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FUNGI ASSOCIATED WITH A DRY INVASIVE WHITE PATCHES ON TRUNKS OF ECONOMIC FRUIT TREES IN SOUTH-WEST NIGERIA

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ABSTRACT

An unprecedented number of fungal and fungal-like diseases are the main threat to the diversity and productivity of economic fruit trees in south-west Nigeria. The objective of this study was to investigate the fungi associated with dry invasive whitish patches, noticeable on trunks and branches of cocoa (*Theobroma cacao*), kola-nut (*Cola* spp.) and sweet orange (*Citrus sinensis*) trees prevalent in south-west Nigeria. A total of 108 whitish patch scraped samples were aseptically obtained from the affected cocoa, kola-nut and sweet orange trees in Osogbo, Ife, Ejigbo and Ilesha plantations in south-west Nigeria for this study. Viable fungal populations, were determined using the spread-plate techniques; and pure fungal isolates were identified through their morphological appearance, microscopic features and sporing structures. Mean viable fungal count in the samples ranged from $3.85 \times 10^4 \pm 0.00$ to $9.75 \times 10^3 \pm 0.00$ CFU g^{-1}. Viable fungal counts were significantly different ($P < 0.05$) between fruit tree species and across locations. A total of 52 fungal species belonging to 21 genera were isolated, with *Aspergillus* and *Penicillium*as predominating and occurring at 23 and 17%, respectively. Other isolated genera included seven from cocoa trees; ten from kola-nut trees and five from sweet orange trees. There is need for appropriate control strategies to prevent further spread in contiguous plantations.

Key Words: *Aspergillus,* kola-nut, sweet orange, *Theobroma cacao*

RÉSUMÉ

Un nombre sans précèdent de champignons et de maladies fongiques sont les menaces principales à la diversité et à la productivité des fruitiers à valeur économique dans le sud-ouest du Nigéria. L'objectif de cette étude était d'investiguer les champignons associés aux taches invasives sèches blanchâtres, visibles sur les troncs et les branches du cacaoyer (*Theobroma cacao*), colatiers (*Cola* spp.) et l'oranger sucré (*Citrus sinensis*) prévalent dans le sud-Ouest du Nigéria. Un total de 108 échantillons de taches blanchâtres raclées a été aseptiquement obtenues des pieds de cacaoyer, colatier et oranger sucré dans les plantations de Osogbo, Ife, Ejigbo et Ilesha dans le sud-Ouest du Nigéria pour cette

étude. Des populations viables de champignons, ont été déterminées en utilisant les techniques de plaque de diffusion ; et des isolats purs de champignons ont été obtenus à travers leur apparence morphologique, les caractéristiques microscopiques et les structures de production des spores. Le nombre moyen de champignon dans les échantillons variait de 3,85 x 104 ± 0,00 au 9,75 x 103 ± 0,00 CFU g^{-1}. Les nombres de champignons viables ont été significativement différents ($P < 0,05$) parmi les espèces fruitières et à travers les localités. Un total de 52 espèces de champignons appartenant à 21 genres ont été isolées, avec *Aspergillus* et *Penicillium*as les plus prédominants et apparaissant à 23 et 17%, respectivement. Autres genres d'isolats comprennent sept de cacaoyers, dix des colatiers et cinq des orangers sucrés. Il y a nécessité de détermines des stratégies appropriées de contrôle pour prévenir la propagation avancée dans les plantations contiguës.

Mots Clés: *Aspergillus,* colatier, orange sucrée, *Theobroma cacao*

INTRODUCTION

Cocoa (*Theobroma cacao*), kola-nut (*Cola* spp.) and sweet orange (*Citrus sinensis*) are important economic cash crops in tropical Africa. Cocoa serves as raw material source for local confectionery, beverage and winery industries in African countries (Adejumo, 2005; Verter and Beèváøová, 2014; 2016). Kola-nut and citrus, on the other hand, are widely cultivated for their euphoriant value, bioactive compounds and pharmacological properties with strong social and traditional significance (Oboh *et al*., 2014a; 2014b; Fallico *et al*., 2017).

South-west Nigeria is the country's main growing region of cocoa, kola-nut and sweet orange trees. The cocoa, kola-nut and sweet orange trees in this region are prone to severe drought, pests and microbial attacks, contributing to low fruit yield (Adejumo, 2005; Asogwa *et al*., 2011). The susceptibility of these important wood plants is further exacerbated by reliance on seedlings from the wild (Bailey *et al*., 2016; Surujdeo-Maharaj *et al*., 2016). South-west Nigeria, like most of the other African countries involved in large-scale cultivation, is yet to benefit from the propagation of improved cash crop seedlings with diseases-prevention potential through genetic modifications and use of relevant biotechnological tools. Furthermore, there have been no contiguous kola-nut and sweet orange plantations in the region; rather they are scattered in cocoa plantations, mixed-crop farmlands and forests (Asogwa *et al*., 2011).

In south-west Nigeria, an increasingly common disease feature on cocoa, kola-nut and sweet orange trees are whitish patches usually noticeable on the tree trunks and branches (Plate 1). Once established on a tree, the whitish patches spread rapidly in the plantation, mainly on trunks and branches of both young and mature cocoa, kola-nut and sweet orange trees. Farmers in the region obtain very low cocoa fruiting, especially on the parts covered by the whitish patches. Although the observed whitish patches on the tree trunks and branches may be the consequence of syntrophic activity of various plant pathogens, the pattern of spread as well as the dry scaly nature of the disease condition is suggestive of predominant fungal attack.

The objective of this study was to investigate the occurrence and characteristics of fungi that are associated with the whitish patches found on stem and branches of the fruit trees in south-west Nigeria.

MATERIALS AND METHODS

Site description. This study was conducted in Osun State in south-west Nigeria. Specifically, four sites, namely Osogbo, Ife, Ejigbo and Ilesha were considered for the study. The study sites fall within the tropical rainforest zone of south-west Nigeria, and were characterised by two rainy seasons

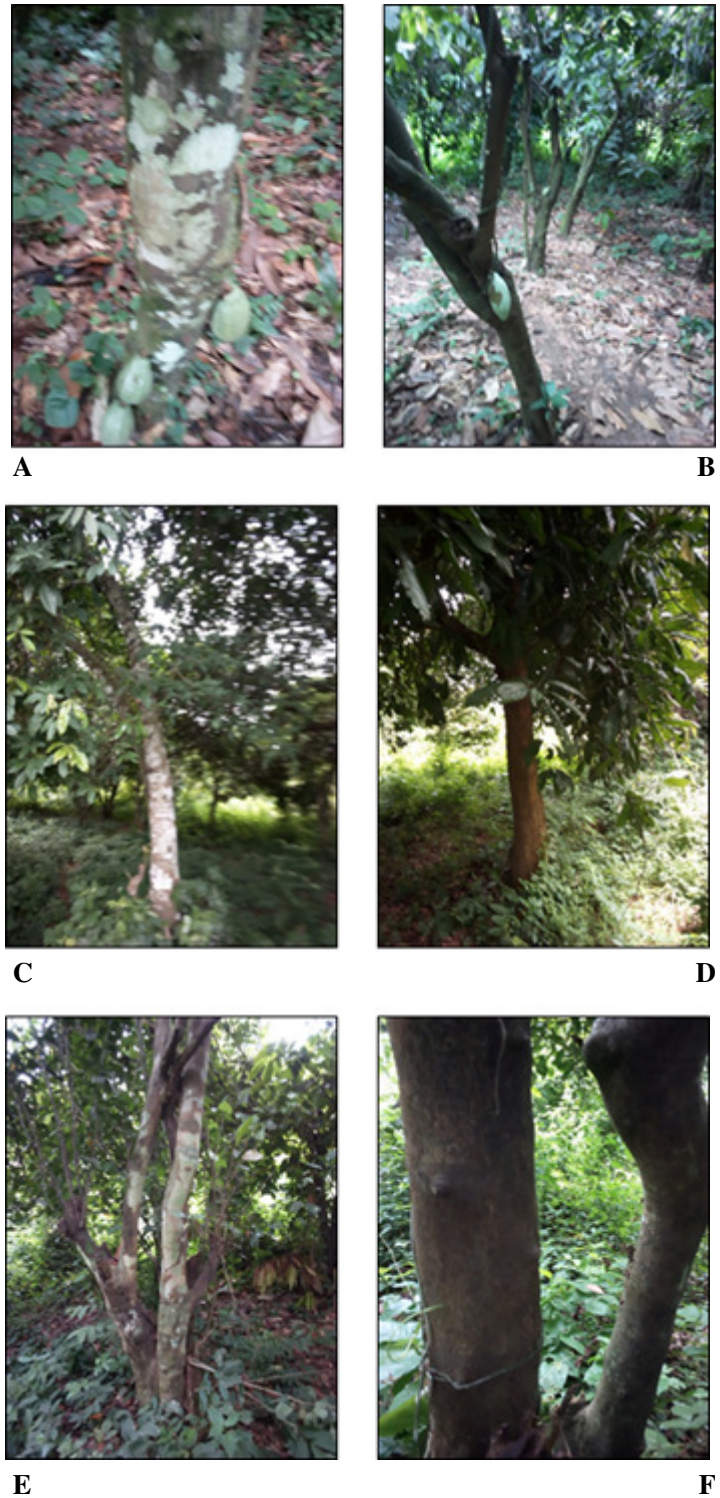

Plate 1. Healthy and white patches affected trees. (A) affected cocoa (no pod grows on the affected parts), (B) healthy cocoa, (C) affected kola-nut, (D) healthy kola-nut, (E) affected sweet orange, and (F) healthy sweet orange tree.

(March - November) and the dry (November - February) seasons. The dry season is also known to bring Harmattan dust.

Collection of samples. Sterilised scalpels were used to scrape the whitish stuff from randomly selected adult (>20 years) tree trunks and branches of cocoa, kola-nut and sweet orange into separate labelled 10 ml sterile screw-capped plastic containers. To eliminate surface contamination of the affected areas, the trunks and branches were surface-sterilised with 0.5% sodium hypochlorite prior to scraping (Mejía *et al*., 2014).

A total of 27 samples were obtained per study site in a 3 (different farms) x 3 (kinds of trees) x 3 (replicates) factorial arrangement as analytical study design. Altogether, 108 samples of the whitish patched stuff were obtained from the four selected sites and used for the study. The samples were preserved in ice boxes at 4 ± 2°C and transported to the laboratory for fungal evaluation.

Enumeration of fungi. Fungal isolates were recovered from the whitish patch scrapings on potato dextrose agar (PDA) plates, fortified against bacterial growth with 0.25 g chloramphenicol. Fungal estimation was carried out by aseptically weighing 1 g of each sample into 9 ml of Ringer's solution. This was followed by ten-fold serial dilution. A 100 µl aliquot from 10^1, 10^2, 10^3 and 10^4 of each serially diluted sample were spread-plated onto PDA plates in triplicates.

The inoculated plates were incubated at 25 ± 2°C for 7 days in a humidified environment. Discrete fungal colonies were counted on each plate and recorded. Counts per gramme of sample were calculated using the formula below and reported as colony forming units (CFU) per gramme (Nwachukwu and Akpata, 2003).

$$CFU/g = \frac{\text{Number of colonies counted x Dilution factor used}}{\text{Volume plated in ml}}$$

..... Equation 1

Pure isolates were then obtained by selecting discrete colonies with distinct morphological differences such as colour, texture, size and shape. They were randomly picked using a mounting needle, and sub-cultured onto fresh PDA plates, and incubated for 5 days. All axenic strains were stored on PDA slants for further characterisation (Akinde *et al*., 2017).

Identification of fungal isolates. The pure fungal isolates were identified through their morphological appearance, microscopic features and sporing structures (Humber, 1997; Campbell and Johnson, 2013). Morphological characterisation of fungi isolates was based on fungal appearance on surface and reverse side, texture and colour of the colony.

Microscopic identification was by using a sterile inoculating needle, whereby a small portion of fungi isolate was picked and placed in a drop of lactophenol cotton blue stain on a clean grease free microscope slide. A clean cover slip was then gently placed on the prepared slide and examined under a light microscope at X40 magnification. The main characteristics considered for their identification included Hyphae: septate or non-septate; Mycelium: coloured or non-coloured; Growth: rapid, slow or moderate; and Texture: powdery, woolly, downy, glabrous or velvety.

Statistical analysis. The viable fungal count results were presented using descriptive statistics (tables, figures and plates). Differences between mean values were compared using ANOVA ($P < 0.05$). Mean separation was done using Least Significant Difference (LSD) at the probability level of 5%. Statistical analysis was carried out using CoStat, CoHort Software, 2014.

RESULTS

The typical appearance and distribution of the whitish fungal-like patches on affected cocoa, kola-nut and sweet orange trees are presented in Figure 1. The mean viable fungal count in

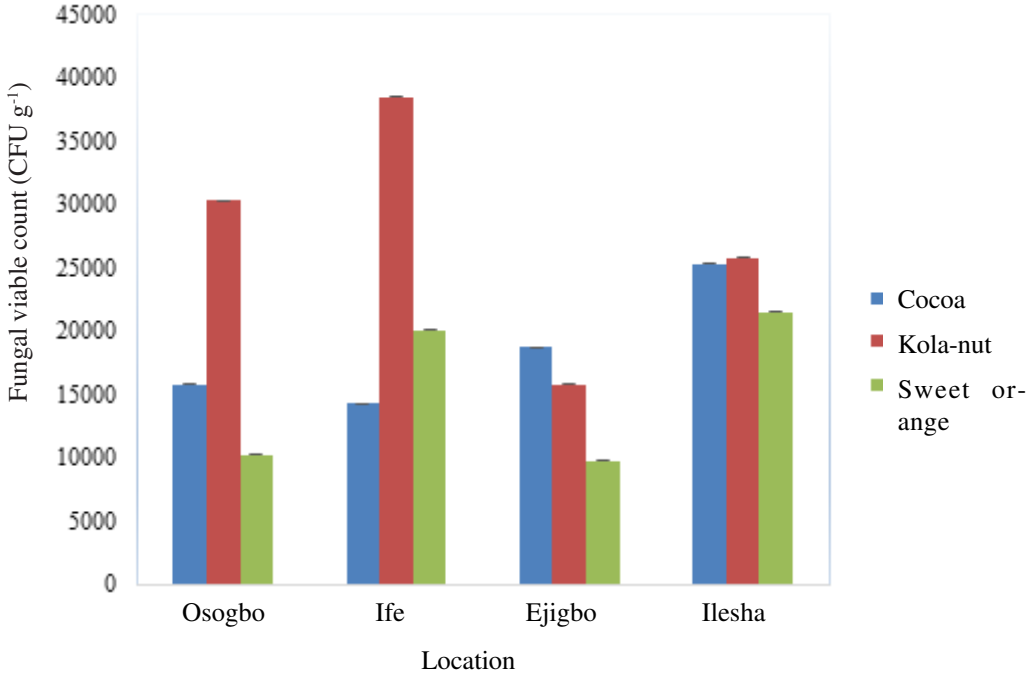

Figure 1. Mean viable fungal count in affected economic trees from different locations.

the whitish patch scrapings varied from one location to another; with Ilesha having the highest viable fungal count of 2.53 x 10^4 ± 0.00 CFU g^{-1}; and Ife having the lowest (1.43 x 10^4 ± 0.01 CFU g^{-1}), particularly from the cocoa trees. The highest viable fungal count (3.85 x 10^4 ± 0.00 CFU g^{-1}) was observed in scrapings from kola-nut trees obtained from Ife; followed by Osogbo and Ilesha; Ejigbo had the lowest count (1.58 x 10^4 ± 0.01 CFU g^{-1}). The viable fungal count in scrapings from sweet orange trees ranged between 9.75 x 10^3 ± 0.00 CFU g^{-1} and 2.15 x 10^4 ± 0.00 CFU g^{-1}, with the highest coming from in Ilesha, and the lowest from Ejigbo site.

Results for the influence of location and tree type, and the interaction between the two on fungal distribution patterns among fruit tree types are presented in Tables 1 - 3. There was a significant ($P<0.05$) difference between fungal counts in different crops and locations (Table 1). Fungal mean viable count of the affected trees was significantly influenced by location, but was highly significantly influenced by tree species and the interaction between the two.

There was no significant difference ($P> 0.05$) between viable counts for fungi in the three crop types across study sites (Table 2). No significant difference ($P>0.05$) also occurred between viable counts of fungi in cocoa and sweet orange. In contrast, kola-nut, significantly more viable fungal counts than cocoa and sweet orange trees (Table 3).

A total of 52 fungal isolates were identified from the different locations and these comprised of 20 genera (Fig. 2). *Aspergillus* and *Penicillium* were the predominant fungal genera in all the samples.

Disaggregation of the fungal genera revealed up to 25 species in all the samples with 10, 15 and 7 species occurring in the scrapings from cocoa, kola-nut and sweet orange trees, respectively (Table 4).

The highest fungal species diversity was noted in the samples obtained from kola-nut trees, followed by cocoa trees. The lowest

TABLE 1. ANOVA table of viable fungal count in different economic trees and locations in south-west Nigeria

S/N	Variables	MS	P-value
1	Block	$1.06 \times 10^{8, ns}$	0.1972
2	Location	$2.94 \times 10^{8, *}$	0.0058
3	Tree type	$9.62 \times 10^{8, **}$	0.0000
4	Location õ tree	$3.11 \times 10^{8, **}$	0.0004

MS = Mean separation, ^{ns} = Non-significant, * = significant, ** = highly significant

TABLE 2. Mean fungal count of white patch samples for different locations in south-west Nigeria

S/N	Location	Mean viable count ($CFUg^{-1}$)
1	Osogbo	2.10×10^{4a}
2	Ife	2.20×10^{4a}
3	Ejigbo	1.48×10^{4b}
4	Ilesha	2.42×10^{4a}

Mean values with the same superscript "a" are statistically comparable but significantly different from superscript "b"

TABLE 3. Mean fungal count of white patch samples for different economic trees in south-west Nigeria

S/N.	Tree type	Mean viable count ($CFU\ g^{-1}$)
1	Cocoa	1.90×10^{4b}
2	Kola-nut	2.76×10^{4a}
3	Sweet orange	1.54×10^{4b}

Mean values with the same superscript "b" are statistically comparable but significantly different from superscript "a"

fungal species diversity was observed from samples from sweet orange trees.

DISCUSSION

The dry scaly nature of the whitish patch lesions and the localised pattern of spread on the outer bark of the affected fruit trees (Plate 1) in the study plantations depicted possible fungal attack. In fact, high fungal densities were observed in the samples across locations (Fig. 1). The observed high fungal densities in the scraping samples of the affected tree parts could be attributed to the cultivation pattern (Asogwa *et al*., 2011), reliance on crop propagation from the wild varieties and low

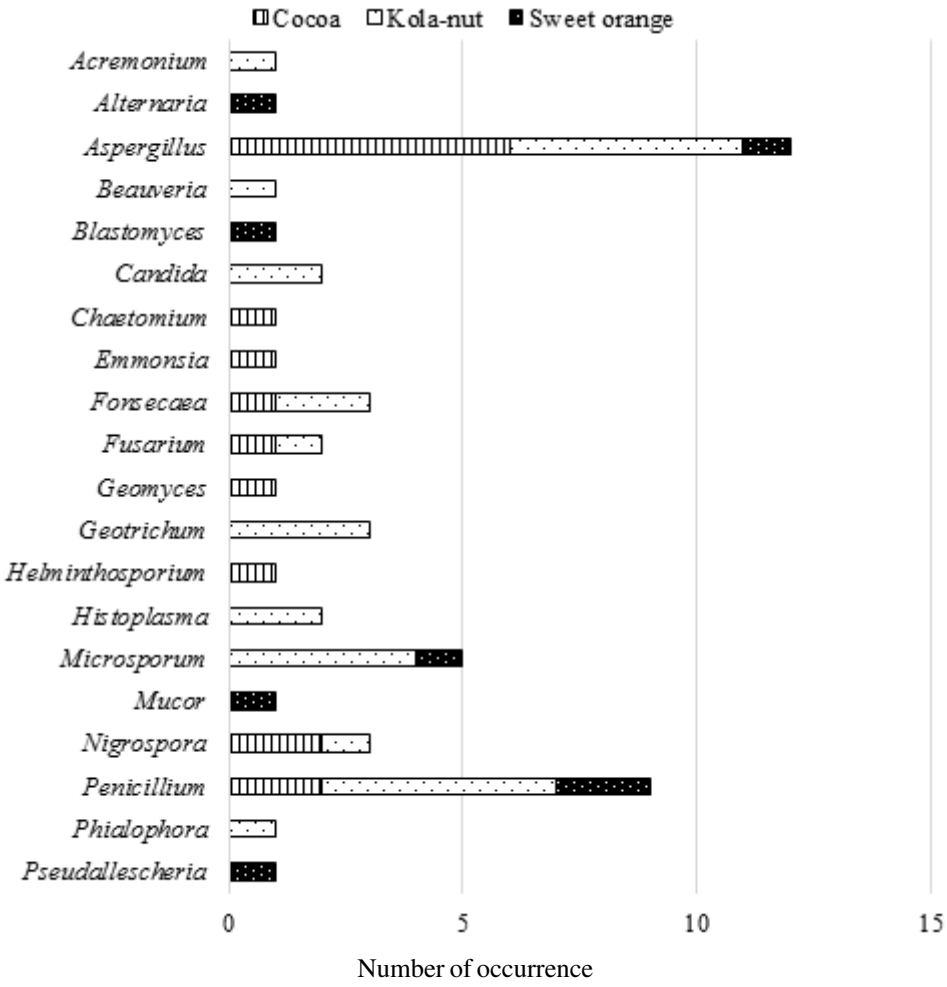

Figure 2. Fungal diversity of white patches from trunk of cocoa, kola-nut and sweet orange trees.

resistance to diseases as the plants age (Etebu and Nwauzoma, 2014; Bailey *et al*., 2016; Surujdeo-Maharaj *et al*., 2016).

The differences in fungal populations among various tree types at the different locations may be attributed to differences in environmental factors such as the local climatic conditions, e.g. wind, rainfall and relative humidity (Table 1). The comparatively lower fungal intensity observed in Ejigbo plantations than in Osogbo, Ife and Ilesha plantations (Table 2) could be attributed to the direction of movement of dust-laden wind, which blows from the Sahara Desert over West Africa, into the Gulf of Guinea (Oladele, 2007; Minka and Ayo, 2014). Ejigbo is farther north of the other three locations.

In the present study, kola-nut trees harboured more fungal load in their whitish patch lesions compared to cocoa and sweet orange trees (Table 3). Fungal exposure to ineffective concentrations of the preformed antifungal compounds, delayed or lack of production of induced antifungal compounds, fungal resistance to the plant's defense mechanisms or combination of any of these might be responsible for the variation in fungal load (Morrissey and Osbourn, 1999; Dixon,2001; Santoso *et al*., 2017; Tiku, 2018). These fungal load could be found in the

TABLE 4. Species distribution of fungi associated with white patches on the trunks of cocoa, kola-nut and sweet orange trees in south-west Nigeria

Sample location	Cocoa		Kola-nut		Sweet orange	
	Fungal species	No. of occurrence	Fungal species	No. of occurrence	Fungal species	No. of occurrence
Osogbo	*Chaetomium globosum*	1	*Beauveria bassiana*	1	*Microsporum nanum*	1
	Emmonsia crescens	1	*Candida albicans*	1		
	Geomyces pannorum	1	*Candida parapsilosis*	1		
	Penicillium verrucosum	1	*Histoplasma capsulatum*	1		
			Microsporum audouinii	1		
			Microsporum nanum	1		
			Penicillium verrucosum	2		
Ife	*Aspergillus nidulans*	1	*Aspergillus nidulans*	1	*Mucor* sp.	1
	Helminthosporium sp.	1	*Aspergillus niger*	1	*Penicillium verrucosum*	1
	Penicillium verrucosum	1	*Geotrichum candidum*	1		
			Penicillium verrucosum	1		
Ejigbo	*Aspergillus niger*	4	*Acremonium murorum*	1	*Pseudallescheria boydii*	1
	Nigrospora sp.	1	*Fonsecaea pedrosoi*	1		
			Geotrichum candidum	1		
Ilesha	*Aspergillus niger*	1	*Aspergillus niger*	3	*Alternaria alternata*	1
	Fonsecaea pedrosoi	1	*Fonsecaea pedrosoi*	1	*Aspergillus niger*	1
	Fusarium oxysporum	1	*Fusarium oxysporum*	1	*Blastomyces dermatitidis*	1
	Nigrospora sp.	1	*Geotrichum candidum*	1	*Penicillium verrucosum*	1
			Histoplasma capsulatum	1		
			Microsporum audouinii	1		
			Microsporum ferrugineum	1		
			Nigrospora sp.	1		
			Penicillium verrucosum	2		
			Phialophora verrucosa	1		

whitish patch samples from the affected kola-nut, cocoa and sweet orange trees.

We noticed that there was absence of pods on the areas covered by the whitish patches on cocoa trees. The powdery nature of the whitish patches formed by the fungal mycelia had potential for prevention of the development of flowers from the affected spots on cocoa trees or cause permanent damage to the flower cushions (Chavez *et al*., 2010; Toledo-Hernández *et al*., 2017), thereby leading to drastic yield and economic losses (Fisher *et al*., 2012). In contrast, fungal presence appeared benign on the trunks and branches of kola-nut and sweet orange trees. Although the exact damage caused by these fungi to the affected kola-nut and sweet orange trees cannot be ascertained, due to lack of physical damage, unlike cocoa which show absence of pods on the affected parts, it might signal a reduction in the production of constitutive plant compounds with antifungal activity in the affected areas as part of the plant's aging process or nutrient deficiency (Minyaka *et al*., 2017; Pusztahelyi *et al*., 2017).

The predominance of *Aspergillus* and *Penicillium* among the general obtained from the samples (Fig. 2) from the study fruit trees, may be attributed to their fungi ability to produce spores that are resistant to various environmental stressors (Bukar *et al.*, 2009). Besides, these two are filamentous fungi with autophagic mechanism for survival nutrient-deficient environment (Richie *et al*, 2007; Pollack *et al*., 2009; Bartoszewska *et al*., 2011).

Aspergillus niger and *Penicillium verrucosum*, unlike *Phytophthora* spp. (Mvondo *et al*., 2017) and *Moniliophthora* spp. (Patrocínio *et al*., 2017), which are established host-specific pathogens of woody plants, are generally found in a saprophytic or endophytic relationship with the plant hosts (Busby *et al*., 2016; Deng and Cao, 2017). Nevertheless, their phytopathogenic potential can manifest in plants with age-induced stress or nutrient deficiency condition (Sánchez-Hervás *et al*., 2008; Bartoszewska *et al*., 2011). Further investigations are required to understand the response of aged woody plants to microbial invasion. In the meantime, appropriate containment measures are required to prevent further spread into unaffected contiguous plantations.

Variability of the fungal communities in composition and structure in the samples from the three trees and across the four locations (Table 4) could be attributed to the fungal spore size and the degree of mycelial fragmentation; and subsequent aerosolisation (Adhikari *et al*., 2009; Gralton *et al*., 2011; Barberán *et al*., 2015; Spilak *et al*., 2015). Most of the isolated fungal species are important causative agents of postharvest diseases of cocoa, kola-nuts and citrus trees (Peever *et al*., 2005; Ezeibekwe *et al*., 2008; Singh *et al*., 2012; Anitha *et al*., 2014; Akinde *et al*., 2017). These key findings are noteworthy for improvement in sanitation and overall management of old plantations.

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