9.38	15	15.63	7	7.29	15	15.63
10	*Verticilium* sp.	13	13.54	0	0	24	25	0	0
		73	76.06 %	53	55.13%	76	79.18 %	42	43.76%

*NOI= Number of isolates +CF% = Colonization Frequency

Acremonium sp colonies usually grow slowly, often compact and moist at first, becoming powdery, suede-like or floccose with age. They may be white, grey, pink, rose or orange in colour. The hyphae are filamentous, septate and hyaline and produce mostly simple awl-shaped erect phialides bearing one-celled conidia on slender conidiophores.

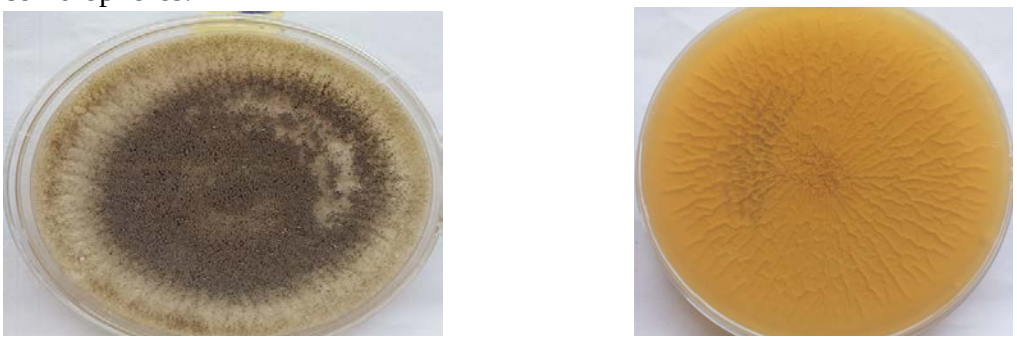

Plate 1: *Acremonium* sp on PDA (top and reverse)

***Aspergillus niger*:** This is one of the commonest and easily identifiable species of the genus *Aspergillus*. Colonies spread rapidly with white mycelium changing to dark brown to black or purple brown when they begin to sporulate producing conidial heads. The conidial heads are globose, radiate, with conidiophores arising from substratum. Vesicles are globose, phialides are borne directly on the vesicles.

Aspergillus parasiticus

A. parasiticus is a member of the *Aspergillus* complex. The conidiophores are upright and simple, ending in a globose or clavate swelling, bearing phialides at the apex or radiating from the apex or the entire surface. Conidia (phialospores) are 1-celled, globose, and often variously coloured in mass, dry basipetal chains. The conidia are rough and thick walled and spherical in shape, with short conidiophores and small vesicles to which the phialides are directly attached. The colonies are dark green in colour (Barnett & Hunter, 1998).

***Candida* sp.**

The mycelium is not extensive; conidia are hyaline, l-celled, ovoid, forming short chains by budding. The conidia are produced apically on the mycelium. It is characterised by globose to elongate yeast-like cells or blastoconidia.

***Epicoccum* sp.**

Sporodochia are dark, more or less cushion-shaped, variable; conidiophores compact dark, rather short; conidia are dark, several-celled (dictyosporous), globose; mostly saprophytic, or weakly parasitic.

Plate 2: *Epicoccum* sp. top and reverse)

***Fusarium* sp**

Mycelium is extensive and cotton-like with yellow colouration on the reverse side of the plate. The conidiopore are inconstant slender and simple with a whorl of philiades; conidia are hyaline, macroconidia are curved

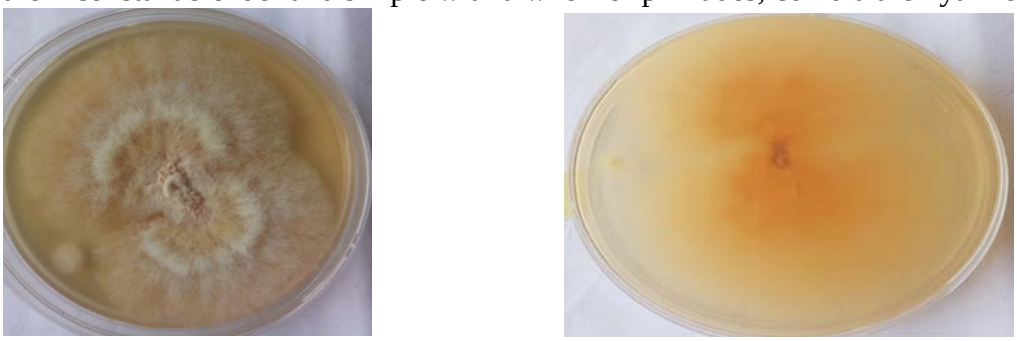

Plate 3: *Fusarium* sp. (top and reverse)

Geotricum candidum

White smooth colony with dichotomously branched hyphae bearing artrosporous cylindrical hyaline spores, without conidiophores

***Penicillium* sp.**

Colonies are fast growing, green in colour with the reverse side off white, have filamentous, septate, brown like hyphae produced in columns. Conidiophores are dense and bear single celled, globose conidia which are produced basipetally

Plate 4: *Penicillium* sp. on PDA (top reverse and Conidiophore

Rhozipus stolonifer
Colony is white in colour with filamentous soma and coenocytic hyphae bearing stolons and rhizoids, has tall sporangiophores in groups, each bearing black brownish, ovoid sporangiopores.

***Verticillium* sp.**
Colony is cottony white, soma filamentous with septate hyphae. Conidia are one-celled and cylindrical, phiiades are solitary without chlamydospores

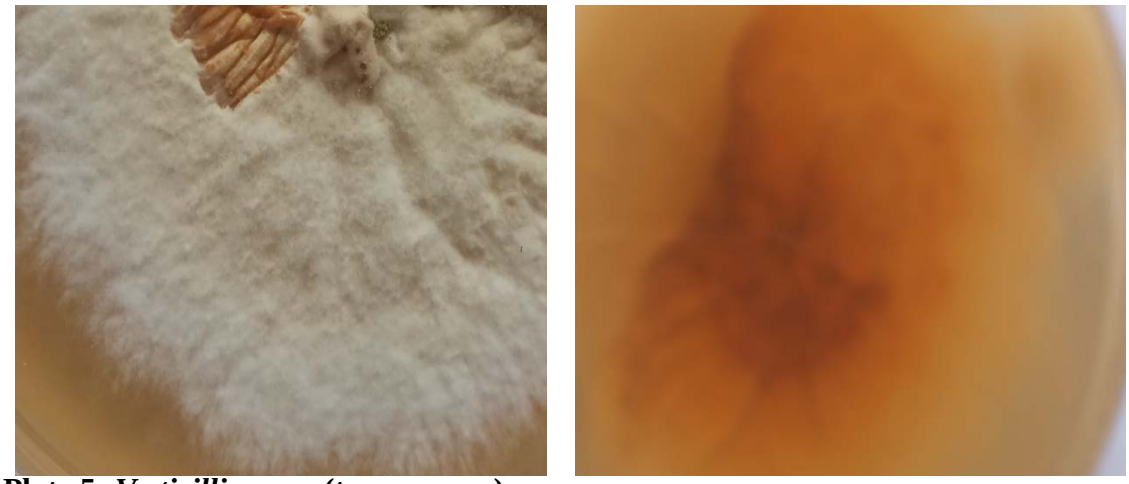

Plate 5: *Verticillium* sp. (top, reverse)

Table 1 shows the colonisation frequency (CF) of endophytic fungi in *G. kola* from Akamkpa and Oban. There were 73 isolates of endophytic fungi for *G. kola* leaves from Akamkpa, giving a CF of 76.04%, the bark produced 53 isolates resulting in a CF of 55.21%; while for Oban there were 76 isolates of endophytic fungi for the leaves and a CF of 79.17% and 42 isolates from bark with a CF of 43.75% for the same species.
The endophytic infection rates (EIR) for *G. kola* are presented in Table 2. *Acremonium* sp. from Akamkpa and Oban had endophytic infection rates of 33.33% (leaves) and 22.91% (barks) respectively; followed by *Rhizopus stolonifer* in barks from Oban (21.88%), *Verticillium* sp. (20.83%), while *Acremonium* sp. and *Rhizopus stolonifer* had a joint highest EIR (17.71%) for the barks. The highest endophytic infection rate in *G.kola* leaves collected from Oban was *Verticillium* sp. (29.17%), followed by *Acremonium* sp. (27.08%).
The highest infection rates came from the barks from Akamkpa, *Acremonium* sp 33.33%, followed by *Veticillium* sp 29.17% for leaves from Oban; *Acremonium* sp. 22.19% for leaves from Oban and *Rhizopus stolonifer* 21.88% for barks from Oban.

Table 2: Endophytic Infection Rates of Endophytic Fungi in *G. kola* from Akamkpa and Oban

S/No	Endophytic Fungi Species	Oban						Akamkpa					
		Leaves			Barks			Leaves			Barks		
		S^1	I^2	EIR^3%	S^1	I^2	EIR^3%	S^1	I^2	EIR^3%	S^1	I^2	EIR^3%
1	*Acremonium* sp.	96	22	22.91	96	19	19.79	96	32	33.33	96	17	17.71
2	*Aspergillus niger*	96	0	0	96	0	0	96	0	0	96	2	2.08
3	*Aspergillus parasiticus*	96	2	2.08	96	0	0	96	1	1.04	96	6	6.25
4	*Candida* sp.	96	0	0	96	0	0	96	2	2.08	96	0	0
5	*Epicoccum* sp.	96	10	10.42	96	0	0	96	4	4.17	96	2	2.08
6	*Fusarium* sp.	96	0	0	96	2	2.08	96	0	0	96	2	2.08
7	*Geotrichum candidum*	96	8	8.33	96	9	9.38	96	6	6.45	96	12	12.5
8	*Penicillium* sp.	96	6	6.25	96	6	6.25	96	1	1.04	96	6	6.25
9	*Rhizopus stolonjifer*	96	15	15.63	96	21	21.88	96	18	18.75	96	17	17.71
10	*Verticillium* sp.	96	23	23.96	96	0	0	96	20	20.83	96	0	0
			84			57			86			64	

S^1 = No of segments screened I^2 = No of infected segments EIR^3 = Endophytic Infection Rates

The relative percentage occurrence (RPO) of the different classes of fungi are presented in Fig. 2. The Hyphomycetes were the most abundant in both locations and plant parts; with 87.67% in *G. kola* leaves from Akamkpa followed by 85.53% in *G. kola* from Oban.For the barks from Akamkpa and Oban, 71.7% and 64.29% respectively. The second most abundant class was Zygomycetes in barks from Oban 35.71%, barks from Akamkpa 28.3% and leaves from Akamkpa 12.33% and 9.21% for leaves from Akamkpa. Saccharomycetes 5.26%, were recorded in only Oban leaves.

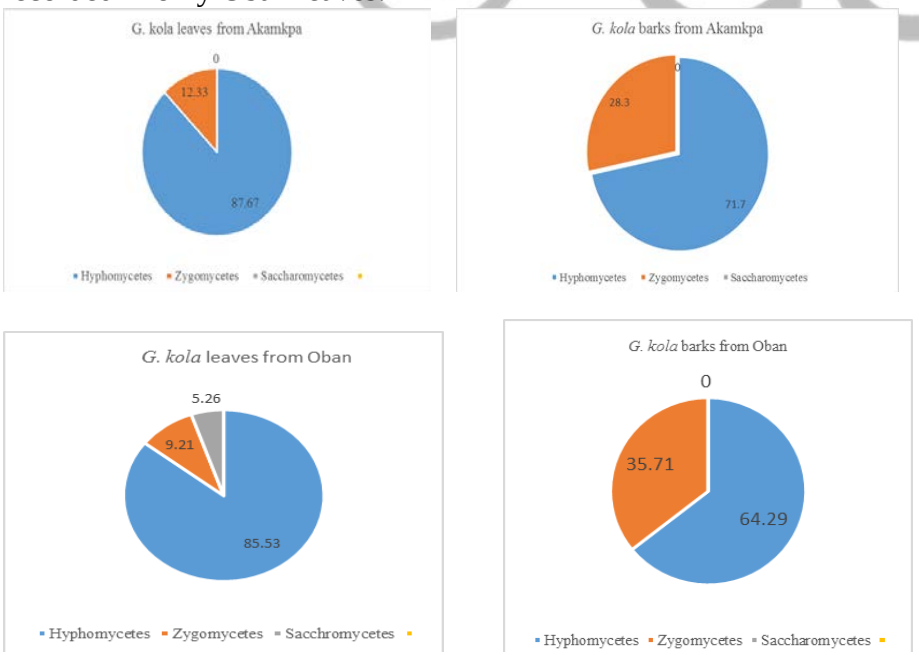

Fig 2: Relative Percentage Occurrence of Different Endophytic Groups of Fungi in *G. kola* from Akamkpa and Oban.

The ecological indices are presented in Table 3. Simpson's diversity index *D* for *G. kola* leaves from Akamkpa was 0.7599 while for Oban it was 0.7628; while for the barks Akamkpa was 0.8120 and Oban 0.7336. For the Shannon-Wiener diversity index H, the values were 1.6331 and 1.6576 for the leaves from Akamkpa and Oban respectively; 1.7450 and 1.2880 for barks from Akamkpa and Oban respectively. The Jaccard similarity index

Cj for leaves from Akamkpa was 0.32 while that of the Barks was 0.25. The same values were also recorded as the Jaccard similarity index Cj for leaves and barks respectively from Oban. Shannon-Wiener Equitability index E_H index for leaves from Akamkpa was 0.8259, while leaves from Oban was 0.7972. For the barks from Akamkpa and Oban the values were 0.8393 and 0.9291 respectively. For species richness, the values for leaves from Akamkpa and Oban were 1.05 and 0.92 respectively; while for barks they were 1.10 and 0.62 respectively.

Table 3: Ecological Indices of Endophytic Fungi from *G. kola* from Akamkpa and Oban

Activity	Akamkpa			Oban		
	Leaves	Barks	Total	Leaves	Barks	Total
Number of segments screened	96	96	192	96	96	192
Number of segments colonized by fungi	73	53	126	72	42	114
Total number of fungal species	7	8		8	4	
Total number of fungal isolates	73	53		76	42	
Colonisation frequency (CF) %	76.06	55.13		79.18	43.76	
Simpson's diversity index *D*	0.7599	0.8120		0.7628	0.7336	
Shannon-Wiener diversity index	1.6331	1.7450		1.6576	1.2880	
Jaccard Similarity Index	0.32	0.25		0.32	0.25	
Shannon-Wiener Equitability index E_H	0.8259	0.8393		0.7972	0.9291	
Species Richness	1.05	1.10		0.92	0.62	

DISCUSSION

Endophytic fungi have been isolated from nearly all plant species ever investigated. While some are beneficial to their hosts helping out in some ecologically vital issues, some may be latent pathogens and some are saprophytes waiting for some environmental stimuli to initiate a change. The most common method of cultivation of endophytic fungi which tends to favour the fast growing species was used in this study. During this study, ten (10) species of endophytic fungi were isolated; Hyphomycetes eight (8), Saccharomycetes one (1) and Zygomycetes one (1). This is in agreement with Arnold *et al*., (2000) which reported hyperdiverse groupings of endophytic fungi in tropical tree species.

Two hundred and forty four (244) isolates of endophytic fungi; yielding ten (10) species were recovered. This agrees with Hawksworth (2004), that endophytic fungi are found in all trees that have been examined. *Acremonium* spp was found to colonize both bark and leaves of *G. kola* in both locations especially in the wet season. However, the level of colonization was significantly ($P< 0.05$) higher in leaves, particularly in the wet season in both locations. This might be due to the large surface area of leaves which tends to provide a better chance for the fungal propagules in the air to shelter hence a higher chance of infecting the internal tissues. This agrees with the work of Suryanarayanan & Thennarasan (2004) that the colonization frequency of the endophytes increased with rainfall.

Aspergillus niger was barely present in the barks of the plant in Akamkpa during the wet season in both plant materials. There were no other reports of *A. niger* colonization in Akamkpa. *Aspergillus parasiticus* was present in leaves and barks of the plant in Akamkpa though not significantly ($P>0.05$), but was absent in the barks from Oban. *Candida* sp was only present in an insignificant ($P>0.05$) level in leaves of the plant in Oban only in the wet season but absent completely in other units. *Epicoccum* sp colonized minimally the leaves of the test plant especially in Akamkpa across seasons, but failed to do same in bark from Oban. *Fusarium* sp could not colonize both plant parts under study in both seasons across locations except for an insignificant ($P>0.05$) presence in bark of *G. kola* in Akamkpa during the wet season. *Geotrichum candidum* was highest in colonizing the bark of *G. kola* in Akamkpa during the two seasons under consideration, but had a considerably lower presence in other

units. *Penicillium* sp was present in the bark of *G. kola* in Oban during the wet season. *Rhizopus stolonifer* leaves from Akamkpa and Oban had significant (P0<0.05) presence of endophytic fungi during the wet season. Leaves of *G. kola* supported the significant (P<0.05) growth of *Verticillium* sp at both locations during the wet season however the barks in Oban showed no growth.

This study agrees with Arnold *et al.*, (2000) that tropical forest trees harbour a high diversity of endophytes, especially where the endophytes are found in aerial tissues as a result of several, independent infections. For *G. kola* leaves from Akamkpa, the EIR ranged from *Acremonium* sp. (33.33%), *Verticillium* sp (20.83%), *R. stolonifer* (18.75%) up to *A. niger* (0%).

The endophytic rates of infection (EIR) were almost always higher in the leaves than the barks. This can be seen in the relatively high values recorded for endophytic fungi in *G kola* leaves from Akamkpa and Oban by *Acremonium* sp (33.33% and 22.91%) respectively; compared to 22.91% and 17.71% for barks from the respective sites. The only deviation was in *R.stolonifer* from Oban 21.88% (barks) was higher than 15.63% (leaves). The EIR for *G. cola* leaves from Oban ranged from *Verticillium* sp. (29.17%), *Acremonium* sp. (27.08%), *R. stolonifer* (15.63%), *Epicoccum* sp. (10.42%) up to *Alternaria* sp. (0%). For the barks from Akamkpa, the EIR ranged from *R. stolonifer* (21.88%), *Acremonium* sp (19.79%), *Geotrichum candidum* (9.38%) up to *A. niger* (0%). The endophytic infection rates (EIR) for *G. kola* leaves from Akamkpa ranged from *Acremonium* sp. (33.33%), *Verticillium* (20.83%), *R. stolonifer* (18.75%) up to *A. niger* (0).

The Simpson diversity index *D* ranges from 0 (no diversity) to 1(maximal diversity), that is, the closer the value is to 1, the greater the diversity of species in a community. For *G. kola* leaves and barks from Akamkpa and Oban, the Simpson diversity indices were high (0.7599 and 0.8120 for leaves and barks from Akamkpa respectively); (0.7336 and 0.7628 barks and leaves respectively) portraying the hyperdiverse nature of endophytic fungi in the tropical forest species.

The Shannon-Wiener diversity index *H* typically ranges from 1.5 to 3.5 and it accounts for both abundance and evenness of species. The highest Shannon-Wiener index was 1.7450 for barks from Akamkpa, on the other hand the least value 1.2880 was in barks from Oban. Shannon-Weier equitability index wich measures evenness was highest 0.9291 in barks from Oban indicating a more uniform community. Species richness is simply a count of species. Generally if the number of species is high, it means a high species richness hence a stable ecosystem. High species richness contributes to increase in biodiversity which is also an important aspect of biodiversity conservation. The values from Akamkpa were; *G. kola* barks 1.10 with eight (8) species; *G. kola* leaves 1.05 with seven (7) species. For Oban the values were *G. kola* leaves (0.92) with eight (8) species and *G. kola* barks (0.62) and four (4) species. These suggest a community with rich biodiversity.

CONCLUSION The result seems to show that *G. kola* from Akamkpa and Oban harbour diverse species of endophytic fungi, with the leaves having more than the barks. The low Jaccard indices seem to suggest that the two populations are not very similar. From literature searches, it appears this is the first survey of this species from CRNP for endophytic fungi. With the importance of *G. kola* in Nigerian herbal medicine, it would be of great benefit if more surveys are carried out. These could report more endophytic fungi and possibly kickstart the search for the bioactive molecules they may habour.

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