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Incidence of Filamentous fungi in some food commodities from Ivory Coast

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ABSTRACT

This study surveyed important food crops consumed in Ivory Coast for fungi. To achieve this, the following local food items (attieke, cassava flakes, chili, gnangnan, haricot, melon, millet, okra, rice, white maize and yellow maize) were sampled from local markets (Adjame, Cocody and Youpougon) in Abidjan, Ivory Coast. These food crops were screened for fungal contaminants, and each sample was serially diluted to a concentration of 10^{-5}. One hundred microliters of each mixture at 10^{-3} 10^{-4} and 10^{-5} were inoculated onto potato dextrose agar (PDA), Czapek yeast agar (CYA), and malt extract agar (MEA). The isolates were identified using morphological characters and confirmed by PCR with the internal transcribed spacer 1 and 4 primers (ITS1 and ITS4). A total of 227 isolates were morphologically identified and confirmed to be in the genera *Aspergillus* (54.9%), *Penicillium* (23.3%) and *Fusarium* (14.3%). Few isolated species were identified as *Alternaria, Chaetomium, Cladosporium, Epicoccum, Emerica, Rhizopus* and Tr*ichoderma* spp. The highest mean fungal load of 5.9 $\log_{10}$ CFU/g was found in maize. Confirmed isolates were dominated by *Aspergillus* species which were frequent in cassava flakes, chili, gnangnan, haricot, rice and yellow maize. *Penicillium* species were found to be frequent in chili, haricot and rice, while *Fusarium* species highly prevalent in melon and millet. Isolates from food commodities in this study were grouping with known toxigenic fungal species.

1. Introduction

Filamentous fungi are the major food contaminants in the world. They are capable of growing on a variety of food crops including like cereals [1], oil seeds [2], roots and tubers, herbs and medicinal plants [3,4], legumes [5], fruits and their products [6]. Invasion by various fungi may result in the deterioration of quality, physical-chemical changes, discoloration of food, and toxin production [7,8]. In Ivory Coast, the presence of fungi species has been reported in food commodities. According to Djossou [9], the most common *Aspergillus* species in Ivory Coast are *A. fumigatus, A. niger*, and *A. tubingensis*. They also found *Aspergillus nigri* in 52% of the coffee beans, with a 20% chance of finding ochratoxin A (OTA) at concentrations ranging from 0.3 to 56 g/kg. *P. chrysogenum* was detected in attieke from Abidjan, Ivory Coast by Jonathan-Segun [10].

Fungi produce mycotoxins that can have adverse effects on animals and humans [11]. Main mycotoxins are produced by five major fungi genera which include *Aspergillus, Penicillium*, *Fusarium, Alternaria* and *Trichoderma* [12,13]. Some of the major toxins produced by these genera include aflatoxins, ochratoxins, deoxynivalenol fumonisins, trichothecenes, and zearalenone. These fungi and their toxins are prevalent in various food commodities from the African continent. Human and animal effects of mycotoxins include nephrotoxic, immunotoxic, teratogenical and mutagenic conditions [14].

Several abiotic factors are necessary for the growth of fungi and the production of mycotoxins. Some of these factors include temperature, humidity, pH and water [14]. These conditions are prevalent in Africa where with poor food storage infrastructure, food commodities are highly exposed to fungi infection. There are limited studies on fungal contamination of food commodities in Ivory Coast [9,15,16]. Therefore, the aim of this study is to investigate fungal contaminants of food commodities sold in some local food markets in Abidjan, Ivory Coast.

2. Materials and methods

2.1. Sample

In this study, a total of 70 food commodities were screened for fungal contamination. Samples were randomly collected and purchased from three local markets (Adjame, Yopougon and Cocody) in Abidjan, Ivory

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Coast as shown in Table 1. Samples were collected based on the availability of the product during the sampling process; some had one sample per market, while some had seven samples per market. Despite these irregularities, each sample had at least one representative from each market, with the exception of cassava flakes and okra, which were only collected from the Adjame market. Fungal culture was isolated from 34, 19 and 17 food commodities from Adjame, Yopougon and Cocody respectively.

Approximately 100 g of individual samples were collected into ziplock bags. Except for "attieke," which was purchased pre-packaged, each sample was thoroughly mixed before being collected into these bags to obtain a representative sample of the food commodities. They were then transported to the laboratory at the University of Johannesburg, South Africa for further processing. The Sample was ground into smaller particles using a mechanical blender. The blender was sterilized in between samples with a 70% ethanol. Grind sample was then stored at −80 °C until further analysis.

2.2. Fungal isolation and enumeration

Fungal isolation and enumeration was done as described by Iheanacho [17] with some modifications of not using Ohio Agricultural Station agar (OAESA) and the plates were incubated at 25 °C in this study not 30 °C. For culture preparation, 1 g of each sample was weighed into a test tube containing 10 ml of sterile ringer's salt solution and vortexed. Each sample was then serially diluted to 10^{-5}. One hundred microliters of each mixture at 10^{-3} 10^{-4} and 10^{-5} was inoculated onto potato dextrose agar (PDA), (potato extract, 4 g/L, dextrose 20 g/L and agar 15 g/L), Czapek yeast agar (CYA), and malt extract agar (MEA). Inoculated plates were then incubated at 25 °C for 5–7 days. Fungal colonies were examined and counted from the different media plates using a colony counter (Gallenkamp, England) in the range of 30–300. The results were expressed as colony-forming units per gram (CFU/g).

CFU/g = Numbers of colonies x reciprocal of the dilution factor/plating volume (1 ml). Single spore colonies were subsequently obtained by sub-culturing on PDA, CYA, and MEA and incubated at 25 °C for 7 days.

2.3. Fungal identification

Fungal culture on PDA, CYA, and MEA was putatively identified based on morphological characters such as colony structure, colour and formation [18,19]. Further morphological characterization of pure colonies was done by mounting mycelium on slides and stained with lactophenol cotton blue. Slides were then observed under an optical microscope (Olympus CX-40, micro-Instruments, New Zealand Ltd) at a magnification of 400**X**.

Further identification was done using DNA sequences. For this purpose, fresh mycelium from pure culture was collected into ZRbashing bead lysis tube for use in extracting genomic DNA. This was done with Quick-DNA™ fungal/bacteria miniprep kit (Zymo Research, The Epigenetics Company, USA) following the manufacturer's instructions. Extracted DNA was then quantified using the ND-1000 spectrophotometer (NanoDrop Technologies) and adjusted to a working concentration of approximately 50 ng/μL. PCR was then done using quantified DNA to amplify sections of the internal transcribed spacer (ITS) regions (including 5.8S rRNA gene). The primer combination used was ITS1 (5′-TCC-GTA-GGT-GAA-CCT-GCG-G-3′ (forward)) and ITS4 (5′- TCC-TCC-GCT-TAT-TGA-TAT-GC-3′ (reverse) [20]. PCR reagents included deoxynucleotide triphosphate (dNTP) (Fermentas life Science, Lithuania). The reaction mixture was a 5 μL $MgCl_2$, 0.5 μL of each primer, 0.5 μL Mytaq, 5 μL buffer, and deionized water. PCR was done on an Eppendorf 96-well Thermocycler (Eppendorf, USA) with an initial denaturation step at 95 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 1 min, an annealing step at 55 °C for 45 s, an extension of primer at 72 °C for 1 min and a final extension at 72 °C for 5 min.

Successful PCR amplification was confirmed by staining 4 μL of PCR product with 1 μL of GelRed (Biotium Inc.) nucleic acid dye and ran on a 2% agarose gel. A DNA molecular ruler (100 bp ladder; Fermentas O'Gene Ruler) was included to determine the base-pair length of DNA amplicons. Generated bands were visualized under Gel IX imager 20–2.8 M Pixel (Bio Olympics, CA, USA) ultraviolet (UV) transilluminator at a wavelength of 312 nm. The DNA ZR-96 sequencing clean-up kit (Applied Biosystem, Foster City, CA) was used to purify amplicons.

Table 1
Food commodities sampled from three local markets in Ivory Coast.

Samples	Scientific name	Adjame	Yopougon	Cocody	Total
Attieke (Steamed grated cassava)	*Manihot esculentus*	3	2	2	7
Cassava flakes	*Manihot esculentus*	6	–	–	6
Chili pepper	*Capsicum annuum*	1	2	2	5
Gnangnan	*Solanum aethiopicum* L	2	2	2	6
Haricot	*Phaseolus vulgaris*	2	1	1	4
Melon	*Citrullus colocynthis*	2	2	1	5
Millet	*Pennisetum glaucum*	5	4	3	12
Okra	*Abelmoschus esculentus*	7	–	–	7
Danane Rice	*Oryza sativa*	2	2	2	6
White maize	*Zea mays I*	2	2	2	6
Yellow maize	*Zea mays I*	2	2	2	6
Total		34	19	17	70

2.4. DNA sequencing and phylogenetic analysis

Sequencing PCR reactions was done at a final volume of 11.5 μL with the same primers as used for PCR amplification. The reaction mixtures included 2.5 μL sequencing buffer, 4 μL PCR grade water, 0.5 μL BigDye terminator cycle sequencing kit (Applied Biosystem, Foster City, CA), 0.5 μL each of ITS1 and ITS4 introduced into separate Eppendorf tubes and 4 μL of the purified PCR product. The sequencing reaction was done on the Eppendorf 96-well Thermocycler using the PCR programme as utilized with the normal PCR. DNA ZR-96 sequencing clean-up kit was then used to clean DNA amplicon and sequenced on Applied Biosystems™ 3730 x l DNA Analyzer (ThermoFisher Science, CA, USA). Sequences were downloaded and assembled using SeqMan Pro v. 15 (DNASTAR). Obtained sequences were blasted against the Gen Bank (http://www.ncbi.nlm.nih.gov/) with BLAST 2.2.31 as described by Altschul [21] to confirm the putative identity of isolates at a similarity index score of greater than 90%. A data set of sequences was also generated from Gen Bank by obtaining sequences with similarity index close to those of isolates from this study. These sequences were aligned using the online alignment Muscle 3.8.31 (doc) phylogeny. fr (www.phylogeny.fr/simple phylogeny. cgi) after which alignments were checked manually. Aligned sequences were then used to perform a Maximum likelihood analysis (ML) using PhyML3.1/3.0 aLRT (doc + aLRT) (www.phylogeny.fr/simple phylogeny.cgi) with the default GTR model. Generated phylogenetic tree using TreeDyn 198.3 (doc) (www.phylogeny.fr/simple phylogeny.cgi) was done and the identity of isolates was confirmed based on taxa grouping.

3. Results

From the food commodities sampled for fungal contamination, cereals had the highest count of fungi present (Table 2), while cassava products had the least contamination level. In white maize, the range was from 4.7 to 5.9$\log_{10}$ and yellow maize which range from 4.9 to

Table 2
Total fungal load and isolated fungal genera from Ivorian food commodities.

Food Samples	Market	+ve sample	CFU/g Range	Mean	Fungal genera
Attieke	Adjame	3	5.5–5.7	5.6	*Aspergillus, Penicillium,* and *Emerica.*
	Yopougon	2	5.0–5.3	5.2	*Aspergillus* and *Penicillium*
	Cocody	2	4.9–5.5	5.3	*Aspergillus, Penicillium* and *Rhizopus*
Chili	Adjame	1	4.0–5.0	4.6	*Aspergillus, Chaetomium, Fusarium,* and *Penicillium*
	Yopougon	2	4.0–5.2	5.9	*Aspergillus* and *Penicillium*
	Cocody	2	3.7–5.1	4.8	*Aspergillus, Chaetomium, Fusarium, Penicillium* and *Rhizopus*
Gnangnan	Adjame	2	3.3–5.0	4.8	*Aspergillus* and *Penicillium*
	Yopougon	2	3.0–5.4	4.9	*Aspergillus, Chaetomium* and *Penicillium*
	Cocody	2	3.4–5.5	4.3	*Aspergillus, Penicillium* and *Trichoderma*
Haricot	Adjame	2	4.5–5.4	5.1	*Aspergillus* and *Penicillium*
	Yopougon	1	4.6–5.4	5.1	*Aspergillus, Fusarium* and *Penicillium*
	Cocody	1	3.6–4.5	4.2	*Aspergillus, Chaetomium* and *Fusarium*
Melon	Adjame	2	5.5–5.8	5.7	*Aspergillus, Fusarium* and *Penicillium*
	Yopougon	2	4.8–5.5	5.5	*Aspergillus, Emerica* and *Penicillium*
	Cocody	1	4.7–5.5	5.3	*Aspergillus, Fusarium, Penicillium* and *Trichoderma*
Millet	Adjame	5	3.5–4.6	4.6	*Aspergillus, Alternaria, Epicoccum and Fusarium*
	Yopougon	4	3.3–5.3	4.6	*Aspergillus, Fusarium* and *Penicillium*
	Cocody	3	4.1–5.6	5.2	*Aspergillus, Cladosporium* and *Penicillium*
Rice	Adjame	2	3.9–5.6	5.2	*Aspergillus, Alternaria, Emerica, Fusarium* and *Penicillium*
	Yopougon	2	3.1–5.1	4.5	*Aspergillus, Alternaria, Chaetomium, Epicoccum, Fusarium and Penicillium*
	Cocody	2	4.0–5.1	4.7	*Aspergillus, Alternaria, Fusarium* and *Penicillium*
White maize	Adjame	2	4.8–5.9	5.4	*Aspergillus, Alternaria, Emerica, Rhizopus* and *Penicillium*
	Yopougon	2	4.7–5.9	6.1	*Aspergillus, Alternaria, Emerica* and *Fusarium*
	Cocody	2	5.0–5.9	5.6	*Aspergillus, Epicoccum* and *Fusarium*
Yellow maize	Adjame	2	4.9–5.7	5.4	*Aspergillus, Chaetomium, Epicoccum, Fusarium, Penicillium* and *Rhizopus*
	Yopougon	2	5.4–5.7	5.5	*Aspergillus, Alternaria, Epicoccum, Fusarium*
	Cocody	2	5.2–5.6	5.5	*Aspergillus* and *Cladosporium*
	Adjame	5	4.7–5.5	4.6	
Cassava flakes	Adjame	7	3.5–5.4	5.0	*Aspergillus* and *Penicillium*
Okra	Adjame	7	3.5–5.4	5.0	*Aspergillus, Cladosporium* and *Penicillium*

5.7log$_{10}$, melon 4.7–5.8 log$_{10}$, *attieke* (4.9–5.7log$_{10}$), millet (3.3–5.6 log$_{10}$) and rice (3.1–5.6 log$_{10}$). High contamination levels were also detected in haricot 3.6–5.4 log$_{10.}$ Samples having low fungal load includes, gnangnan and dried okra. The fungal load (CFU/g) of the species were extremely variable among the markets. The fungal load (CFU/g) recorded in Cocody ranged from 3.4 to 5.9 log$_{10}$ followed by 3.3–5.9 log$_{10}$ CFU/g in Adjame and 3.0–5.9 log$_{10}$ CFU/g in Yopougon.

Using morphological characterization, a total of 227 fungal species were confirmed from the 70 food commodities considered in this study (Table 3). These include fungi belonging to genera *Aspergillus, Fusarium* and *Penicillium*. *Aspergillus* species were found in 92% of analysed food commodities and 121 (53%) of the main contaminants recovered in this study followed by *Penicillium* 40 (18%) isolates and *Fusarium* species 27 (12%). Incidence rate of 17% (39 isolates) was recorded for other genera, which included *Alternaria*, *Cladosporium, Chaetomium, Epicoccum, Emerica, Tricoderma* and *Rhizopus*.

In terms of prevalence, *Aspergillus niger* (30 isolates) was the most common contaminant recovered from the analysis, followed by *Aspergillus flavus* (24 isolates) and *Aspergillus fumigatus* (15 isolates). The highest levels of *A. niger* contamination were found in rice, gnangnan, and cassava flakes. Furthermore, A. flavus was found more frequently in white maize than in other analysed cereals in this study. Although *A. niger* and *A. flavus* were found in high concentrations in chilli pepper, gnangnan, haricot, danane rice, and white maize, these species were not found in melon, millet, okra, or yellow maize. Millet was found to contain 50% of the isolated *A. clavatus* in this study. Other less frequently isolated *Aspergillus* species that contaminated the samples include *A. aculeatus*, *A. candidus, A. ochraceus, A. parasiticus, A. tamarii, A. terreus,* and *A. tubingensis*.

The highest recorded *Penicillium* species in this study were *P. phirophilum* and *P. restricum,* with an incidence rate of 23% (9 isolates) and 20% (8 isolates), respectively. *P. restricum* was found to be more abundant in Chili, followed by haricot than in other samples (Table 3). Despite the fact that *P. restricum* and *P. phirophilum* were the most isolated from the analyzed samples; their absence was noted in food commodities like cassava flakes and *attieke*. Also, 75% of all isolated *P. crustosum* were discovered in rice. *Penicillium* species discovered at a lower level include *P. brevicompactum, P. chrysogenum, P. citrinum, P. expansum, P. griseofulvum, P. italicum,* and *P. paneum*.

This survey also revealed the presence of *Fusarium* species, which occur at a low rate, with 27 isolates from the samples tested. *F. proliferatum* was found more frequently, followed by *F. oxysporum*, then *F. solani,* and *F. graminearum*. In millet, *F. proliferatum* and *F. oxysporum* were common, whereas *F. graminearum* was prevalent in rice. In addition, *F. proliferatum* and *F. solani* were found more frequently in haricot samples than in other samples. Chili, millet, and rice, on the other hand, are among the samples that contain both *F. proliferatum* and *F. solani*. Other isolated *Fusarium* species with low contamination levels include *F. culmorum, F. graminearum, F. monoliforme, F. oxysporum*, and *F. poae*.

As shown in Table 4, Adjame had 96 fungal isolates, Yopougon had 67 isolates, and Cocody had 64 isolates. In Adjame, millet, okra, and white maize were the samples with the most contamination. Rice and millet had the highest isolate records in Yopougon, with 13 (19%) and 11 (16%) isolates, respectively, whereas in Cocody, chili and rice had 11 (17%) isolates, millet had 10 (16%) isolates, and yellow maize had 10

Table 3
Occurrence of fungal species in food commodities from Ivory Coast.

Isolates	*Attieke*	Cassava	Chili	*Gnangnan*	*Haricot*	Melon	Millet	Okra	Rice	Wmaize	Ymaize	Total
***Aspergillus* species**												
A. aculeatus	–	–	–	–	–	2	2	1	2	1	3	11
A. candidus	–	–	–	–	–	–	2	1	–	–	–	3
A. clavatus	–	1	1	–	–	2	5	–	1	–	–	10
A. flavus	3	1	3	3	3	–	2	1	2	4	2	24
A. fumigatus		–	1	3	2	2	–	2	3	1	1	15
A. niger	2	4	3	3	3	2	–	4	4	2	3	30
A. ochraceus	–	–	–	–	–	–	2	–	–	1	–	3
A. parasiticus	–	–	3	1	1	–	3	2	1	–	–	11
A. tamarii	–	–	–	–	–	–	1	1	–	–	–	2
A. terreus	–	–	1	1	–	1	3	–	–	2	–	8
A. tubingensis	–	–	1	–	–	–	–	1	1	–	1	4
***Penicillium* species**												
P. brevicompatum	–	–	–	–	–	1	–	–	–	–	–	1
P. chrysogenum	–	1	–	–	–	1	1	–	–	–	–	3
P. citrinum	1	–	–	1	–	–	–	–	–	–	–	2
P. crutosum	–	–	–	1	–	–	–	–	3	–	–	4
P. decumbens	–	–	–	–	1	–	1	1	–	1	–	4
P. expansum	–	–	–	–	–	–	1	–	1	–	–	2
P. griseofulvum	–	1	–	–	–	–	–	–	–	–	–	1
P. italicum	–	–	–	–	–	–	1	–	–	–	–	1
P. oxalicum	–	–	–	1	–	–	1	–	1	1	–	4
P. panuem	1	–	–	–	–	–	–	–	–	–	–	1
P. phirophilum	–	–	1	1	2	1	–	1	1	1	1	9
P. restricum	–	–	4	–	2	–	1	–	–	–	1	8
***Fusarium* species**												
F. culmorum	–	–	–	–	–	–	–	–	1	1	–	2
F. graminearum	–	–	–	–	–	–	1	–	2	1	1	5
F. moniliforme	–	–	–	–	–	–	–	–	–	–	1	1
F. oxysporum	–	–	–	–	–	1	3	–	–	1	1	6
F. poae	–	–	–	–	–	–	1	–	–	–	–	1
F. proliferatum	–	–	–	–	2	1	3	–	1	–	–	7
F. solani	–	–	1	–	2	–	–	–	2	–	–	5
Other identified species												
A. alternata	–	–	1	–	–	–	1	–	3	2	1	8
E. nidulan	1	–	–	–	–	1	1	–	1	2	–	6
E. nigrum	–	–	–	–	–	–	1	–	1	1	2	5
Cladosporium sp.	–	–	–	–	–	–	1	1	–	–	1	3
C. brasilenses	–	–	3	2	1	–	–	–	1	–	1	8
R. stolonifer	3	–	1	–	–	–	–	–	–	1	2	7
T. lixii.	–	–	–	1	–	1	–	–	–	–	–	2
	11	8	24	18	19	16	38	16	32	23	22	227

Note: Total number of *Aspergillus* species is 121; *Penicillium* species (40); *Fusarium* species (27) and other identified species (39) which includes *Alternaria alternata, Cladosporium* species*, Chaetomium brasilenses*'*Emerica. Nidulans, Epicoccun nigrum, Rhizopus stolonifer* and *Trichoderma lixii.*

(16%) isolates (10%). In all markets, *Aspergillus* species were found to be more common than other fungal genera.

CFU/g: Colony forming unit per gram of sample. +ve samples are the total number of samples contaminated with fungi per market.

Based on phylogenetic analysis as shown in Fig. 1, the isolates AAF105 and AAFP1015 were grouped in the same clade with confirmed *A. fumigatus* (MN634635) in the section *fumigati.* The isolates AAFP106 and AAFP1061 were grouped with *A. niger* (GU951769) under subgenus *circumdati*, section *nigri*. AAFP1014, AAF109 and AAF1011 were all grouped together with isolates within the section *terrei*. AAFP1020, AAFP101, and AAFP103 were grouped with what is known as *A. flavus* (MG659630) in the section *flavi*. In a similar analysis, AAF106 was grouped in the same clade as *P. crustosum* (MH345861) at the bootstrap value of 93% (Fig. 2). The isolate AAF1017 was grouped with a confirmed *P. chrysogenum* (JX156372) under the section *chrysogena*. In a clade having *P. brevicompactum* (KX067822), AAF1019 was grouped at 100% bootstrap value. Lastly, AAFP1014 was grouped with a confirmed *P. panuem* (KX664410) under section *roquefortorum*.

4. Discussion

Food safety is a global concern because many food commodities are contaminated with microorganisms that can cause harm if consumed. Fungi are among the important microorganisms that affect food commodities due to their ubiquitous nature [7]. As a result, the presence of fungi in Ivory Coast food commodities was investigated in this study. Because information on the distribution and contamination levels of fungi in Ivory Coast is limited, this study focused on the identification of fungi associated with food commodities consumed in Ivory Coast in order to provide more information to reduce the problems caused by these microorganisms. Many of the analysed foods, including melon, haricot, okra, cassava, maize, millet, rice, and vegetables, were found to be susceptible to fungi. This study provided the first report on the presence of fungi in some food commodities such as gnangnan, millet, okra, melon, and chilli in Abidjan, Ivory Coast.

The most important and predominant toxigenic fungi isolated from this study include *Aspegillus* (54.9%) followed by *Penicillium* (23.3%) and *Fusarium* (14.3%). The high occurrence rate of *Aspergillus* agrees with several other pieces of research who reported the high incidences of *Aspergillus* species in grains and grains based products [22–24]. Occurrences of these species have been reported in various agricultural and agricultural-based products [24,25]. Most of these fungi are widely recognized as producers of mycotoxin in the field and under storage conditions [22]. The existence of these fungal genera in cereals could be due to post-harvest conditions such as storage, mode of transport, since most of the storehouses are not well ventilated, thus improving the growth of these toxigenic fungi [26]. Also, climatic conditions are of paramount importance in fungal contamination of food commodities

Table 4
Absolute and relative % frequencies of fungal isolates from Ivory Coast food commodities per markets.

Isolates	Attieke	Cassava	Chili	*Gnangnan*	*Haricot*	Melon	Millet	Okra	Rice	Wmaize	Ymaize
Adjame	(n = 3)	(n = 6)	(n = 1)	(n = 2)	(n = 2)	(n = 2)	(n = 5)	(n = 7)	(n = 2)	(n = 2)	(n = 2)
A. clavatus	–	1(16)	–	–	–	1(50)	2(40)	–	–	–	–
A. flavus	1(33)	1(16)	1(100)	1(50)	2(100)	–	–	1(14)	1(50)	2(100)	1(50)
A. fumigatus	–	–	–	1(50)	2(100)	–	–	2(29)	–	–	–
A. niger	–	4(66)	1(100)	2(100)	2(100)	1(50)	–	4(57)	1(50)	1(50)	–
A. parasiticus	–	–	–	–	–	–	2(40)	2(29)	–	–	–
Other *A.* spp	–	–	1(100)	–	–	1(50)	3(60)	4(57)	1(50)	1(50)	1(50)
P. chrysogenum	–	1(16)	–	–	–	1(50)	1(20)	–	–	–	–
P. decumbens	–	–	–	–	1(50)	–	1(20)	1(14)	–	1(50)	–
P. phirophilum	–	–	–	–	1(50)	–	–	1(14)	1(50)	1(50)	1(50)
P. restricum	–	–	1(100)	–	1(50)	–	–	–	–	–	1(50)
Other *P.* spp	–	1(16)	–	2(100)	–	–	1(20)	–	1(50)	1(50)	–
F. oxysporum	–	–	–	–	–	–	2(40)	–	–	–	1(50)
F. proliferatum	–	–	–	–	–	1(50)	1(20)	–	–	–	–
Other *F.* spp	–	–	1(100)	–	–	–	1(20)	–	1(50)	–	–
R. stolonifer	2(66)	–	–	–	–	–	–	–	–	1(50)	1(50)
Other spp	1(33)	–	1(100)	–	–	–	2(40)	1(14)	2(100)	2(100)	2(100)
	Attieke	**Cassava**	**Chili**	***Gnangnan***	***Haricot***	**Melon**	**Millet**	**Okra**	**Rice**	**Wmaize**	**Ymaize**
Yopougon	**(n = 2)**	**(n = 0)**	**(n = 2)**	**(n = 2)**	**(n = 1)**	**(n = 2)**	**(n = 4)**	**(n = 0)**	**(n = 2)**	**(n = 2)**	**(n = 2)**
A. clavatus	–	–	–	–	–	1(50)	2(50)	–	–	–	–
A. flavus	1(50)	–	2(100)	1(50)	1(100)	–	1(25)	–	1(50)	–	1(50)
A. fumigatus	–	–	–	1(50)	–	1(50)	–	–	2(100)	–	–
A. niger	1(50)	–	–	–	–	1(50)	–	–	2(100)	1(50)	1(50)
A. parasiticus	–	–	2(100)	1(50)	–	–	–	–	1(50)	–	–
Other *A.* spp	–	–	–	1(50)	–	1(50)	3(75)	–	1(50)	1(50)	–
P. oxalicum	–	–	–	1(50)	–	–	1(25)	–	–	–	–
P. phirophilum	–	–	1(50)	–	1(100)	1(50)	–	–	–	–	–
P. restricum	–	–	1(50)	–	1(100)	–	–	–	–	–	–
Other *P.* spp	1(50)	–	–	–	–	–	–	–	1(50)	–	–
F. graminearum	–	–	–	–	–	–	1(25)	–	1(50)	1(50)	1(50)
F. proliferatum	–	–	–	–	1(100)	–	2(50)	–	–	–	
Other *F.* spp	–	–	–	–	1(100)	–	1(25)	–	1(50)	1(50)	1(50)
A. alternata	–	–	1(50)	–	–	–	–	–	1(50)	1(50)	1(50)
Other spp	–	–	–	2(100)	–	1(50)	1(25)	–	2(50)	1(50)	2(100)
	Attieke	**Cassava**	**Chili**	***Gnangnan***	***Haricot***	**Melon**	**Millet**	**Okra**	**Rice**	**Wmaize**	**Ymaize**
Cocody	**(n = 2)**	**(n = 0)**	**(n = 2)**	**(n = 2)**	**(n = 1)**	**(n = 1)**	**(n = 3)**	**(n = 0)**	**(n = 2)**	**(n = 2)**	**(n = 2)**
A. aculeatus	–	–	–	–	–	1(100)	1(33)	–	1(50)	–	2(100)
A. clavatus	–	–	1(50)	–	–	–	1(33)	–	–	–	–
A. flavus	1(50)	–	–	1(100)	–	–	1(33)	–	–	2(100)	
A. fumigatus	–	–	1(50)	1(100)	–	1(100)	–	–	1(50)	1(50)	1(50)
A. niger	1(50)	–	1(50)	1(100)	1(100)	–	–	–	1(50)	–	2(100)
A. parasiticus	–	–	1(50)	–	1(100)	–	1(33)	–	–	–	–
Other *A.* spp	–	–	1(50)	–	–	–	3(100)	–	1(50)	1(50)	1(50)
P. expansum	–	–	–	–	–	–	1(33)	–	1(50)	–	–
P. restricum	–	–	2(100)	–	–	–	1(33)	–	–	–	–
Other *P.* spp	1(50)	–	–	1(50)	–	1(100)	–	–	2(100)	–	–
F. proliferatum	–	–	1(50)	–	1(100)	–	–	–	1(50)	–	–
F. solani	–	–	–	–	1(100)	–	–	–	2(100)	–	–
Other *F.* spp	–	–	–	–		1(100)	–	–	–	1(50)	–
R. stolonifer	1(50)	–	1(50)	–	–	–	–	–	–	–	–
Other spp	–	–	2(100)	1(50)	1(100)	1(100)	1(33)	–	1(50)	1(50)	1(50)

Note: Total number of fungal isolated from food commodities from Adjame market is 96, Yopougon (67) and Cocody (64). Values in the brackets are relative % frequency of occurrence, **n** is the number of analysed food samples per markets. Other A. spp (*Aspergillus* species) includes *Aspergillus candidus, Aspergillus tamarii, Aspergillus terreus* and *Aspergillus tubingensis*. Other *P.* spp (*Penicillium* species) includes *Penicillium brevicompatum, Penicillium citrinum, Penicillium crustosum, Penicillium griseofulvum, Penicillium italicum, Penicillium oxalicum, and Penicillium panuem.* Other *F.* spp *(Fusarium* species) includes *Fusarium culmorum, Fusarium moniliforme* and *Fusarium poae*. Other species includes *Cladosporium* specie, *Chaetomium brasilenses.' E. nidulans.' Epicoccun nigrum and Trichoderma lixii.*

[27].

The survival of fungal species on food products is well known and the use of microbiological techniques to determine the quality of the products is important. The population of isolated mycoflora was ranged between (3.0–5.9log$_{10}$ Cfu/g). Using three different media (CYA, MEA, and PDA) gave a wider spectrum of fungi. *Aspergillus* sp. (*A. niger, A. flavus, A. fumigatus* and *A. parasiticus*) isolated from this study are of toxicological important. *A. flavus* was one of the most frequently isolated species after *A. niger* in all the samples. The findings are in accordance with Désiré [28] research in Ivory Coast, which identified *A. flavus* and *A. niger*, then *A. versicolor* as the most isolated *Aspergillus* species from maize. The isolation of these toxigenic fungi is in accordance with the work several authors who discovered the enormous presence of *A. flavus* and *A. niger* as the major *Aspergillus* species in food commodities in Africa [29–32]. *A. fumigatus, A. flavi, A. niger, A. ochraceus* and A. *parasicticus* are known to be producers of Aflatoxins and ochratoxins [9, 33–36] which are carcinogens. The health implications of consuming such contaminated products are enormous especially in Africa where there is correlation between Hepatitis B and aflatoxins [30]. The co-occurrence of toxigenic fungi, as presented in this report, indicates how humans are exposed to these toxigenic fungi. This inappropriate could enhance possible health effects on consumers of the food [26].

Penicillium species are known to be ubiquitous and nutritionally undemanding as opportunist saprophytes and their identification from

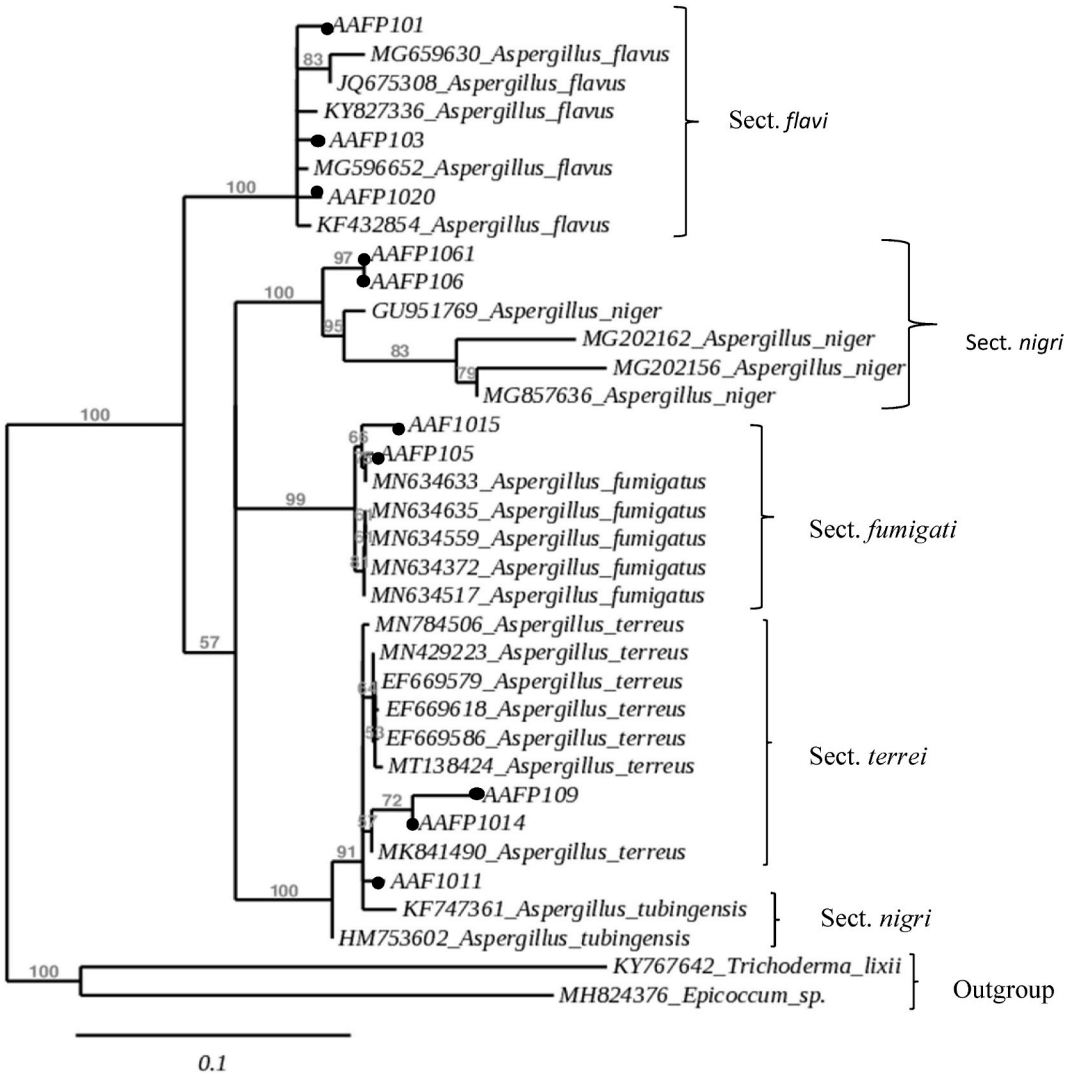

Fig. 1. Phylogeny for ITS data set showing the phylogenetic relation of species and section within the *Aspergillus* subgenus *Circumdati* and *Fumigati*. The isolated species from this study were indicated with bold round bullet and represented with code AAF. Bootstrap percentages of the maximum likelihood (ML) are presented at the nodes. The bar indicates the number of substitutions per site. The phylogram is rooted (outgroup) with *Epicoccum* species and *Trichoderma lixii*.

foods to species level has only been done in few studies. In this study we are able to isolate and identify *P. phirophilum* and *P. restricum* as the most frequently species. A slightly high level of contamination in chilli and rice *P. restricum* and *P. crustosum* were detected. Other species include *P. crustosum, P. chrysogenum, P. expansum, P. oxalicum* which are known to producers of roquefortine and *P. brevicompactum* produces mycophenolic acid also *P. italicum* produces tryptoquivalins [37]. Also *P. citrinum* and *P. verrucosum* are major producers of citrinin and OTA respectively [11,35]. Some of the mycotoxins that can be produced by the *Penicillium* species detected in this study as reported by Rundberge [37] include Penitrems, patulin, and chaetoglobosin. However, Ochratoxin A and patulin are the most important of all toxin produced by *Penicillium* species and regulations for these toxins have been imposed in many countries [38]. Occasionally, the maximum limits set by relevant food safety regulators have been exceeded by many of the toxins associated with these fungi [39]. The presence of *Penicillium* species in the food sample could indicate that the consumers might be at risk of exposure to acute or chronic toxicity when sufficient amounts of contaminated food are consumed.

Fusarium species confirmed at a low rate compare to *Aspergillus,* this means most *Fusarium* species are more active in the field than the stores [40]. Often, fungal growth can start at the field and during storages; a sharp detection of where contamination occurred is impossible [41]. Nonetheless, *Fusarium* species including *F. proliferatum, F. oxysporum*, and *F. solani* were isolated more frequently than other F. species especially in millet, haricot and rice whereas total absence of *Fusarium* species were recorded in *attieke*, cassava flakes, *gnangnan*. Similarly, a number of researchers have reported their presence in food commodities, particularly in Africa [16,22,42,43] and some of these species are mycotoxigenic which can pose a dangerous effect on consumers. *F. moniliforme* and *F. verticilloides* produces fumonisins [35,44]. *F. graminearum, F. culmorum* produces deoxynivalenol, nivalenol and zearalenone [33,35].

This study showed the minimal contamination of some food commodities by *Alternaria, Chaetomium, Cladosporium, Epicoccum, Emerica, Trichoderma,* and *Rhizopus* species which could be explained by the facts that these genera are less occurring in the environment compare to *Aspergillus, Penicillium* and *Fusarium.* Several of the species were confirmed in the Gen bank with previously identified *Aspergillus* species (*A. flavus, A. niger*, and *A. fumigatus*) by various researchers [45–47] as well as *P. brevicompactum, P. crustosum, P. chysogenum*, and *P. paneum* [48–51].

The phylogenetics deduced from the ITS genes showed the genetic association, clonal relationship and divergence among the *Aspergillus*

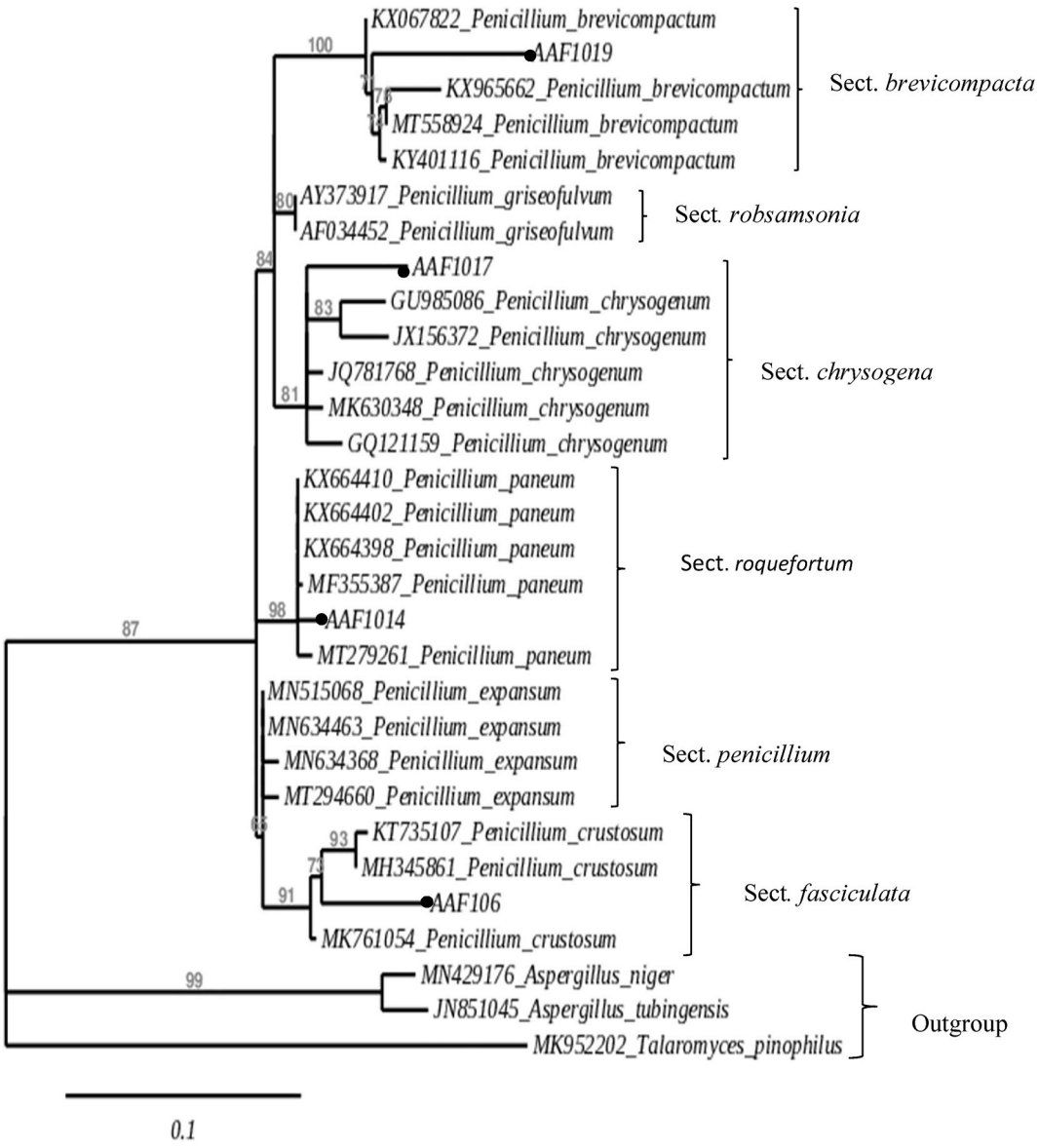

Fig. 2. Phylogeny for ITS data set showing the phylogenetic relation of species and section within the *Penicillium* subgenus *Aspergilloides* and *Penicillium*. The isolated species from this study were indicated with bold round bullet represented with code AAF. Bootstrap percentages of the maximum likelihood (ML) are presented at the nodes. The bar indicates the number of substitution per site. The phylogram is rooted (outgroup) with, *Aspergillus niger, Aspergillus tubingensis* and *Talaromyces pinophilisi*.

especially sub section *Circumdati* and *Fumigati*. Also a tree was drawn among the sub genus *Aspergilloides* and *Penicillium* under the genera *Penicillium.* The tree revealed the genetic variation among species which is important in basic and translational research [52]. The wide diversity of these fungi and variation in their relationships shows that fungal identification by conventional means must be complemented with molecular identification for proper identification [53].

Mycotoxigenic fungi have also been found to contaminate cereals, nuts, cowpea, cassava products and vegetables in their raw form and by-products obtained from three markets, which means none of the three markets is void of fungal species. Therefore, In Ivory Coast, more attention needs to be paid to the influence of fungi and their metabolites on food commodities. As presently, there is not enough research focus on that direction, which is detrimental to community health and the economy. The study recommends increased monitoring, more public awareness campaigns, and the availability of research equipment in regional laboratories.

5. Conclusions

The presence of toxigenic fungi with a diverse species where genera *Aspergillus, Penicillium,* and *Fusarium* were the most representative fungal population were recorded in this study. These three genera have economic and scientific importance recognized by scientific researches especially co-contamination of such fungi. The high incidences of toxigenic fungi co-contamination recorded in this study are cause for concern, particularly among toxigenic members of *Aspergillus* subgenus *circumdati* and *fumigati,* as well as *Penicillium* and *Fusarium.* Their occurrence in food commodities reveals a major problem that opens up multiple fields of study, including control approaches such as biological, chemical, and physical. Natural co-occurrences of unrelated fungal species have been found in many food samples, particularly cereals, increasing the severity of health-related problems caused by such contamination. Therefore, adequate quality control measures, good processing practices, transport and storage facilities must be adopted to curb the incidence of these micro-organisms in food commodities.

Finally, more research is needed in this country on the presence of toxigenic fungi in food commodities.

Declaration of competing interest

There is no conflict of interest.

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