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Fungal Diversity and Mycotoxin Contamination of some selected Food Commodities from Ivory Coast

A dissertation submitted to the

Faculty of Science, University of Johannesburg, South Africa,

In fulfilment of the Requirement of an award of Masters of Science:

Biotechnology

By

Adeola Oluwakemi Aasa

Student number: 218088043

Supervisor: Prof. PB Njobeh

Co-supervisor: Dr. FF Fru

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ABSTRACT

This study surveyed fungi and mycotoxins in important food crops consumed in Ivory Coast. To achieve this, the following local food items (attieke, cassava flakes, chilli, gnangnan, haricot, melon, millet, okra, rice, white maize and yellow maize) were sampled from local markets (Adjame, Cocody and Youpougon) in Abidjan, Ivory Coast. They were screened for fungal contamination based on morphological characters and confirmed by PCR using the internal transcribed spacer 1 and 4 primers (ITS1 and ITS4). A total of 227 isolates were morphologically identified withisolates dominated by species within the genera *Aspegillus* (54.9 %) followed by *Penicillium* (23.3 %) and *Fusarium* (14.3 %). Few isolated species were confirmed in other genera which include *Alternaria, Chaetomium*, *Cladosporium, Epicoccum, Emerica, Rhizopus* and Tr*ichoderma* spp.The highest mean fungal load of 6.85 x 10^5 CFU/g was found in white maize while the lowest mean level of 4.39x10^4CFU/g was recorded in cassava flakes. The subset of isolates were identified using ITS1 and ITS4, as *Aspergillus* species found to be most frequentin cassava flakes, chilli, gnangnan, haricot, rice and yellow maize. *Penicillium* species were also found frequent in chilli, haricot and rice, while *Fusarium* species were frequent in melon and millet. A sample of each food commodities was subjected to mycotoxin analysis, 3-acetyldeoxynivalenol (3-ACDON), 15-acetyldeoxynivalenol (15-ACDON), aflatoxins (AFs): aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), and aflatoxin G_2 (AFG_2), ochratoxin A (OTA) and Toxin-2 (T-2) were confirmed. The overall limits of detection (LOD) and quantification (LOQ) ranged from 0.024 - 14.00 µg/kg and 0.13 - 47.33 µg/kg, respectively. Concentrations ofAFB_1 were from (0.04 - 17.52µg/kg), AFB_2 (<LOQ-39.20 µg/kg), AFG_1 (<LOQ - 3.89 µg/kg), AFG_2 (<LOQ-66.80µg/kg), total AFs (0.44 - 153.21 µg/kg), 3-ACDON (<LOQ-64.30µg/kg), 15-ACDON (<LOQ-40.40 µg/kg), OTA (0.17- 25.49µg/kg), and T-2 (<LOQ-32.23µg/kg). Co-occurrence of mycotoxins was established especially for AFs in attieke, cassava flakes, gnangnan, haricot, millet, rice, white maize and yellow maize. Other mycotoxins that co-occuring with AFs include 3-ACDON, 15-ACDON and T-2. This was mostly found in attieke samples, gnangnan, haricot, millet, okra and white maize.AFB_1 and OTA concentrations were higher than the maximum level of 2 µg/kg and 5 µg/kg respectively set by European Union. It is therefore important that farm produce should be regularly monitored to reduce fungal and mycotoxin contamination.

Keywords: Food safety, fungi, mycotoxin, UHPLC-MS, agricultural products, and Ivory Coast

DECLARATION

I, Adeola Oluwakemi Aasa declare that this dissertation is a product of my work under the supervision of Prof. Patrick B. Njobeh and that of Dr. Felix F. Fru submitted for the degree of Master of Sciencein Biotechnology at the University of Johannesburg. This work has not been submitted for any degree at any other university.

DEDICATION

This thesis is dedicated to Almighty God

My husband, Samson A. Aasa and my children, J. Toluwalade Aasa and S. Adeoluwa Aasa. Thank you for your persistence and love.

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Special thanks to my supervisors, Prof. P.B. Njobeh and Dr. F.F. Fru.

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I acknowledge my family; you all have been so real and supportive.

All glory to God for the strength and opportunity throughout the time of the program.

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LIST OF ABBREVIATIONS

3-ACDON	3-acetyldeoxynivalenol
15-ACDON	15-acetyldeoxynivalenol
AFs	Aflatoxins
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AOH	Alternariol
AME	Alternariol monomethyl ether
ASHC	Africa Soil Health Consortium
A_W	Water activity
PBS	Phosphate buffer saline
CFU	Colony forming unit
CIT	Citrinin
CYA	Czapek yeast agar
DNA	Deoxyribonucleic acid
DON	Deoxynivalenol
DMSO	Dimethylsulphoxide
dNTP	Deoxynucleotide triphosphate
EC	European Commission
EFSA	European Food Safety Authority
ESI	Electron ionization
EU	European Union

FAO	Food and Agriculture Organization
FBs	Fumonisins
FB_1	Fumonisin B_1
FB_2	Fumonisin B_2
g	Gram
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
ITS	Internal transcribed spacer
JECFA	Joint Expert Committee on Food Additives
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MEA	Malt extract agar
MRM	Multiple reactions monitoring
ML	Maximum likelihood analyses
NCBI	National Center for Biotechnology Information
OTs	Ochratoxins
OTA	Ochratoxin A
OTB	Ochratoxin B
OTC	Ochratoxin C
PDA	Potato dextrose agar
PCR	Polymerase chain reaction
R^2	Coefficient of determination
RNA	Ribonucleic acid
Sp.	Specie

Spp.	Species
TEA	Tetramic acid-tenuazonic acid
WHO	World Health Organization
ZEN	Zearalenone

UNITS

%	Percentage
CFU	Colony forming unit
g	Gram
µL	Microlitre
µg	Microgram
kg	Kilogram
µg/kg	Microgram/kilogram
mL	Millilitre
rpm	Revolution per minutes
nm	Nanometer
ng	Nanogram
M	Molar
v	Volume
ºC	Degree celsius
β	Beta
α	Alpha
γ	Gamma
kg	Kilogram

kV	Kilovolts
V	Volts

CHAPTER ONE
GENERAL INTRODUCTION

1.1 Background

Fungi are ubiquitous eukaryotic microorganism that can survive on dead and living things. They cannot manufacture their food, so they depend solely on their environments, with various surface materials such as animal, plant debris, soil and water (Hernandez and Matnez, 2018).The absorption of nutrients from their environment makes it difficult to separate fungi existence and the climate reaction(Wu *et al*., 2011). Fungal growth and mycotoxin production depend on various factors including climate, agricultural practices, type of substrate and geographical location (Gareis *et al*., 2001). Fungi in the quest for survival frequently invade plants with the help of threadlike structures (mycelia and hyphae) and in so doing, infect agricultural crops during sporulation and thereafter, secrete secondary metabolites, some being mycotoxins. Globally, foodspoilageis often caused by the presence of fungi which can produce secondary metabolites that have serious economic and health effects on humans (Avery *et al*., 2019). Food can be exposed to fungi and their metabolites through improper handling, harvesting, storage, packaging and distribution (Rawat, 2015).

Mycotoxins are secondary metabolites produced by some fungi, which can be found in food and feed (Vasconcelos de Medeiros *et al*., 2012). Mycotoxins are produced mainly by fungal genera *Aspergillus*, *Alternaria*, *Fusarium* and *Penicillium.*These genera are not only toxigenic; they are also pathogenic as they cause a number of diseases in both plants and animals. Contamination by these micro-organisms and the production of their metabolites can take place on the farm, during harvest, transportation and in storage(Bryden, 2009; Ukwuru *et al*., 2017) if the climatic conditions arefavourable.Generally, mycotoxins occur more frequently under tropical conditions.Therefore in most African countries, crops are susceptible to mycotoxins due to thetropical hot to humid conditions that favour fungal growth.Mycotoxins that occur frequently in food commoditiesinclude aflatoxins (AFs), ochratoxins (OTs), fumonisins (FBs), trichothecenes (TCs) and zearalenone (ZEN)and consumption by animalsand

humans may lead to diseases generally referred to asmycotoxicoses (Koppen *et al*., 2010).The effect of these diseases on the economy and health of animals and humans can be enormous (Inglis*et al*., 2013).

In Africa, fungal and mycotoxin food contamination is a chronic problem. Various food commodities are affected by different fungal strains, leading to serious economic losses, resulting from the reduction in food quality accompanied by a decline in healthy living conditions of the population (Streit *et al*., 2013; Clarke *et al*., 2014). However, information on the distribution and contamination levels of fungi and mycotoxins in African countries and especially Ivory Coast is scarce. In order to provide more information, to reduce the problems caused by these microorganisms and their metabolites, this study focused on identification of fungal and mycotoxin contamination associated with food commodities consumed in Ivory Coast. Many of these foods such as melon, haricot, okra, cassava, maize, millet, rice, and vegetables are susceptible to fungi and mycotoxin contamination.

1.2 Problem statement

Fungal contamination has become a threat to food safety and security in many parts of Africa. It has implications for food and feed safety (Jimoh and Kolapo, 2008). Most importantly, the productions of mycotoxins in these commodities make it an important consideration during food production (Bryden, 2007; Zain, 2011). Fungi and mycotoxins are commonly encountered in food commodities in Africa. This could be the result of inadequate infrastructure,which can lead to exposure of stored food to toxigenic fungal colonization (Aristil *et al.,* 2017). Food commodities that frequently harbor fungi and mycotoxin include cereals such as maize, rice sorghum, and millet. Other susceptible food commodities include legumes, vegetables, fruits, spices, and nuts etc. Most of these foods are consumed regularly especially in sub-sahara Africa. Such high dietary exposure to fungi can have an adverse effect on humans and animals. Due to the health impact of these mycotoxins on humans and animals (Yogendrarajah *et al*., 2013), mycotoxins have attracted global attention (Abdallah *et al*., 2018). More to their significance is that these mycotoxins are often reported in food (Darwish *et al.,* 2014; Nguyen*et al*.,

2017).Considering the health implications of fungal and associated mycotoxins in humans and animals, it is important to regularly monitor their exposure through food and feed.

1.3 Hypothesis

Fungi and mycotoxin are ubiquitous and they are regarded as common cause of food spoilage in Sub-Sahara Africa. It is hypothesized in this study that Ivorian food commodities such as attieke, cassava flakes, chilli, maize, millet, gnangnan, haricot, okra, and rice, can be contaminated with diverse fungi species that can produce various mycotoxins.

1.4 Research questions

In this study, the following research questions were addressed;

1.4.1 What are the fungal species commonly found in food commodities in Ivory Coast?

1.4.2 What is the level and frequency of fungal contamination in food commodities in that country?

1.4.3 What types of mycotoxins are present in these food commodities?

1.4.4 What are the mycotoxin contamination levels in these food commodities and do they meet regulatory standards enforced by the European Commission?

1.5 Aims and objectives

1.5.1 Aim

The overall aim of this study was to investigate the incidence and degree of contamination of fungi and mycotoxins occurring in some food commodities in Ivory Coast.

1.5.2 Objectives

To achieve the aim, the following objectives were addressed:

1. To establish the identities, frequency and levels of fungal contamination in selected food commodities from Ivory Coast.

2. To detect and establishthe mycotoxin contamination levels in similar samples.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter summarizes the literature on naturally occurring fungal and their secondary metabolites (mycotoxins) in food as well as the human health implication associated with these food contaminants. The role of environmental conditions necessary for the production of mycotoxins and measures adopted to inhibit the prevalence of fungal and mycotoxins in food are reviewed. In addition, the geography, climate, vegetation and staple foods in Ivory Coast are discussed to finalize this review.

2.2 Fungi

Fungi are ubiquitous eukaryotic organisms found in the soil, plants, animals and humans. They adopt different relationships with their host being saprophytic and parasitic to name a few (Leake, 2005; Zeilinger *et al.*, 2016) and found in sub-tropical, tropical and temperate environments (Mateo *et al*., 2011; Cabral *et al*., 2016). It is known that there are about 1.5 million fungal species in existence (O'Brien and Dietrich, 2005) and about 70,000 species are described and identified as either pathogen, toxin producers or both (Blackwell, 2011). Fungi presence in crops can result in the reduction in product yield and value (Bennett and Klich, 2003; Ban Koffi *et al.,* 2017) in the field, at harvest, during transportation or in storage. Food commodities including maize, rice, bean, yam, cassava, peanut, spices, and chillies are known to have been affected by toxigenic fungi (Abiala *et al.,* 2011). When these occur, mycotoxins are often produced with more than 25 % of world's agricultural products affected (Vasconcelos de Medeiros *et al.,* 2012). These mycotoxins include aflatoxins, citrinin, ochratoxins, fumonisins, and trichothecenes.

Exposure to mycotoxins by animals and humans can result in acute or chronic illnesses (Koppen *et al*., 2010). Long term exposure might result in damage to the immune system, nervous system, liver cells, modulates human immunity or even cause cancer in humans (Jolly *et al.,* 2008; Dragan *et al.,* 2010). Acute toxicity starts from exposure to high doses within a short period and such cases can resultin death

(Escriva *et al*., 2015). Due to the impact of mycotoxins on health and the economy, many countries have established food and feed regulatory regimes to monitor their occurrence. Maximum limits for different mycotoxins vary from one country to country in Africa (FAO, 2004).

Over 100 countries have developed specific limits for mycotoxins in foodstuffs (FAO, 2004; Wu and Mitchell, 2016; Eskola *et al*., 2018). These are exemplified in countries such as Ghana, South Africa, and Nigeria (FAO, 2004; Adetunji *et al.,*2014; Matumba *et al*., 2014; Agbetiameh *et al.,* 2018). For instance, the standards for aflatoxins in Ghana, for maize and groundnuts are 15 µg/kg and 20 µg/kg respectively (Agbetiameh *et al.,* 2018). In Nigeria, the acceptable regulatory standards for aflatoxins in foodstuffs is 20 µg/kg, while in Ivory Coast, 10 µg/kg is accepted for aflatoxins in feed (FAO, 2004). Also, in South Africa the acceptable maximum limit for AFB1 in all foodstuffs is 5 µg/kg, total aflatoxins (10 µg/kg), patulin(50 µg/kg) and aflatoxin M_1 (AFM_1) in milk(0.05 µg/kg) (FAO,2004). Developing countries often benchmark regulatory standards in developed countries (Matumba *et al*., 2015). Therefore, there is a need for frequent monitoring and assessment of these secondary metabolites based on conditions in developing countries (Deduke *et al*., 2012).

2.3 Occurrence of fungi in food commodities

Pathogenic fungi invade agricultural products and cause various modifications during developmental stages and after harvest (Olivera *etal*., 2014).Food types infected include cereals, fruits, spices, tubers and vegetables (Bottalico and Logrieco, 2001). The effects of fungal invasion on agricultural products include discolouration and unpleasant odour chemical and nutritional changes, loss of quality and production of mycotoxins (Fapohunda *et al*., 2012; Hossain *et al*., 2014). Researches from various countries in Africa have confirmed the presence of *Aspergillus, Fusarium* and *Penicillium* in food commodities. Tang *et al*. (2019) reported the occurrence of *Aspergillus* species in rice samples from Senegal .*Aspergillus flavus, A. niger, A. parasiticus, A. fumigatus, A. clavatus, A. ochraceus, A. tamari, A. candidus,* and *A. versicolor* were found in smoked or dried fish and peanut cake sold in markets Benin (Adjou *et al*. 2012; Adjovi *et al*., 2019). Incidences of *Aspergillus* species have also been reported on

ugba, ogi, ogi baba and iru inNigeria (Adekoya *et al*., 2017). Frimpong *et al.* (2019) confirmed the presence of *Aspergillus* and *Fusarium* species in fresh pepper consumed in Ghana and Nigeria.

Fusarium species are one of the dominant mycoflora isolated from peanut cake in the Benin Republic (Adjou *et al*., 2012). The occurrence of *Fusarium* species in spices (aniseed and rosemary) and spice products in Ghana was recorded by Ahene *et al*. (2011). Also, high incidences of *Penicillium* species have been reported on farm and storage maize with *P.notatum* being the most isolates recovered from this food crop (Makun *et al*., 2010; Egbuta *et al*., 2015). Incidences of *Penicillium* species have also been reported on smoked or dried fish in Cotonou, Benin (Adjovi *et al.,* 2019). A similar report by Tovide *et al.* (2018) confirmed the presence of *Penicillium* species in millet and sorghum produced in Benin. Adekoya *et al*. (2017) reported the occurrence of *Penicillium* species in ugba and ogi from Nigeria.

2.3.1 *Aspergillus*

Aspergillus species are filamentous fungi characterized by a unique spore-bearing structure, called the conidiophore (Fig 2.1) (Klich, 2009). They are ubiquitous and can grow on plants in the field, stored foods and animal feeds (Areo, 2017). They cause food spoilage and are exceptionally common in stored cereals, nuts, herbs and spices (Toldra *et al*., 2015; Paterson and Lima, 2017). They thrive better in sub-tropical and tropical conditions compared to temperate climates (Pitt and Hocking, 2009).

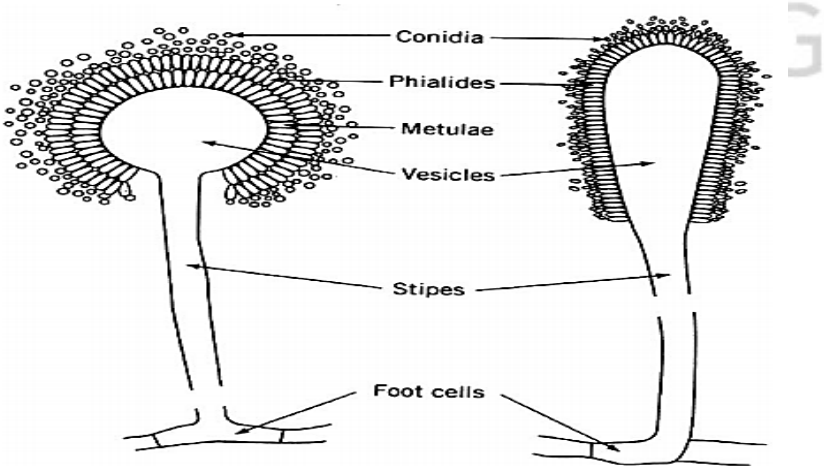

Figure2.1Microscopic structure of *Aspergillus* conidiophores (Source: Klich, 2009)

Some frequently isolated *Aspergillus* species include *A. clavatus, A.flavus, A. fumigatus, A. oryzae, A. niger,* and *A. parasiticus* reported as producers of aflatoxins (AFs), ochratoxins (OT), patulin (PAT) and fumonisins (FBs) where applicable. According to Djossou *et al.* (2015), the most prevalent *Aspergillus* species in Ivory Coast are *A. fumigatus, A. niger* and *A. tubingensis*with 52 % of *Aspergillus nigri* in coffee beans and ochratoxin A (OTA) detected at concentrations ranging from 0.3 - 56 µg/kg in 20% of analysed samples.

In another study in Ivory Coast by Boli *et al*. (2013), the predominant fungi isolated from the peanut butter samples selected from the nine communes of Abidjan District belonged to *Aspergillus* species with incidence rate ranging from 20.22 – 51.65%.*Aspergillus* species were found to be the predominant fungi isolated from Ivorian cowpea by Koffi-Nevry *et al*. (2013). Occurrence of *A. flavus*, *A. niger* and *A. parasiticus* were found in peanut paste consumed in Ivory Coast (Boli *et al*., 2020).In a study of Njobeh *et al.*(2009),*A.parasiticus* and *A.flavus* were found to be responsible for the production of mycotoxins in maize, beans and peanuts from Cameroon. These two species are commonly reported to be responsible for the production of aflatoxins. In that study,*A. fumigatus, A. niger*, *A. flavus* and *A. parasiticus* were the most prevalent *Aspergillus*species recovered from these commodities.

Aspergillus species are capable producing secondary metabolites on various food commodities. The most common toxins produced by the genus include aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2, AFM_1 AFM_2, AFQ_1, and AFL)(Bennett and Cahill, 2016) and ochratoxins(OTA, OTB, OTC, α, and β) (Arroyo-Manzanares *etal*., 2017). *Aspergillus* species produce aflatoxins at optimal a_w of 0.98 and 25 – 30 ºC (Mousa *et al*., 2016) and ochratoxins produce at 0.94 – 0.99 a_w and 25 - 32 ºC (Oliveira *et al*., 2019). Other important mycotoxins produced by *Aspergillus*species include 3-O-methylsterigmatocystin, cyclopiazonic acid, kojic acid, speradine A, tenuazonic acid and versicolorins (Frisvad *et al*., 2019). Dietary exposure to *Aspergillus* toxins can be carcinogenic, mutagenic in human (Coppock *et al*., 2018), nephropathy, immunosuppressive (Djossou *et al*., 2015). It can cause hormonal imbalance in children (Jonsyn-Ellis, 2012).

2.3.2 *Alternaria*

Alternaria species are mainly saprophytic fungi. One of the main features of *Alternaria* is the presence of longitudinal and transverse septa borne from inconspicuous conidiophores (Fig 2.2). The septa are conical thinning or beak at the apical end and produce large brown conidia, often formed in chains (Pitt and Hocking, 2009). *Alternaria* species have been reported to be responsible for the decay of cereals and vegetable products in the field, during processing and storage (Patriarca, 2016). *Alternaria* can withstand low temperatures, and therefore can thrive in temperate regions. Agricultural products affected by *Alternaria* include citrus fruits (Bottalico and Logrieco, 2001), eggplant, rice (Webley *et al.*, 1997), cereals, tomato fruits, bell pepper, olives, berries, and their derived products (Patriarca, 2016). Few reports on occurrence of *Alternaria* species in food commodities from Ivory Coast are available. However, a survey carried out by Boli *et al*. (2013), revealed the mean concentration of *Alternaria* species isolated from peanut butter in Adjame to be 21.05 ± 1, Yopougon (20.45 ± 1) while the total absent of these species was recorded in the samples collected from Cocody city in Ivory Coast.

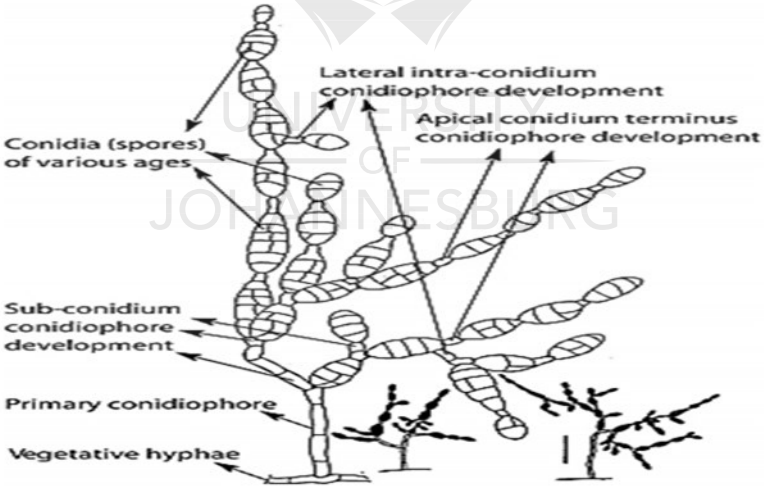

Figure2.2Typical hyphae with spores of *Alternaria* species (Source: Taralova *et al*., 2011)

According to Moretti andSarrocco (2016), the mycotoxins produced by *Alternaria* are divided into three main chemical groups: (1) The dibenzo-α-pyrones-alternariol (AOH), alternariol monomethyl ether (AME) and altenuene; (2) The tetramic acid-tenuazonic acid (TeA) and (3) the altertoxins having three main members including ATX I, ATX II and ATX III (Amatulli *etal.*, 2013).Altertoxins I – III are perylene quinone-type alternaria toxinsthat aremutagenic in nature especially ATX II. They have a 50-

fold mutagenic potential than AOH and AME (Fleck *et al*., 2012). AOH and AME are usually found in combination in the host. *Alternaria* produce altenuene at 25 ºC and 0.97 a_w(Sanchis and Magan, 2004) and tenuazonic acid produced by different strains at 0.98 a_w and 25 to 30 ºC (Oviedo *et al*., 2009). Other important mycotoxins produced by *Alternaria* include ALL toxins (*Alternaria f.sp. Lycopersici* toxins) and stemphyltoxin III. These toxins are produced in both pre and post-harvest phase of crops, hence human are exposed to these toxins. Dietary exposure to *Alternaria* toxins are related to grain, beverages, vegetables, oilseeds, fruits and their products (Amatulli *et al*., 2013). *Alternaria* toxins are mutagen and can cause DNA strand in human to breaks in V79 cell (Fleck *et al*., 2012). These toxins can also induce esophageal cancer, cytotoxicity in human colon carcinonoma cells, proponing a mechanisms mediated by activation of the mitochondria pathway of apoptosis (Bensassi *et al*., 2009).

2.3.3 *Fusarium*

Fusarium is a large fungal genus with a confused and unstable taxonomic history. Production of septate and fusiform to crescent conidia (macroconidia) with a foot-shaped basal cell and beaked apical cell are the main features of the genus (Fig 2.3) (Pitt and Hocking, 2009). They are capable of producing different spores, which can be exploitedfor their morphological identification. The major spores produced by these fungi are chlamydosphores, microconidia and macroconidia (Scauflaire *et al.,* 2011). Some other secondary characters unique to *Fusarium* are the nature of matured hyphae when cultured, pigmentation, detectable odour, growth rate and their secondary metabolites (Jacobs *et al.,* 2010).

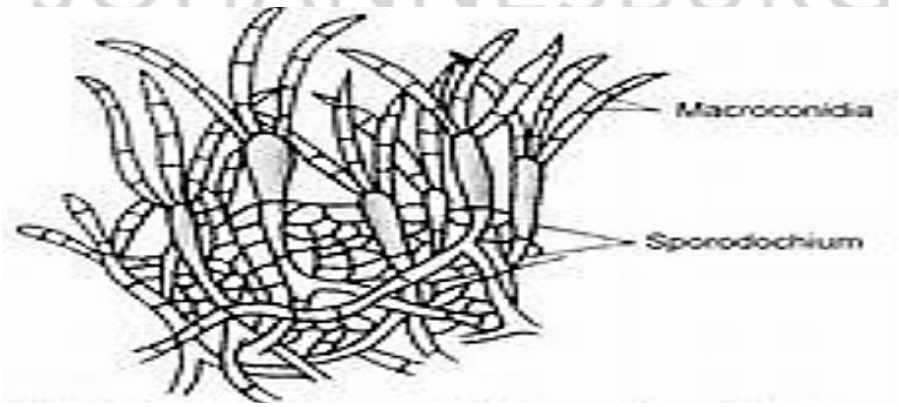

Figure 2.3Conidia structure of *Fusarium* species (Source: Sempere and Santamarina, 2009)

Fusarium can be found in soils, plants, among others. In plants, some species in this genus are known to be producers of secondary metabolites (Gareis *et al*., 2001; Larsen *et al*., 2004; Asam *et al*., 2017). The most prevalent toxins produced by these species include trichothecenes produced by *F. culmorum* and *F. graminearum* (Llorens *et al*., 2006; Winiewska *et al.,* 2017), fumonisins by *F. verticilloides* and *F. proliferatum* (Dragan *et al*., 2010; Ferrigo *et al*., 2016; Phoku *et al.,* 2017) and beauvericin, zearalenone by *F. avenacum, F. proliferatum, F. sublutinans* and *F. oxysporum* (Nazari *et al*., 2015). The trichothecenes are commonly found in cereals such as maize, sorghum, millet and rice (Larsen *et al*., 2004; Chu, 2006; Prandini *et al*., 2009; Nguyen *et al*., 2017).

The occurrence of *Fusarium* species has been reported in maize from Kogi state, Nigeria, with a 38.4% presence of *F. oxysporum* contamination (Makun *et al*., 2010). High incidences of *F. verticilloides* and *F. proliferatum* were also recorded in stored maize in Nigeria (Egbuta *et al*., 2015). In another study from the same country, occurrence of *Fusarium oxysporum* was reported in "solom" (a Ghanaian traditional beverage of millet) (Kortei *et al*., 2018). Also, peanut butter sold in retail markets in Ivory Coast was non-susceptible to *Fusarium* species as revealed by Boli*et al*. (2013). Occurrence of *Fusarium* species was recorded in coffee beans analysed in Ivory Coast by Djossou *et al*. (2015).

Exposure to *Fusarium* and their mycotoxins can result to inflammatory bowel disease (IBD), chronic bronchitis, accelerated decline of lung function (Dorribo *et al*., 2015) and immunostimulatory or immunosuppressive effects on the human. Also, consumption of *Fusarium* and their metabolites can be associated with high rates of oesophagus cancer (Wild and Gong, 2010). Adverse health effects of mycotoxins on human in developing countries have been reported by Shephard (2008), with that T-2 toxin produced by *Fusarium sporotrichioides* and *F.poae* causes hemorrhagic syndrome known as alimentary toxic aleukia. Further discussion on this is provided subsequently under the effects of fumonisins in this chapter.

2.3.4 *Penicillium*

Penicillium species are ubiquitous saprophytes colonizing different environments especially soils, foods and drinks (Leitao, 2009). Species in this genus do exceptionally well where there are trace amounts of minerals, and also survive in environments with a wide range of physico-chemical (water activity, temperature, pH and redox potential) characteristics. Those species that are soil fungi like *Penicillium janczewskii, P. janthinellum, P. paxilli* and *P. raistrickii,* seem to be rare in food and feed (Pitt and Hocking, 2009). *Penicillium* produces conidia structures called penicilli (meaning little brush), which consist of a well-defined cluster of philiades or similar cells bearing small, single-celled dry conidia in chains and a dense brush-like structure (Pitt and Hocking, 2009) mostly used as mode of recognition as shown in Fig 2.4. They could also be identified based on the structure of their branches as monoverticillate, biverticillate or terverticillate (Pitt and Hocking, 2009).

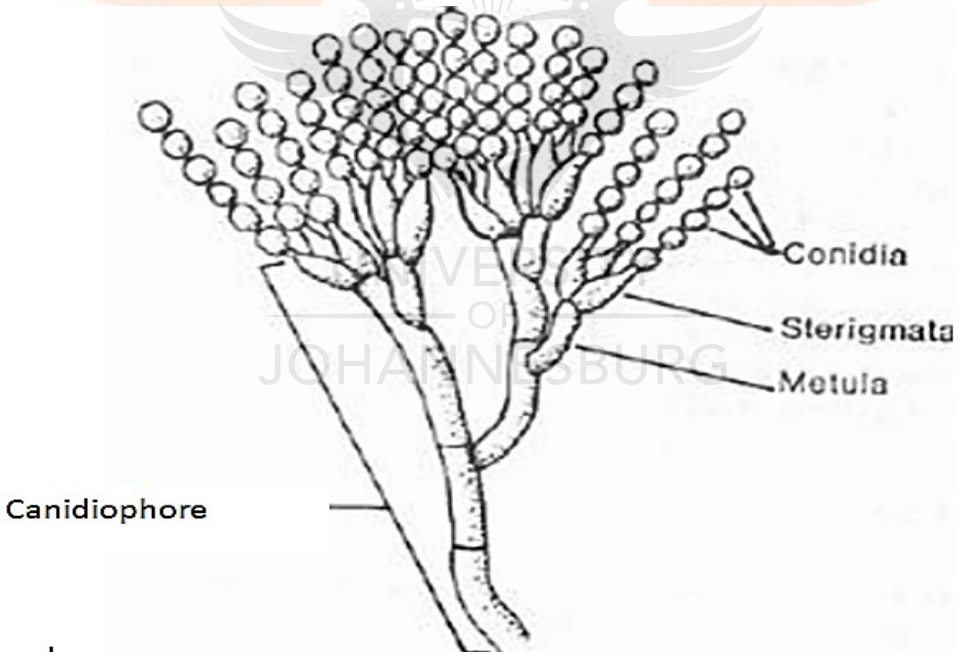

Figure 2.4 Microscopic structure of *Penicillium* Species (Source: Samson *et al.*, 2004)

This genus is a large group of fungal genera, which contains over 354 species (Visagie *et al*., 2014) with over 150 species characterized, 50 of which which are recognized and commonly cause food spoilage (Njobeh *et al*., 2009; Nigam *et al*., 2018).The most prevalent foodborne *Penicillium* species are *P. digitatum, P. citrinum, expansum, P. verrucosum, P. nurdicum, P. expansum, P. polonicum,* and *P.*

italicum (Frisvad and Thrane, 2000). Common food commodities affected by *Penicillium* species include maize and maize products (Pitt and Hocking, 2009), coffee, dairy products, fish, meat, spices (Konietzny and Greiner, 2003), citrus, pear and vegetables (Rawat, 2015). Some *Penicillium* species can affect processed and refrigerated foods like margarine and jams (Rawat, 2015). The occurrences of *Penicillium* species have been reported in food commodities. *P. chrysogenum* was found to be the most common in attieke from Abidjan, Ivory Coast and Osun state, Nigeria. Occurrence of *Penicillium* species in cowpea and peanut paste was established in Ivory Coast (Koffi-Nevry *et al.*, 2013; Boli *et al*., 2020).

Those mycotoxins produced by *Penicillium* include citrinin by *P. citrinum,* ochratoxin A by *P. verrucosum, P. verruculogen* and *P. vanthinellum* (Klaric *et al*., 2013; Oplatowska-Stachowiak *et al*., 2015), and penicillic acid by *P. aurantiogriseum*. Exposure to *Penicillium* and their mycotoxins can cause respiratory problems such as sneezing, runny nose, coughing, shortness of breath and chronic sinusitis (John, 2018). Therefore, *Penicillium* species are considered capable of posing a threat to human health when present in food (Tian *et al.,* 2017). *Penicillium* species causes known allergies that result in worsen asthma, hay fever and pneumonitis hypersensitivity.

2.4 Mycotoxins

Mycotoxins are naturally occurring low molecular weight compounds produced by filamentous fungi as secondary metabolites. These fungi are toxigenic and chemically grouped since members cause diseases and eventually death in human and other vertebrates. Toxins produced by these fungi are classified into various groups (Matumba *et al.*, 2015). However, classification schemes tend to reflect on the field of specialization because of their diverse chemical structures and sources, effects and their development by a large number of fungal species. Organic chemists categorize mycotoxins as lactone or coumarins based on their chemical properties, while mycologists categorized them as toxins e.g. *Aspergillus* toxins, *Alternaria* toxins, *Fusarium* toxins and *Penicillium* toxins.There are several fungal toxins, five of which are agriculturally important, i.e, zearalenone produced by *Fusarium* species; fumonisins by *Fusarium verticilioides*; ochratoxins produced by *Aspergillus ochraceus* and *Penicillium verrucosum*

(Yogendrarajah *et al*., 2013); deoxynivalenol produced by *Fusarium graminearum* and *F. culmorum* and the aflatoxins propagated by *Aspergillus flavus* and *A. parasiticus* (Ukwuru *et al*., 2017). Malfunctioning of human cells associated with mycotoxins are categorized as carcinogens, mutagens, and teratogens (Alghuthaymi *et al.,* 2020). Some of the mycotoxins (classes) that are frequently studied include aflatoxins, ergot alkaloids, fumonisins, ochratoxins, trichothecenes and zearalenone (De Ruyck *et al*., 2015). Much attention has recently been paid to the so-called 'alien-emerging *Fusarium*-mycotoxins' including fusaproliferin, beauvericin and moniliformin (Jestoi, 2008).

Generally, the toxic effect caused by mycotoxins is referred to as mycotoxicoses, affecting various organs of the body including kidney, liver, lungs, nervous, endocrine, and immune systems. Mycotoxicoses mostly occur in tropical regions with high temperature and humidity which encourage fungal growth and mycotoxin production (Peraica, 2016). Also, mycotoxicoses are widely distributed in developing countries mostly on the African countries where foods are exposed to microbial contamination due to inadequate handling and storage methods. Aflatoxins, ochratoxins, and fumonisin are the major causes of mycotoxicoses and they are the common mycotoxins in Africa relative to other mycotoxins as shown in Fig 2.5 (Darwish *et al*., 2014).Even though mycotoxins are rampant in food commodities from Africa yet mycotoxin regulationsare very uncommon in the continent with only 14 of the 55 countries having regulation for at least one mycotoxin(Kebede *et al*., 2020).

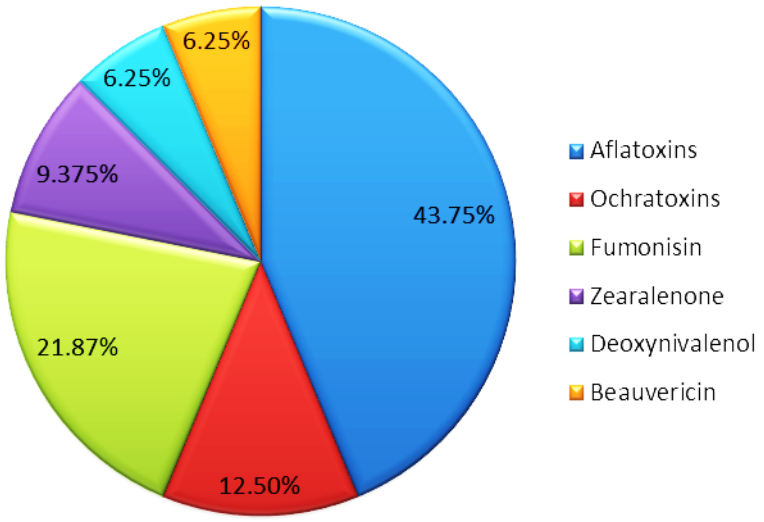

Figure 2.5 Distribution of mycotoxins in Africa countries (Source: Darwish *et al*., 2014)

2.4.1 Aflatoxins

Aflatoxins are mostly produced by species in the genus *Aspergillus* (Omar, 2013) especially *A. flavus* and *A. parasiticus.*They represents a global threat to human health due to their frequent existence in agricultural products. Aflatoxins generate interest in research since they were discovered in the 1960s with the outbreak of Turkey X disease, which caused the death of a hundred thousand turkey poults due to consumption of feed produced with contaminated peanut meal (Bennett and Klich, 2003). Aflatoxins productions are enhanced in environments with high humidity and temperature (Rachaputi *et al*., 2002) and are known to be the most prevalent of all mycotoxins produced by *Aspergillus.* Aflatoxins are classified into two according to their chemical structure, i.e., difurocoumarocyclopentenone including AFB_1, AFB_2, AFM_2, AFQ_1, and AFL and difurocoumarolactone such as AFG_1, and AFG_2 (Bennett and Cahill, 2016). They are composed of two furan rings and are linked together by coumarin moiety. Furofuran rings have been recognized as the structures responsible for the toxic and carcinogenic activity upon metabolic activation (IARC, 2012). Typical chemical structures of AFs are shown in Figure 2.6.

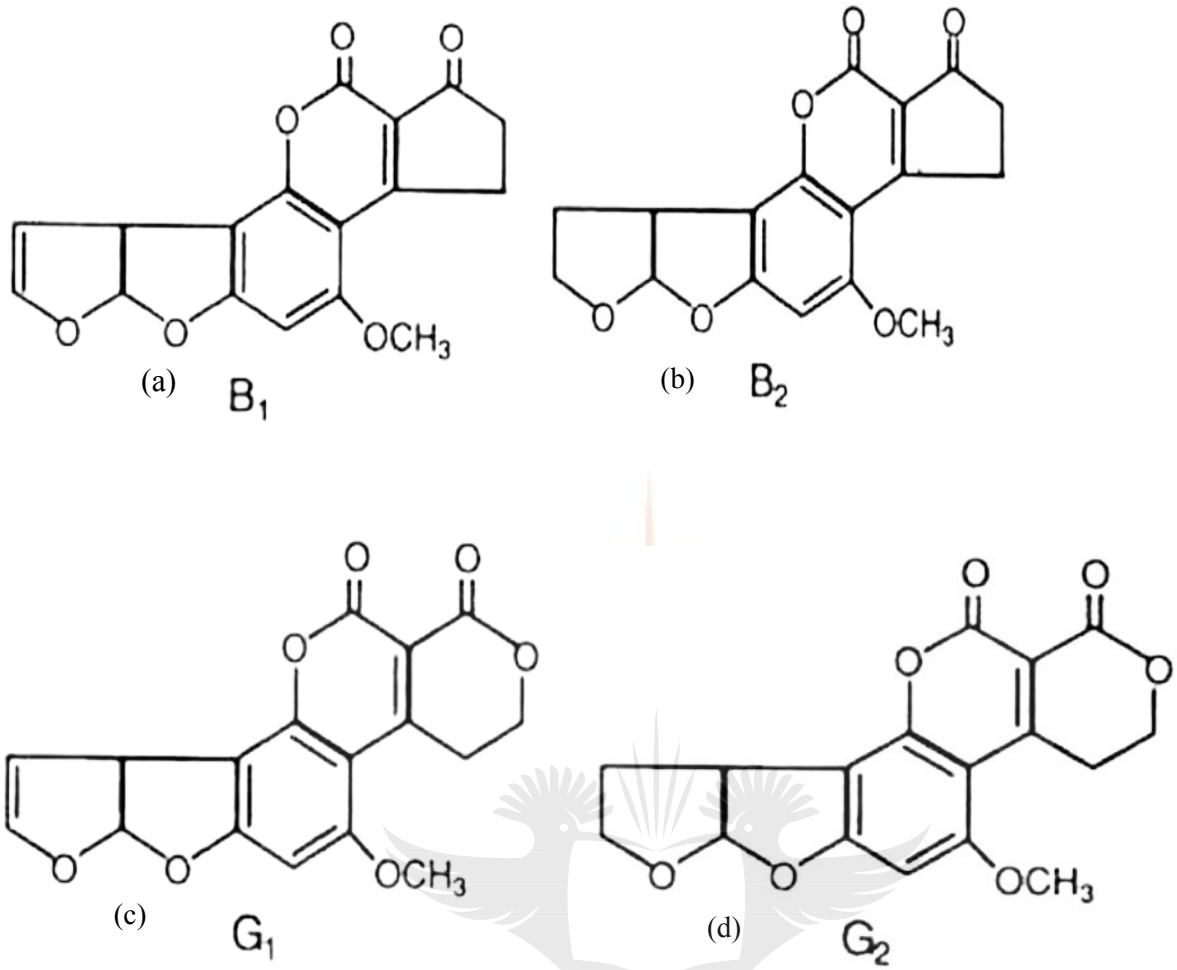

Figure 2.6Chemical structures of aflatoxins (a) AFB_1 (b) AFB_2 (c) AFG_1 and (d) AFG_2 (Source: Fratamico *et al*., 2008)

Aflatoxins have found in various agricultural products in developing country like Ivory Coast. Research from Ivory Coast by Manizan *et al*. (2018) confirmed the presence of aflatoxins in imported rice and native rice, cracked maize, peanut paste, and maize flour with/without potash. The average concentration of total aflatoxins in imported rice and native rice was 4.1 µg/kg and 3.3 µg/kg respectively while cracked maize have 0.3 – 173 µg/kg, peanut paste 1.4 – 8094 µg/kg and maize flour without potash 0.8 – 67 µg/kg. In another study from the same country, AFB_1 concentrations of 20 µg/kg were reported in maize (Sangare-Tigori *et al*., 2006). Total aflatoxins (AFs) were also found on dried kernels ranging from 2 – 162 µg/kg in Ivory Coast (Probst *et al*., 2014). Analysis of contaminated "attieke" from Adjame in Ivory Coast showed AFB_1 and AFB_2 concentrations ranging between 1.64 – 6.47 µg/kg and 2.48 – 2.53 µg/kg respectively.

2.4.1.1 Effects of aflatoxins

Exposures to aflatoxins can lead to aflatoxicosis, which accounts for a high percentage of diseases in developing countries (Atanda *et al*., 2013). Acute aflatoxicosis can cause death to humans and animals while chronic high levels can lead to a gradual deterioration of health through liver damage and immunosuppression (Negash, 2018). Aflatoxins have been categorized as Group-1 carcinogens, most especially aflatoxin B_1, G_1, and M_1. There is limited evidencethat aflatoxin B_2andG_2inducecancer (IARC, 2012). Moreover, aflatoxins have been associated with an increased number of hepatocellular carcinoma (HCC-liver cancer) cases globally (Liu and Wu 2010; Atanda *et al.,* 2013). Commonly, AFB_1 is the most carcinogenic, mutagenic, and teratogenic compound of AFs occurring naturally in foods and feeds (Coppock *et al.,* 2018). Aflatoxin B_1 obstructs DNA, RNA and protein synthesis, causing immuno-suppressive, hormonal and teratogenic effects (Cavaliere *et al.,* 2010; IARC, 2012; Okafor and Eni, 2018). Exposures to aflatoxins have been found to cause hormonal imbalance in children, leading stunting/growth faltering in children in Sierra Leone (Jonsyn-Ellis, 2012).

2.4.2 Citrinin

Citrinin was first discovered in 1931 as the pure metabolites of *P. citrinum,* also isolated from yellow-coloured rice (*Oryza sativa*) transported from Thailand to Japan (Gupta *et al*., 2017). It isalso produced by several filamentous fungi of the genus *Aspergillus*, *Monascus* and *Penicillium*. Among the *Penicillium*species, *Penicillium citrinum* remains a major producer of citrinin (EFSA, 2012). Other important *Penicillium* species that produce citrinin include *P. verrucosum* and *P. expansum* (Doughari, 2015). These speciesare natural contaminants of foods such as grains, fruits, vegetables (EFSA, 2012), peanut, and beans (Ostry *et al*., 2013) yet literature data are scare on natural occurrence of citrinin on food commodities. However, in Ivory Coast, occurrence of citrinin was reported in imported rice at concentration level of 29 µg/kg, local rice (<LOQ), cracked maize (<LOQ-146 µg/kg), maize flour with potash (<LOQ - 2349 µg/kg) and maize flour without potash (52 – 122 µg/kg) (Manizan *et al*., 2018).

Citrinin is a polyketide with a quinone and two intramolecular hydrogen bonds (Fig 2.7) (He and Cox, 2016). It forms acidic lemon-yellow crystals with maximal ultra-violet absorption. Citrinin is capable of forming chelate complexes and can be degraded by heating, acidic or alkaline solutions but it is insoluble in water (Doughari, 2015). It has a conjugated, planar structure, which gives its natural fluorescenes (Franco *et al*., 1996; Filho *et al.,* 2017).

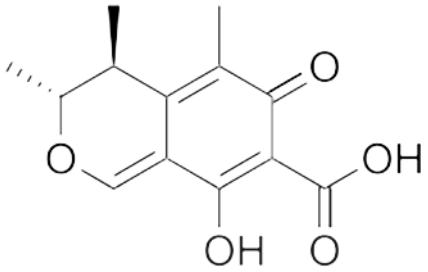

Figure 2.7Chemical structure of citrinin (Source: He and Cox, 2016)

2.4.2.1 Effects of citrinin

Exposure to citrinin through consumption of contaminated food can lead to teratogenic and immunotoxic (Dalefield, 2017) conditions. Researches have shown that citrinin has toxic effects on liver, kidney, gastrointestinal tract and reproductive system. The most sensitive part usually targeted by citrinin is the kidney. Citrinin is a powerful nephrotoxin capable of inhibiting the synthesis of nucleic acid, microtubule assembly, tubulin polymerization and alteration of mitochondria functions (Ostry *et al*., 2013). Citrinin cause ROS-mediated DNA damage, the damage can directly or exerted through intrinsic pathway: DNA and RNA synthesis inhibition, inhibition of microtubule assembly, alteration of mitochondrial functionality which lead to cell death (EFSA, 2012; Gayathri *et al*., 2015). In animals, citrinin can induce chromosome abnormalities and hydiploidy in the bone marrow (EFSA, 2012). In vivo and invitro studies have shown that citrinin stimulates chromosomal abnormality, micronuclei and teratogenic in humans and animals.

2.4.3 Fumonisins

Fumonisins were first characterized and described in 1988 by a group of South African scientists. They were first discovered as metabolites of *F. verticilloides* in mouldy corn, associated with a field outbreak

of equine leukoencephalomalacia in South Africa (Marasas, 2001). They are produced by at least 14 species of *Fusarium* with *F. verticilloides* and *F. proliferatum* identified as the most prominent producers (Dragan *et al*., 2010; El-sheikha, 2019). Fumonisins are diesters of propane-1, 2, 3-tricarboxylic acid and have similar long-chain aminopolyol backbones (Loi *et al*., 2017). Fumonisins resemble the sphingoid bases sphinganine and sphingosine with tricarboxylic acid groups added at the C14 and C15 positions (Fig 2.8) (Loi *et al*., 2017).Thus, they are capable of interfering with sphingolipid metabolism by inhibiting ceramide kinase (Aiko and Metha, 2015), leading to sphinganine accumulation in cells and tissues (Soriano *et al*., 2005). Fumonisins are grouped into four designated series: A, B, C and P. FB (B-series) is the most prevalent toxin (Rheeder*et al.,* 2002). FB_1FB_2 and FB_3 are toxicologically important fumonisins, although FB_1 frequently occurs in food and feedstuffs than FB_2.

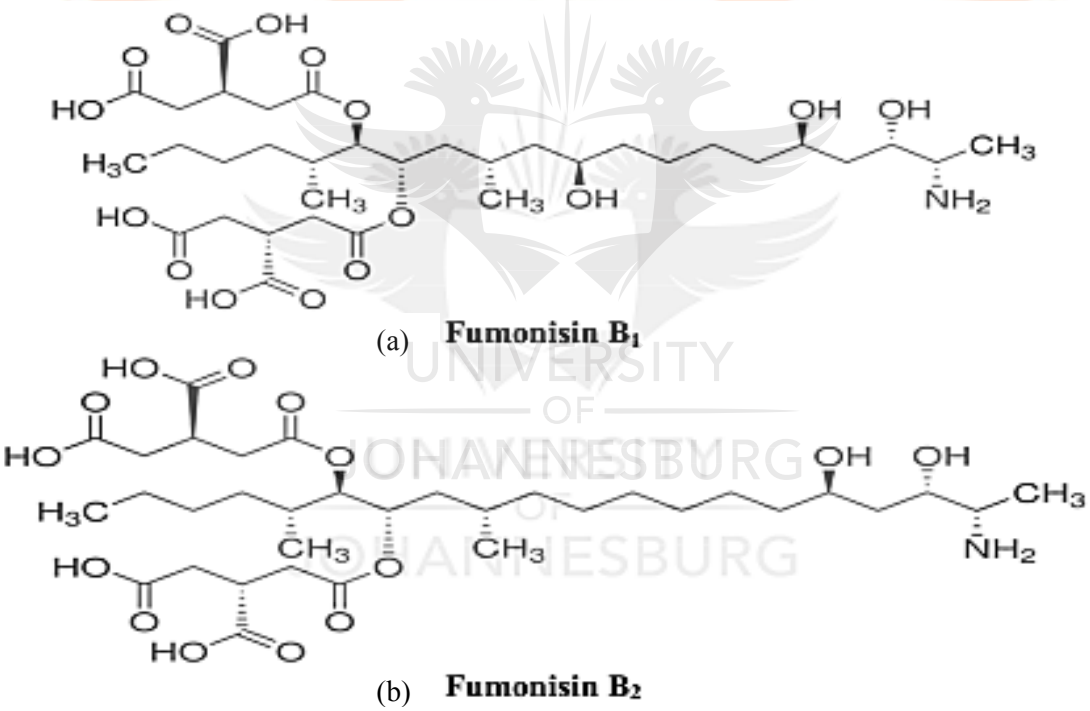

Figure 2.8 Chemical structure of fumonisins (a) FB_1 (b) FB_2 (Source: Phoku, 2015)

Globally, fumonisins contamination of cereals and other food is a serious problem particularly in developing countries where cereals are highly consumed (Chu, 2006). Contamination takes place in the field, during transportation and in storage even at low temperatures (Mannaa and Kim, 2013). A survey carried out by Manizan *et al*. (2018) showed that fumonisins are present in maize, peanut paste and rice

consumed in Ivory Coast. FB_1 and FB_2were detected in 32% of local rice with levels ranging from 2.7-13 µg/kg. Also, 100% of analysed maize flour without potash was shown to be contaminated with FB_1 and B_2 with concentration at 2.3 - 319 µg/kg. Cracked maize and maize flour with potash was found to contain FBs at 11-706 µg/kg and 14 -214 µg/kg concentrations respectively. Sangare-Tigori *et al*. (2006) reported the contamination of fumonisins in maize at the range of 0.3 – 1.5 µg/kg and <0.3– 6 µg/kg in peanut. A similar report of fumonisins in Ivory Coast by Kouadio *et al*. (2014) has confirmed the presence of fumonisins in maize flour. FB_1 and FB_2 occurrence were reported in roasted meat spice consumed in Ivory Coast within the range of 10.0 µg/kg to 33.30 µg/kg (Yapo *et al.,* 2020).

2.4.3.1 Effects of Fumonisins

Fumonisins have been classified as Group 2B carcinogen that is possibly carcinogenic to humans and animals (IARC, 2012). A link between oesophagal cancer and FB_1 occurrence in humans has been established in South Africa (Sydenham *et al.,* 1991) and was confirmed by Trung *et al*. (2008) in Vietnam, who demonstrated that FB_1 potentially promote this cancer in humans. In China, Yoshizawa *et al*. (1994) reported the incidence of human oesophagus cancer associated with the consumption of maize contaminated with $FB_1$and FB_2. Strong and rapid hepatic effects of FB1, which impaired the membrane phospholipid in rats was reported by Szabo *et al*. (2016). Fumonisins can obstruct cell production, increase cell penetrability and cell mortality (Yu *et al*., 2020). They cause various cell damages such as morphogenetic changes, transcriptome and proteome alteration (Fernandez-Blanco *et al*., 2016), the decrease membrane potential in mitochondria (Zhang *et al*., 2017). Fumonisins majorly cause damages to several cells in humans and animals.

2.4.4 Ochratoxins

Ochratoxins are a group of structurally related metabolites. They were first discovered in 1965 in South Africa as the metabolites of *A. ochraceus*in a corn meal that was intentionally inoculated with this micro fungus (Van Der Merwe, 1965). Recently, reports have shown that ochratoxins are major mycotoxins usually produced by the fungal genera *Aspergillus* and *Penicillium* (Koszegi and Poór, 2016), which are

found in food such as cereals. Ochratoxins especially ochratoxin A, comprises of a para-chlorophenolic moiety enclosing a hydroiso-coumarin group that is amide-linked to L-phenylalanine (Fig 2.9).Several derivatives of ochratoxins occur naturally or molded in the body after biotransformation,some are hydroxylated, others lack phenylalanine moiety (Heussner and Binge, 2015; Yang *et al*., 2015).Several analogues of ochratoxins including ochratoxin A (OTA), B (OTB), C (OTC), alpha (α), and beta (β) (Arroyo-Manzanares *etal*., 2017; Viaro *et al.,* 2017). OTA is the most toxic and prevalent one in foods such as coffee, cereals, spices, vegetables, dried fruits and medicinal plants (Pitt *et al*., 1998; Magnoli *et al*., 2006).

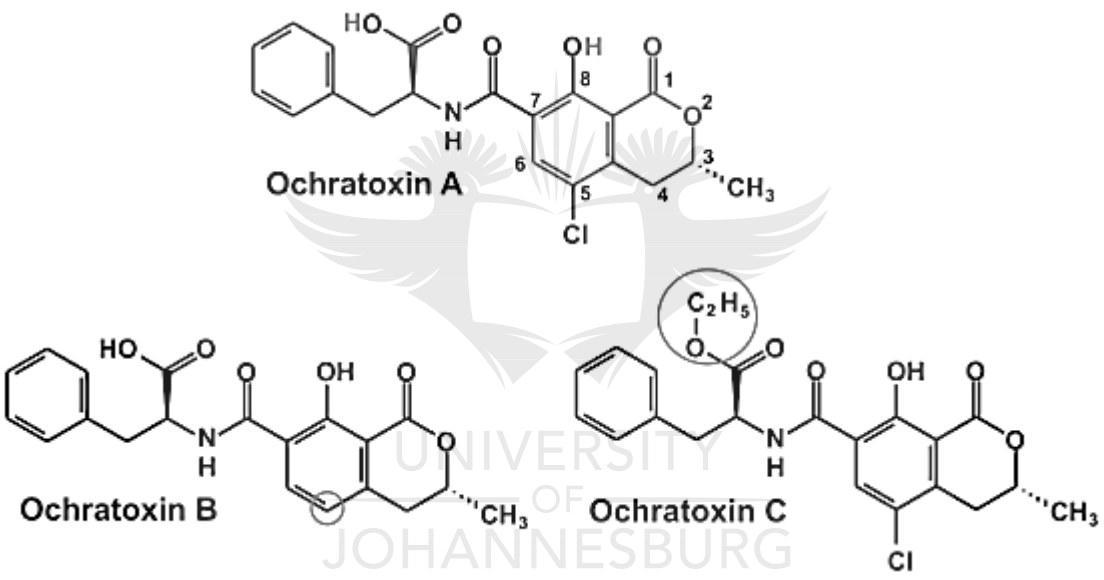

Figure 2. 9Chemical structure of ochratoxin A, B and C (Source: Koszegi and Poór, 2016)

Several researchers have reported the incidence of OTA in various food commodities. In Ivory Coast, occurrences of OTAare common in cassava flour across the six geographical zones of the country. Concentrations of OTA in this food product ranged from 0.49-1.21 µg/kg for the main season (November to March) to 0.43 - 0.80 µg/kg in April to July (Coulibaly *et al*., 2012). In another study, Manizan *et al*. (2018) reported the presence of this toxin in rice, peanut paste and maize from Ivory Coast. They indicated OTA concentrations of 7.8 µg/kg to as high as 147 µg/kg across these different food products. Presence of OTA has also been confirmed in spices (Chilli pepper and ginger) in this

country (Manda *et al*., 2016). This was also confirmed by the report from Kedjebo*et al*. (2015) in cocoa beans, both in bad and good state condition containing maximum concentration of 39.2 and 11.2 µg/kg respectively. Occurrence of OTA was reported by Yapo *et al*. (2020) in kankankan (spices used for roasted meat) consumed in Ivory Coast. Frequent detection of OTA in various food commodities from Ivory Coast revealed how the Ivorians are daily exposed to OTA.

2.4.4.1 Effects of ochratoxins

Ochratoxins have been described as Group 2 carcinogens (Dragan *et al*., 2010). Their toxicity involves in suppressing the human immune system (Aiko and Metha, 2015) and the development of certain kidney diseases through generation of oxidative stress at high concentrations (Jamkhande*et al*., 2014). Direct genotoxin mechanisms and oxidative stress can also encourage OTA to acts as a nephrotoxin and urothelial carcinogen (Malir *et al*., 2016). Effects of OTA on humans and animals include renal toxicity, nephropathy, immunosuppressive (Djossou *et al.,* 2015), teratogenic and cause tumors in urinary tract (Jamkhande*et al*., 2014; Wang *et al.,* 2019). Dietary exposure to OTA can induces micronuclei and delays DNA repair kinetics (Gonzalez-Arias *et al*., 2014). OTA can induce or support the development of fibrotic kidney disease by involving post transcriptional regulation mechanisms (Hennemeier *et al*., 2014).

2.5 Factors influencing the fungal growth and mycotoxin production

Many factors are influencing fungal growth and mycotoxins production. They are grouped as biological and physical factors, while some as biotic and abiotic factors (Bouras *et al*., 2009). Other scientists classify them as intrinsic, extrinsic and implicit microbial factors (Magan *et al*., 2004), post-harvest processing include handling of crops, drying, blending, addition of preservatives and packaging.

Intrinsic factors responsible for the development of fungi and production of mycotoxins include the availability of nutrient, substrate (proteins, fats, carbohydrates) (Miedaner, 2004), type and amount of trace element (organic or inorganic element) in food (Atanda *et al*., 2013). Even though fungi have genetic potential to grow and produce mycotoxins in certain food, the availability of nutrients determines

their survival. Fungal species subjected to the same environmental conditions but different substrates (nutrients) will grow at different rates (Pardo *et al*., 2006).

The presence of fungi in a given locality implies that the host and the environmental conditions are conducive for their growth and development. Extrinsic factors are environmental conditions, which have great influence on the fungal growth and mycotoxin production (Bouras *et al*., 2009). These factors include temperature, water activity (humidity) and pH (Hungaro *et al*., 2015). The fungal population and mycotoxin production are known to vary based on the range of temperature and humidity in the locations they have been identified (Marie-Helene *et al.,* 2013).

Temperature is the most important factor affecting fungal growth. It is a limiting factor which could either favour or inhibit the fungal growth and mycotoxin production (Fapohunda *et al*., 2012; Taheu *et al.,* 2019). Most of the foodborne fungal pathogens are mesophilic growing well at temperatures between 20-45ºC. Food storage at low temperature is one of the most effective ways of slowing down microbial metabolic activity (Hungaro *et al*., 2015). Warmer weather and inadequate refrigeration may increase the level of fungal and mycotoxincontamination (Moreira *et al.,* 2001).

Another factor influencing the fungal growth and mycotoxin production is the water availability for the metabolic reaction. The average water activity that affects fungal growth depends on the species. Some species thrive well at 0.97- 0.58 a_w whereas some have shown to produce mycotoxins only at 0.76 a_w (Hungaro *et al*., 2015). Reduction of humidity below the optimal range in food will inhibit the growth of microorganisms.

The pH level of the food plays an important role in fungal growth because fungi are more sensitive to internal and external changes. It is well established that most fungi thrive well in the absence of oxygen and at pH close to neutral, although a few can grow at pH below 4.0 (Whitlow *et al*., 2010). Many filamentous fungi and yeasts associated with spoilage of foods and food products made with acidic fruits can survive at low pH.

2.6 Control of fungal growth and mycotoxin contamination on food commodities

Contamination of food commodities by fungi and their mycotoxins is a threat to global food security and safety. In order to prevent these problems, fungi and mycotoxins eradication must be considered. Mycotoxin controls are grouped into two approaches (1) prevention of fungal growth and mycotoxin production, the removal of fungal agent, growth inhibition and microbial inactivation (Hungaro *et al*., 2015), and (2) detoxification of existing mycotoxins in the food commodities (Krnjaja *et al*., 2009). Along the food production chain, management of fungi and their mycotoxins can be handled as pre-harvest and/or post-harvest strategies (Devreese *et al.,* 2013). Pre-harvest strategies of inhibiting the fungal growth include good agricultural practices (GAP) such as crop rotation, tillage and irrigation (Devreese *et al*., 2013).Also, planting a crop with genotypic resistance to the fungi can help in inhibiting their growth. Avoid high cropping density, good balance fertilization and effective control of insect and pests. Ensure that the plant matured before harvest, high level of agro-technical measures by controlling the health of the seed and separate the seeds with pathological changes (Krnjaja *et al*., 2009).

Post-harvest strategies are applied to inhibit further growth of fungi and mycotoxins production on farm products during harvesting, processing or storage. These strategies can be divided into three main methods,the first post harvest strategies are physical approach.This involves the mechanical removal of contaminated plant/seed from healthy and intact products. This is done by sorting, cleaning, milling and dehulling (Schrodter, 2004). This also involves proper storage condition at the farm e.g. cooled dry environment may also lead to reduced fungal contamination (Stankvoic *et al.,* 2007). Furthermore, these strategies also involve heat application (Park, 2002), irradiation, and extrusion (Vanhoutte *et al*., 2016). Application of cold plasma (Bong *et al.*, 2007) and microwave treatment have been proposed in food processing as sterilization or degrading methods (Bretz*et al*., 2006).

Another approach to preventing fungal growth and mycotoxin synthesis is the use of chemicals. Several chemicals have been used to reduce mycotoxin contamination in foods and feeds. These chemicals include hydrogen peroxide, ozonation (Prudente and King, 2002), ammonia (Mishra and Das, 2003) and

lactic acid (Aiko and Metha, 2015). Salicyclic, silphamine, sulposalicyclic, anthrailic, benzoic, boric, propionic acids are known to be effective in reducing AFB_1 in agricultural products (Hasan, 1996). Application of fungicides is also encouraged for controlling fungal growth, though synthetic fungicides are expensive, many accompanied with some negative effects on humans and animals (Jard *et al*., 2011; Loi *et al*., 2017).

Alternatively, plant extracts with bioactive compounds such as phenols, flavonoids, quinones, alkaloids, tannins, saponins and sterols (Burt, 2004; Chhetri and Jha, 2016) have been demonstrated to be effective antifungal agents (Burt, 2004). They are considered safe and easily degradable. Their use has been implemented with the inhibition of *Aspergillus flavus* growth and degradation of aflatoxins in maize (Asghar *et al*., 2016)in the case of an extract from *Azadirachta indica* and *Morinda lucida* (Bankole *et al*., 2006). Ferreira *et al*. (2018) reported that *Zingiber offinale* essential oil inhibited ergosterol production at a concentration of 1000 µg/mL and DON production at a concentration of 500 µg/mL. In-vitro and in-vivo test have been done where *Nigella sativa* oil, extracts and some other active component were used to inhibit the growth of pathogenic yeast and filamentous fungi (Shokri, 2016).

With the biological approach, harmless bacteria or fungi and their enzymes are used to prevent the fungalgrowth (Gebremeskel*et al*., 2019) and mycotoxin production. It is an ecologically friendly method, effective with no or little effects on the physical form of the products, food sensory and nutritional quality (Samuel *et al*., 2014; Loi *et al*., 2017). Different microorganisms and their enzymes have been used as antifungal agents. A survey carried out by Velmourougane *et al*. (2011) using dip treatment containing yeast suspension on coffee resulted in drastic reduction of fungal (*Aspergillus niger,* and *Aspergillus ochraceus*) incidence and ochratoxin A production without affecting the quality of the crop. Pure enzymes from microorganisms like bacteria and fungi can degrade mycotoxins (Loi *et al.*, 2017). Lactic acid bacteria and *Actinomycetes* species (*Rhodococcus erythropolis*ATCC 4277 strain, *Streptomyces lividans* TK 24, and *S. aeurofaciens*) can degrade AFB_1 at the optimum temperature of 30ºC (Zinedine *et al*., 2005; Eshelli *et al*., 2015). A research carried out by Pauls and Zhou (2010),

showed that gene from *F. graminearum* Tri 101 gene used to detoxify DON on maize produces a promising result in reducing the toxin. Also, a report from Zhou *et al*. (2008) revealed that *Citrobacter* sp. ADS47 (from soil), *Bacillus* sp. LS100 and *Lactobacillus* sp. (from sheep rumen fluid) detoxified mycotoxin in food sample. Toxin binders like mannan-oligosaccharides can also be used, as this substance binds with toxins in alimentary canal and eliminate through faeces (Carter, 2001).

2.7 Food crop production their susceptibility to fungi in Ivory Coast

The climatic conditions in Ivory Coast are generally warm and humid. There are three major seasons in the country; warm and dry (November to March), hot and dry (March to May), and hot and wet (June to October). The average temperature is between 25 and 30ºC and relative humidity range from 85 and 71 %.These climatic conditions favour agricultural processes in Ivory Coast.

Agriculture accounts for 30 % of the gross domestic products of Ivory Coast. It is the primary source of income for two-thirds of the Ivorian household. Agriculture is relatively more diversified but with two major cash crops that include cocoa and coffee (Daddieh and Mundt, 2016). The country also produces high volumes of pineapples, bananas, coconut, kola, palm oil, cashew, melon, haricot, cotton, sugar, okra, rubber, cassava, maize, millet, rice, yams, plantains and vegetables. Many of such foods are susceptible to fungi examples include Haricot (Beans - *Phaseolus vulgaris),* maize *(Zea mays),*melon (*Citrullus colocynthis*), attieke (Steamed grated cassava: *Manihot esculentus),*okra (*Abelmoschus esculentus*) and gnangnan (Eggplant: *Solanum aethiopicum L*).

Haricot (white beans) is a leguminous plant, which serves as a source of food for household in Ivory Coast (Kouakou *et al.*, 2018). It has high nutrients with relatively high protein, vitamins, and other mineral content. According to FAO (2009), beans are regarded as one of the major agricultural products in the economy of Ivory Coast and contribute to basic household income. Despite its importance, beans can be contaminated by various fungi like *Fusarium, Penicillium, Aspergillus* and *Mucor* (Djossou *et al*., 2015), and *Colletotrichum*spp. (ASHC, 2015). Common diseases of beans include anthracnose caused by

Colletotrichum lindemutheanum and Cercospora leaf spot caused by *Mycosphaerella cruenta* causing leaves to fall off and serious yield loss of up to 40 % (ASHC, 2015).

Maize is a cereal used for food, animal feed and industrial purpose to manufacture different goods for human consumption (FAO, 2010). It is consumed as fresh maize cob, used as daily diet in form of flour in making bread for feeding children and animals and also used in making local beer in Ivory Coast (Ekou, 2015). It is considered a crop of significant importance in Africa and particularly in Ivory Coast. It is important crops in Ivory Coast because it is used for income generation (Ekou, 2015). Maize however, is affected by various fungal genera including *Acremonium, Aspergillus, Diplodia, Fusarium* and *Penicillium* both in the field and storage. Some of these fungi are capable of producing mycotoxins for examples, *A. flavus* and *A. parasiticus* produce aflatoxins, *A. ochraceus* produce ochratoxins*, and F. verticilloides* produce fumonisins and *P. citrinum* produce citrinin, as earlier reviewed in this chapter.

Melon is a seed of Africa cucurbits widely grown around the tropics and temperate regions. Several species of melon identified in Ivory Coast based on researches are *Lagenaria siceraria, Cucumeropsis manni, Cucumis melo, Cucurbita moschata,* and *Citrillus lanatus* (Zoro Bi *et al*., 2003). It is an essential part of traditional food for Ivorians and also serves as the source of income to communities growing this seed. Oil extracted from the seed of this crop in Ivory Coast is used in wedding, birth and house warming ceremonies (Zoro Bi *et al.,* 2003). Though melon is an important plant in terms of its nutritional and socio-economic value, it is exposed to the pathogenic pressure that reduces seed production (Vodouhe *et al*., 2000). Amongst the fungal pathogens that cause diseases and reduce melon production, are *Pseudoperonospora cubensis* that causes downy mildew disease (Choi and Shin, 2006), *Podosphaera xanthii* causing powdery mildew and *Fusarium oxysporum* causing *Fusarium* wilt (Trionfetti-Nisini *et al*., 2002). Marie-Helene *et al*. (2013) reported the presence of *Aspergillus, Botryosphaeria, Cochliobolus, Colletotrichum, Lasiodiplodia, Phoma* and *Fusarium* spp. in melon from Ivory Coast.

Attieke is a locally fermented cassava product consumed in Ivory Coast. It is processed by the natural fermentation of cassava (Soro-Yao *et al.*, 2013). It is whitish with faintly sour taste consumed as a staple food and used as halting food for babies. Attieke is exposed to microorganisms like *Bacillus* sp, coliforms, yeast, lactic acid bacteria and fungi (*Aspergillus,* and *Rhizopus* spp.) due to poor cassava processing in the local communities (Assanvo *et al.,* 2009). This is similar to the findingsof Kouame*et al*. (2012) who reported the presence of *Mucor, Rhizopus, Fusarium* and *Thamnidium* spp. in attieke from Ivory Coast. It was revealed that fungi are also capable of affecting the fermented foods. Therefore, the processing method of attieke must be improved to ensure its safety for human consumption.

Okra (*Abelmoschus* spp.) is a traditional vegetable crop in Africa and Asia. It is regarded as the regularly cultivated and widely consumed traditional vegetable in West and Central Africa. It has huge socio-economic potential (Kumar *et al*., 2009) because of its robust nature, nutritive nature, dietary fibres and distinct seed protein with stable in both lysine and tryptophan amino acids (NAP, 2006). Fresh pods of okra have low calories with nearly no fat, rich in fibre, and have several valuable nutrients such as vitamin C, folate, and vitamin A. The seeds also contain some principal elements like potassium, sodium, magnesium and calcium (Avallone *et al*., 2008). There are reports of high losses of okra due to fungal infection (Kumar *et al*., 2010) such as Cercospora leaf spot by *Cescospora abelmosch, C. malayensis, and C. hibisci*, *Fusarium* wilt by *Fusarium oxysporum* and Damping-off disease by *Pythium* and *Rhizoctonia* spp., which may kill the okra seed before or soon after germination (Ek-amnuay, 2010).

Gnangnan is one of the common edible non-tuberferous cultivated *Solanum* species (Sekara *et al*., 2007) in Ivory Coast. It has a high nutritional value i.e. low in calories and fats (Toldra *et al*., 2015), also contains essential amino acids such as leucine, lysine, isoleucine and valine that can aid thelowering of cholesterol in the human body. The fruit has a bitter taste but medicinal features with high levels of antioxidant bioactive compounds (Lim, 2013). The medicinal feature of this fruit makes it useful in treating malaria (Kouassi *et al.,* 2014). *Solanum aethiopicum* is susceptible to many pathogenic fungal

like *Aspergillus* but rarely contaminated by *Fusarium* spp, bacteria (*Ralstonia solanacearium*) and root knot nematodes (*Meloidogyne*spp.) (Toppino *et al*., 2008).

2.9 Conclusions

From the literature reviewed in this chapter, it is clear that toxigenic fungi and mycotoxins occur in various food commodities at a global level, especially in developing African countries like Ivory Coast. The most prevalent fungal genera found in food commodities, especially in tropical areas are *Aspergillus, Penicillium, Fusarium, Mucor,* and *Rhizopus.* Species in the different groups are capable of producing secondary metabolites some of which are mycotoxins including AFs, DON, FBs, OTs, T-2 and ZEN. Mycotoxins have been demonstrated to affect human and animal health. Few studies have been done on mycotoxin contamination in Ivory Coast.Continuous monitoring is important as it is the case in South Africa. In this way, more insight is gained into food safety and provides adequate information on fungi and their secondary metabolites that may contribute to the implementation of management strategies to alleviate the health and economic problems facing that country.

CHAPTER THREE
MATERIALS AND METHODOLOGY

3.1 Sampling and sample preparation

Various food commodities were selected randomly from three local markets (Adjame, Yopougon, and Cocody) in Ivory Coast between February and May 2018.

3.1.1 Sampling

In this study, a total of 70 food commodities were screened for fungal and mycotoxin contamination. The samples were randomly selected and purchased from three local markets (Adjame, Yopougon and Cocody) as shown in Table 4.1. All the samples had a minimum of one representative from each market expect for cassava flakes and okra which were selected from Adjame market only based on availability during the sampling process. Fungi were isolated from 34, 19 and 17 food commodities from Adjame, Yopougon and Cocody respectively, representing a cross-section of what is sold in the local markets in Ivory Coast.

Table 3.1 Food commodities collected 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
Chilli 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

3.1.2 Sample preparation

Approximately 100g of 'haricot', yellow maize, white maize, 'attieke', millet, dried okra; cassava flakes, melon, 'gnangnan', dedane rice and chilli pepper were collected into ziplock bags. Each sample was thoroughly mixed to obtain a representative sample, except for "attieke", which was already packed in a plastic bag. The samples were then conveyed to the Laboratory of the University of Johannesburg for further processing. In the lab, samples were milled using a mechanical blender. A 70% ethanolwas used to sterilize the blender after grinding each sample. Grind samples were then stored at -80^{o}C until further analysis.

3.2 Methodology

3.2.1 Fungal isolation

Fungal isolation and enumeration were done as described by Iheanacho *et al*. (2014) with some modifications. For culture preparation, 1 g of each sample was weighed into the test tube containing 10 ml of sterile ringer's salt solution and vortexed. Each sample was then serially diluted to 10^{-5}. One millilitre of each mixture (10^{-3} 10^{-4} and 10^{-5})was inoculated onto potato dextrose agar (PDA), cyapeck yeast agar (CYA) and malt extract agar (MEA)[Merck KGaA, Darmstadt, Germany]. The inoculated plates were incubated at 25^{o}C for 5 – 7 days. Fungal colonies were examined and counted from the different media plates using a colony counter (Gallenkamp, England) were the colonies were counted within the range of 30 – 300. The results were expressed as colony-forming units per gram (CFU/g).

CFU/g = Numbers of colonies xreciprocal 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^{o}C for 7 days.

3.2.2 Fungal identification: Morphological characterization

Fungi culture on PDA, CYA and MEA was putatively identified based morphological characters such as colony structure, colour and formation characters (Klich, 2002; Pitt and Hocking, 2009). Further

morphological characterization of pure colonies of culture was done by mounting mycelia on slides and stained with lactophenol cotton blue. These were then covered with coverslips and observed under an optical microscope (Olympus CX-40, micro-Instruments, New Zealand Ltd) with magnification of 400**X**.

3.2.3 DNA extraction and PCR analysis

Further identification was done using DNA sequences. For this purpose, fresh mycelia from pure cultureswerecollected into the ZRbashing bead lysis tube for use in extracting genomic DNA. This was done with Quick-DNA^{TM} 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 approximately50 ng/µL.

PCR was done using quantified DNA to amplify sections 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) (White *et al*., 1990). PCR reagents include deoxynucleotide triphosphate (dNTP) (Fermentas life Science, Lithuania). 5µL $MgCl_2$, 0.5 µL of each primer, 0.5 µL Mytaq, 5 µL buffer, and deionized water. PCR wasdone on an Eppendorf 96-well Thermocycler (Eppendorf, USA) set with an initial denaturation step at 95ºC for 3 minutes followed by 35 cycles of denaturation at 94 ºC for 1 minute, an annealing step at 55 ºC for 45 seconds, an extension of primer at 72 ºC for 1 minute and a final extension at 72 ºC for 5 minutes.

3.2.4 Agarose gel DNA electrophoresis

Successful PCR amplifications were confirmed by staining 4 uL of PCR product with 2 µL of GelRed (Biotium Inc.) nucleic acid dye and running the mixture on 2% agarose gel. A DNA molecular ruler (100 bp ladder; Fermentas O'Gene Ruler) was included in the mixture to determine the base-pair length. Generated bands on the gels were visualized under Gel IX imager 20-2.8M Pixel (Bio Olympics, CA,

USA) ultraviolet (UV) transilluminator with wavelength of 312nm.The DNA ZR-96 sequencing clean-up kit (Applied Biosystem, Foster City, CA) was used to purify amplicons.

3.2.5 DNA sequencing and phylogenetic analysis

Sequencing PCR reactions were done at a final volume of 12 µL with the same primers as used for the PCR amplification. The reaction mixtures included 2.5 µL sequencing buffer, 4 µL PCR grade water, 0.5µL BigDye terminator cycle sequencing kit (Applied Biosystem, Foster City, CA), 1 µL of the ITS primers (ITS1 and ITS4) and 4µL purified PCR product. PCR mixtures containing dimethylsulfoxide (DMSO) was run at an annealing temperature of 48°C; mixes containing bovine serum albumin (BSA) at 55°C. Each of the amplicons was sequenced in both directions using the PCR primers and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's guidelines. Sequencing was performed using the Applied Biosystems^{TM} 3730xl DNA Analyzer (ThermoFisher Science, CA, USA). Consensus sequences for each locus have been assembled using SeqMan Pro v.15(DNASTAR). The sequencing product was cleaned using the DNA ZR-96 sequencing clean up kit (Applied Biosystems, Foster City, CA). The DNA sequence for each fragment was edited using MEGA V.5.2. Obtained sequences were then blasted against the Gen Bank (http://www.ncbi.nlm.nih.gov/) with BLAST 2.2.31 as described by Altschul *et al*. (1997) to confirm the putative identity of culture using similarity index score obtained from blast results. A data set was generated by obtaining the sequence of closely related species to the ones from this study in GenBank. 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 analyses (ML) analysis usingPhyML3.1/3.0 aLRT (doc+aLRT) (www.phylogeny.fr/simple phylogeny.cgi) with the default GTR model. Phylogenetic trees were generated using TreeDyn 198.3 (doc)(www.phylogeny.fr/simple phylogeny.cgi). The phylogenetic trees generated were used to confirm the evolutionary relationship of the isolated fungi from this study with existing relatives in the Gen Bank.

3.2.6 Multi-mycotoxins extraction

The seventy samples screened for fungal contaminations were also screened for mycotoxin contamination. Samples preparations were done following the extraction method used by Malachova *et al*. (2015). Five grams of the ground food samples were homogenized in 20 mL extraction solvent (acetonitrile/water/ acetic acid, 79:20:1, v/v/v) on a mechanical shaker (LABCON, GmbH, Heppenheim, Germany) for 90 minutes at 180 rpm and then centrifuged (Eppendorf Millipore Laboratory Centrifuge, Merck South Africa) at 3000 rpm for 5 minutes. The extracts were filtered using 0.22 µm particle size PTFE syringe filter units. Five-hundred microlitres (500 µL) of extract were transferred into a 1.5 mL glass vial having equal volumes of dilution solvent and then vortexed for 30 seconds and 5 µL of the extracts were injected into the UHPLC-LM/MS system.

3.2.7 LC-MS/MS analysis

Chromatographic separation, detection and quantification of mycotoxins were performed with Water ACQUITY UPLC SYNAPT high definition mass spectrometer coupledwith G2 QTOF instrument (Waters, MilfordMassachusetts, USA). The system is equipped with symmetry guard column 3.5 µm, 10x2.1 mm (Waters, Milford Massachusetts, USA) and a Waters UPLC® C_{18} ethylene bridged hybrid column (1.7 µm, 100 x2.1mm)(Waters, MilfordMassachusetts, USA). Elution was carried out in binary gradient mode. The methanol/water/ formic acid (10:89:1, v/v/v) was used as mobile phase A and methanol/water/formic acid (97:2:1, v/v/v) as mobile phase B with 5Mm ammonium acetate. A gradient initialling with 50% was maintained for 2minutes and then it linearly increased to 100% B after 2minutes. The injection volume was 5µl and the flow rate was kept constant at 0.3 ml with the column and sample temperature maintained at 4 ºC room temperature. Separated analytes were delivered to Quatro Premier^{TM}x E Tandem quadrupole mass spectrometer supplied by Waters (Milford, Massachusetts, USA). The mass spectrometer (MS) was operated in both positive (ESI^+) and negative (ESI^-) electrospray ionization. High-definition multiple reaction monitoring was used to detect and quantify the analytes of interest. Source and desolvation temperatures were set at 350 and 130 ºC,

respectively. N_2 gas was used as cone and nebulizing gas. The cone gas flows as well as desolvation gas flow was maintained at 100 and 800L/h, respectively. The capillary voltage was set at 3.5kV, while the one for extractor cone voltage at 650V. The total run time was 20 minutes. Masslynx^{TM} software V4.1 (molecular weight calculator and Quanlynx^{TM})(Waters, Milford Massachusetts, U.S.A) was used to assess and process data.

3.2.8 Spiking experiments

The mycotoxin standard solutions were prepared for calibration purpose and spikes using serial dilution. The working standards were prepared from standard stock solution to make five standard concentrations to established calibration curves. The prepared working solution was also meant for recovery analysis. The apparent recovery experiments were determined on the least contaminated samples in triplicates, the matrices were grouped into three to achieve this, i.e,cereals (millet, rice, white maize, and yellow maize), cassava (attieke and cassava flakes) and vegetables (melon, okra, gnangnan, haricot and chilli). Exactly 0.5 g for each sample (considered as blank) wasspiked with 100 µl of multi-analytes standard, thoroughly mixed and kept at room temperature in a fume cupboard for at least 30 minutes to establish equilibration between the analytes and the matrix. Subsequently, 2 ml of extraction solvent was added and placed on rotary shaker for 90 minutes. Afterward, 300 µl of extracts were diluted with equal volume of dilution solvent, then vortexed and 5 µl was injected into LC-MS/MS. Linearity was determined by least square regression of a 5-point matrix calibration curve. Coefficient of determination (R^2) and retention time (RT) for each mycotoxin were evaluated. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the signal-noise ratio of the matrix-matched sample. All the analytes detected were quantified by comparing their peak area on the calibration curve of the equivalent mycotoxin standard. LOD and LOQ were estimated using the lowest concentration of the spiked sample estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively. The apparent recovery for each mycotoxin was calculated using Equation 1

$$\%Recovery = \frac{A - B}{C} \times 100 \qquad 1$$

Where A is the concentration of the toxins in spiked samples

B is the concentration of the toxins in non-spiked samples

C is the concentration of the toxin spiked

3.2.9 Data analysis

Concentrations of fungi were calculated for all the food commodities by dividing the total number of CFU by the number of plate volume. The colonies were evaluated and expressed as CFU/g. Mean, range and percentage values of mycotoxins generated in this study were evaluated using SPSS version 15.0 for Windows (15.0, Vista Hotfix Applied) and Microsoft Excel 2010.

CHAPTER FOUR
RESULTS

This chapter presents a summary of the fungi identified and mycotoxins they produce in food commodities purchased from local markets in Ivory Coast. Co-occurrences of fungi as well as mycotoxin are also reported.

4.1 Isolation and identification of fungi

Using morphological character, a total of 227 fungi species were isolated from the 70 food commodities considered in this study from local markets in Ivory Coast (Table 4.1). These include fungi belonging to genera *Aspergillus, Fusarium* and *Penicillium* (with raw data presented in Appendix A, Table 1). Some of the 227 fungal isolates were screened using ITS to confirm the identity of culture as presented in Figs. 4.2 and 4.3 and Tables 4.3 and 4.4.

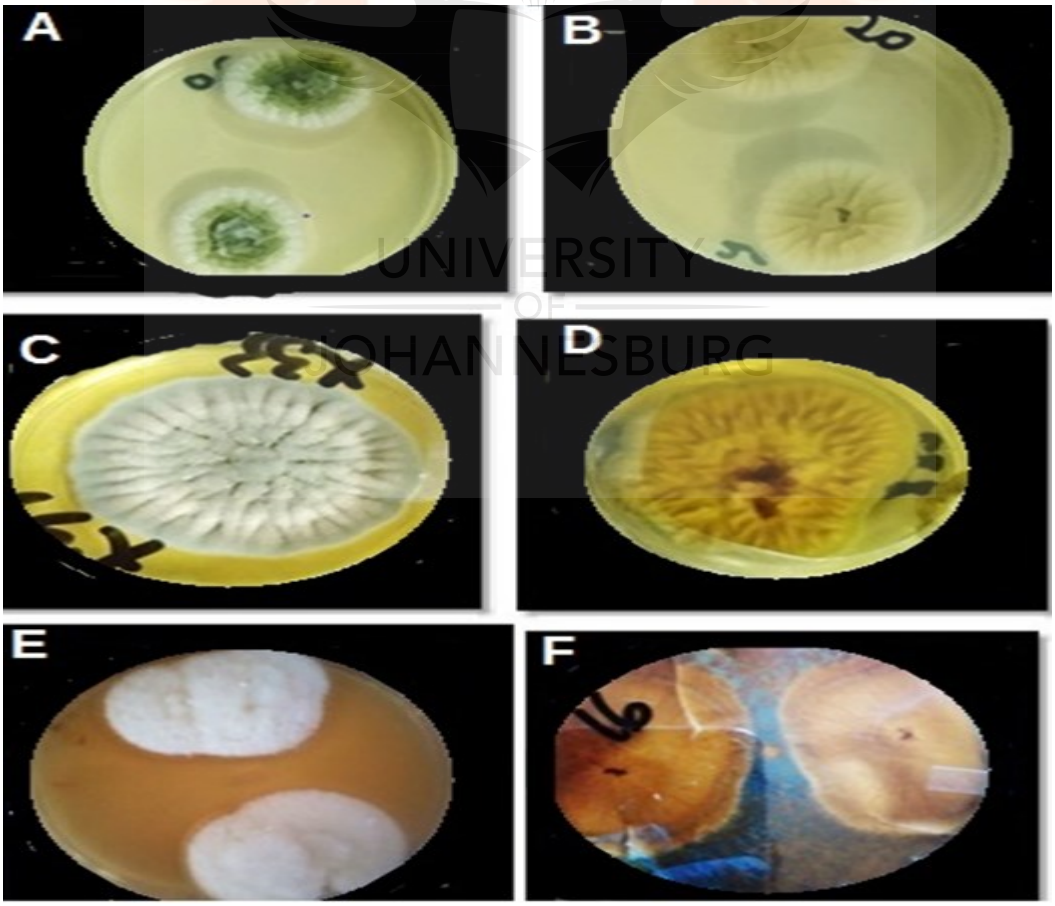

Figure 4.1 Macroscopical characteristics of isolated fungi on media (A-B): *A. flavus* colony features on PDA medium, front and reverse, (C-D): P. *expansum* colony features on MEA medium, front and reverse, (E-F): F. *oxysporum* colony features on CYA medium, front and reverse

Mycological analysis revealed the contamination levels of fungi in food commodities. The total fungal counts represented as colony forming units and the mean for various foodcommodities are shown in Table 4.2. The fungal load (CFU/g) of the species were extremely variable among the markets, ranging from 1.0×10^3– 8.6×10^5 CFU/g. Highest fungal load (CFU/g) was recorded in Cocody (8.6×10^5 CFU/g) followed by 7.5×10^5 CFU/gin Yopougon and 7.0×10^5 CFU/g in Adjame.

The fungal load in all the food samples were determined and the highest contamination levels were found in white maize ranging from 5.0×10^4– 8.6×10^6 followed by yellow maize which ranged from 7.0×10^4 – 5.4×10^5, rice (1.3×10^3 – 4.0×10^5) and millet (2.0×10^3 – 4.0×10^5). High contamination levels were also detected in melon (4.7×10^4 – 5.8×10^5) and haricot (4.0×10^3 – 2.5×10^5). The samples with low fungal load included cassava products, gnangnan and dried okra.

Table 4.1Total fungal load and isolated fungal genera from Ivorian food commodities

Food Samples	Market	+ve sample	CFU/g Range	Mean	Isolated fungal genera
Attieke	Adjame	3	3.5×10^5 - 5.0×10^5	4.15×10^5	*Aspergillus, Penicillium,* and *Emerica.*
	Yopougon	2	1.1×10^5 - 2.3×10^5	1.70×10^5	*Aspergillus* and *Penicillium*
	Cocody	2	8.0×10^4 - 3.1×10^5	2.30×10^5	*Aspergillus, Penicillium* and *Rhizopus*
Chilli	Adjame	1	1.0×10^4 - 1.0×10^5	4.20×10^4	*Aspergillus, Chaetomium, Fusarium*, and *Penicillium*
	Yopougon	2	1.0×10^4 - 1.4×10^5	7.15×10^5	*Aspergillus* and *Penicillium*
	Cocody	2	5.0×10^3 - 1.2×10^5	5.59×10^4	*Aspergillus, Chaetomium, Fusarium, Penicillium* and *Rhizopus*
Gnangnan	Adjame	2	2.0×10^3 - 1.1×10^5	7.03×10^4	*Aspergillus* and *Penicillium*
	Yopougon	2	1.0×10^3 - 2.5×10^5	8.70×10^4	*Aspergillus, Chaetomium* and *Penicillium*
	Cocody	2	3.0×10^3 - 3.3×10^4	1.88×10^4	*Aspergillus, Penicillium* and *Trichoderma*
Haricot	Adjame	2	3.1×10^4 - 2.4×10^5	1.33×10^5	*Aspergillus* and *Penicillium*
	Yopougon	1	4.0×10^4 - 2.5×10^5	1.20×10^5	*Aspergillus, Fusarium* and *Penicillium*
	Cocody	1	4.0×10^3 - 3.2×10^4	1.44×10^4	*Aspergillus, Chaetomium* and *Fusarium*
Melon	Adjame	2	3.1×10^5 - 5.8×10^5	4.46×10^5	*Aspergillus, Fusarium* and *Penicillium*
	Yopougon	2	6.1×10^4 - 3.1×10^5	3.07×10^5	*Aspergillus, Emerica* and *Penicillium*
	Cocody	1	4.7×10^4 - 3.2×10^5	2.07×10^5	*Aspergillus, Fusarium, Penicillium* and *Trichoderma*
Millet	Adjame	5	3.0×10^3 - 3.9×10^4	3.94×10^4	*Aspergillus, Alternaria, Epicoccum* and *Fusarium*
	Yopougon	4	2.0×10^3 - 2.1×10^5	4.05×10^4	*Aspergillus, Fusarium* and *Penicillium*
	Cocody	3	1.3×10^4 - 4.0×10^5	1.42×10^5	*Aspergillus, Cladosporium* and *Penicillium*
Rice	Adjame	2	9.0×10^3 - 4.0×10^5	1.70×10^5	*Aspergillus, Alternaria, Emerica, Fusarium* and *Penicillium*
	Yopougon	2	1.3×10^3 - 1.2×10^5	3.50×10^4	*Aspergillus, Alternaria, Chaetomium, Epicoccum, Fusarium* and *Penicillium*
	Cocody	2	1.0×10^4 - 1.2×10^5	5.46×10^4	*Aspergillus, Alternaria, Fusarium* and

					Penicillium
White maize	Adjame	2	6.5×10^4 - 7.0×10^5	2.67×10^5	*Aspergillus, Alternaria, Emerica, Rhizopus* and *Penicillium*
	Yopougon	2	5.0×10^4 - 7.5×10^5	1.42×10^6	*Aspergillus, Alternaria, Emerica* and *Fusarium*
	Cocody	2	9.0×10^4 - 8.6×10^5	3.67×10^5	*Aspergillus, Epicoccum* and *Fusarium*
Yellow maize	Adjame	2	7.0×10^4 - 5.2×10^5	2.56×10^5	*Aspergillus, Chaetomium, Epicoccum, Fusarium, Penicillium* and *Rhizopus*
	Yopougon	2	2.5×10^5 - 5.4×10^5	3.23×10^5	*Aspergillus, Alternaria, Epicoccum, Fusarium* and *Rhizopus*
	Cocody	2	1.7×10^5 - 4.1×10^5	2.80×10^5	*Aspergillus* and *Cladosporium*
Cassava flakes	Adjame	5	5.1×10^4 - 3.1×10^5	4.39×10^4	*Aspergillus* and *Penicillium*
Okra	Adjame	7	3.0×10^3 - 2.3×10^5	9.02×10^4	*Aspergillus, Cladosporium* and *Penicillium*

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

Table 4.2 Incidence rate of fungal contamination of food commodities from Ivory Coast

Isolates	Location	Attieke	Cassava	Chilli	Gnangnan	Haricot	Melon	Millet	Okra	Rice	Wmaize	Ymaize	Total
Aspergillus species	Adjame	1(25)	6(75)	3(50)	4(67)	6(67)	3(60)	7(44)	13(81)	3(37)	4(40)	2(25)	52
Penicillium species	Adjame	-	2(25)	1(17)	2(33)	3(33)	1(20)	3(18)	2(13)	2(25)	3(30)	2(25)	21
Fusarium species	Adjame	-	-	1(17)	-	-	1(20)	4(25)	-	1(13)	-	1(12)	8
Other species	Adjame	3(75)	-	1(17)	-	-	-	2(13)	1(6)	2(25)	3(30)	3(38)	15
		4	8	6	6	9	5	16	16	8	10	8	96
Aspergillus species	Yopougon	2(67)	-	4(57)	4(57)	1(20)	4(67)	6(50)	-	7(54)	3(42)	2(29)	33
Penicillium species	Yopougon	1(33)	-	2(29)	1(14)	2(40)	1(17)	1(8)	-	1(8)	-	-	9
Fusarium species	Yopougon		-	-	-	2(40)	-	4(33)	-	2(15)	2(29)	2(29)	12
Other species	Yopougon	-	-	1(14)	2(29)	-	1(17)	1(8)	-	3(23)	2(29)	3(42)	13
		3	-	7	7	5	6	12	-	13	7	7	67
Aspergillus species	Cocody	2(50)	-	5(46)	3(60)	2(40)	2(40)	7(70)	-	4(36)	4(67)	6(86)	35
Penicillium species	Cocody	1(25)	-	2(18)	1(20)	-	1(20)	2(20)	-	3(27)	-	-	10
Fusarium species	Cocody	-	-	1(9)	-	2(40)	1(20)	-	-	3(27)	1(16)	-	8
Other species	Cocody	1(25)	-	3(27)	1(20)	1(20)	1(20)	1(10)	-	1(9)	1(16)	1(14)	11
		4	-	11	5	5	5	10	-	11	6	7	64

Aspergillus species:*A. aculeatus, A. candidus, A. clavatus, A. flavus, A. fumigatus, A. niger, A. ochraceus, A. parasiticus, A. tamari, A. terreus, and A. tubingensis. Penicillium* species*: P. brevicompatum, P. chrysogenum, P. citrinum, P. crutosum, P. expansum, P. griseofulvum, P. italicum, P. oxalicum, P. paneum, P. restricum. Fusarium* species:*F. oxysporum, F. Poae, F. culmorum, F. moniliforme.* Other species*:Alternaria alternate.Cladosporium. C. brasilenses., E. nidulans., Epicoccun nigrum., Rhizopus stolonifer.Trichoderma lixii.* The figures in brackets are the % relative frequency of the isolates.

Aspergillus species were found to be present in 92 % of analysed food commodities. In order of prevalence, *Aspergillus s*pecies (53 %) were the main contaminants recovered in this study followed by *Penicillium* (18 %) and *Fusarium* species (12 %). Incidence rate of 17% was recorded for all other genera, which include *Alternaria*, *Cladosporium, Chaetomium, Epicoccum, Emerica, Tricoderma* and *Rhizopus.* The highest incidences of *Aspergilus* species were recorded in yellow maize and okra with 86 and 81 % respectively (Table 4.2). *Penicillium* species were detected in haricot at an incidence rate of 40 %, which was the highest incidence recorded among the *Penicillium* species in this study. Table 4.3 present the relative frequency of the isolates per each market. The total number of fungal isolates from Adjame was 96, while Yopougon had 67 isolates and Cocody 64 isolates. Samples that had the highest number of isolates in Adjame were millet and okra with 16 (17 %) fungal isolates each followed by white maize with 10 (10.4 %) isolates. In Yopougon, millet and rice had highest isolates record of 12 (18 %) and 13(19.4 %) isolates, respectively, while in Cocody, chilli and rice had 11(17 %) isolates followed by millet with 10 (16 %) isolates. In all the markets, *Aspergillus* species were found to be more prevalent than other fungal genera.

Based on phylogenetic analysis as shown in Fig 4.2, the isolates AAF105 and AAFP1015 were grouped in the same clade with confirmed *A. fumigatus* (MN634633) in the section *fumigati.* The isolate AAFP106 and AAFP1061were 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* (JQ675308) in the section *flavi*. In a similar analysis, AAF106 was grouped in the same clade as *P. crustosum* (KT735107) at the bootstrap value of 86 % (Fig 4.3). 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*.

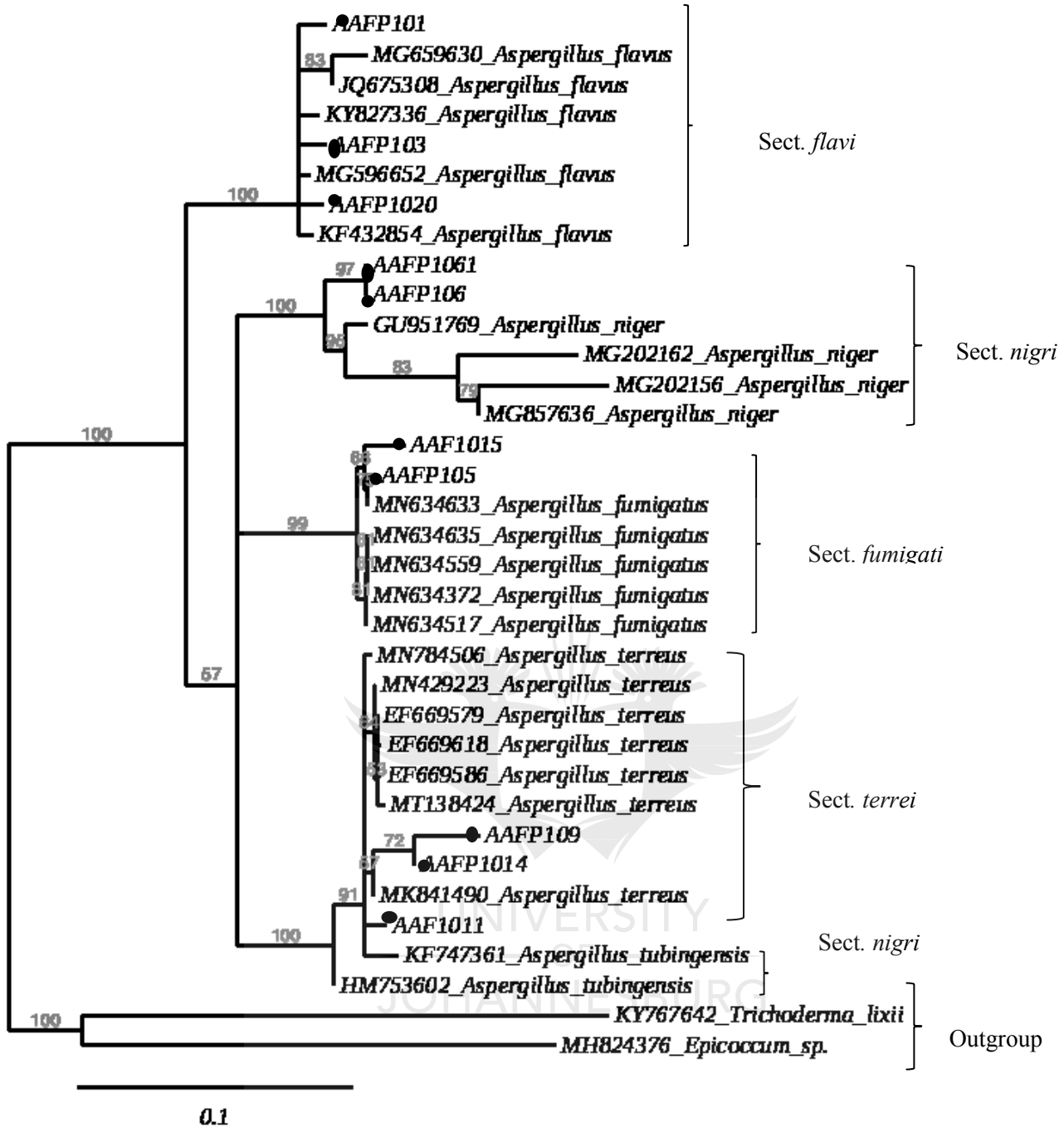

Figure 4.2Phylogeny of ITS data set showing the phylogenetic relationship of species and section within the *Aspergillus* subgenus *Circumdati* and *Fumigati*. The isolated species from this study were indicated with bold round bullet and with code prefixed with AAF. Bootstrappercentages of the maximum 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*.

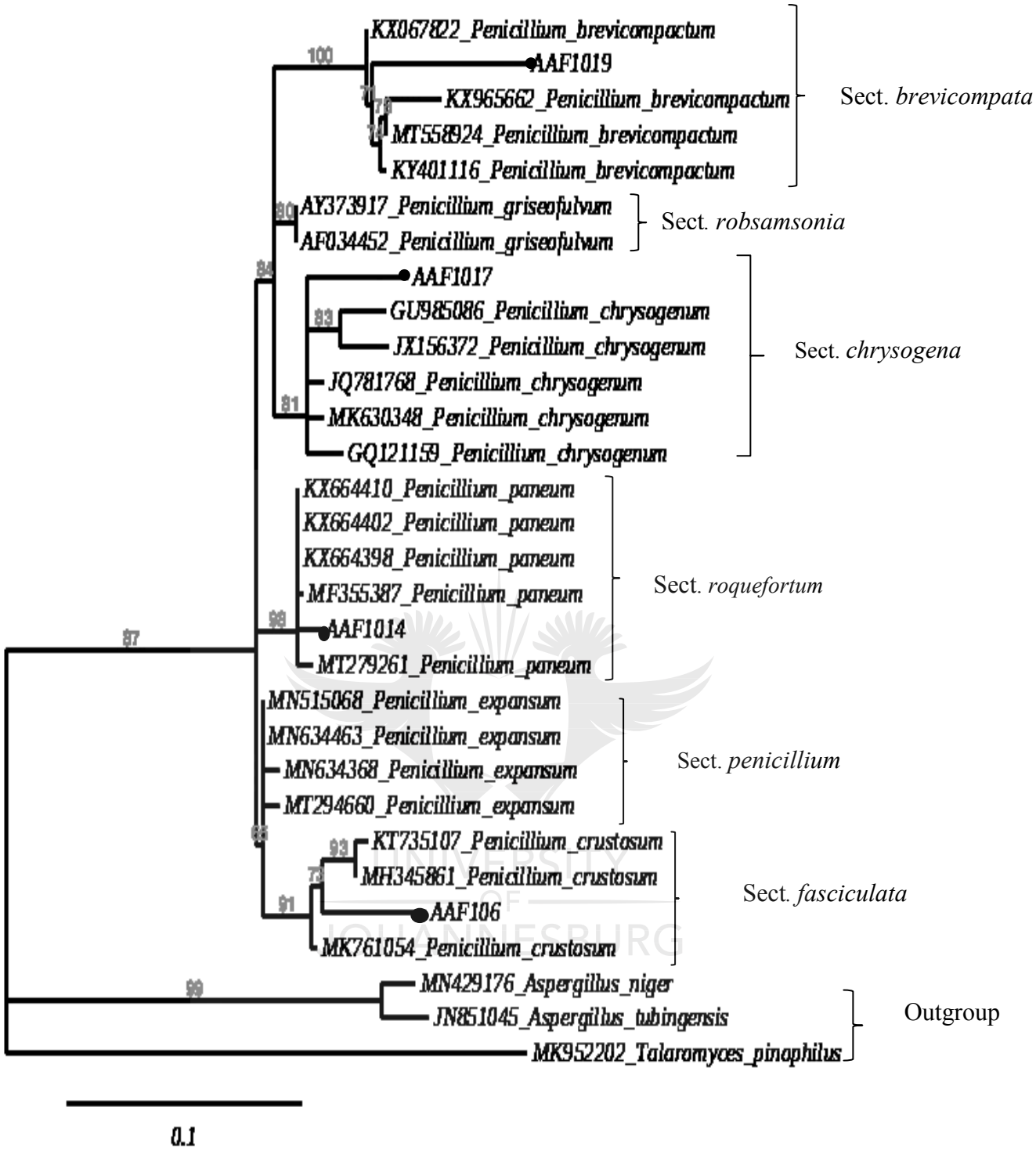

Figure 4.3 Phylogeny ofITS data set showing the phylogenetic relation of species and section within the *Penicillium* subgenus*Aspergilloides* and *Penicillium*. The isolated species from this study were indicated with bold round bullet represented with code AAF. Bootstrap percentages of the maximum 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.*

Table 4.3 ITS-based identification of some *Aspergillus* species isolates analysed in this study in relation to Gen Bank

Species name	Accession No	Geographic Region	Reference
Africa clade			
A. flavus	MG596652	South Africa	Adetunji *et al*. (2017)
A. flavus	MG659630	Zimbabwe	Nleya *et al*. (2018)
	AAFP101	Ivory Coast	
	AAFP103	Ivory Coast	
	AAFP1020	Ivory Coast	
A. fumigatus	MN634635	South Africa	Selvarajan *et al*. (2019)
A. fumigatus	MN634559	South Africa	Selvarajan *et al*. (2019)
A. fumigatus	MN634372	South Africa	Selvarajan *et al*. (2019)
A. fumigatus	MN634517	South Africa	Selvarajan *et al*. (2019)
	AAF105	Ivory Coast	
	AAFP105	Ivory Coast	
	AAF1015	Ivory Coast	
A. niger	MG857636	Nigeria	Mgbeahuruike *et al*. (2018)
A. niger	MG202162	Egypt	Hamad *et al*. (2017)
A. niger	MG202156	Egypt	Hamad *et al*. (2017)
	AAFP106	Ivory Coast	
	AAFP1014	Ivory Coast	
A. terreus	MN784506	Egypt	El-Khouly *et al*. (2019)
A. terreus	MT138424	Egypt	El-Sayed and Mohamed (2017)
	AAFP109	Ivory Coast	
	AAF1011	Ivory Coast	
Asia clade			
A. flavus	KY827336	China	Zhang (2017)
A. flavus	KF432854	India	Divakara *et al*. (2015)
A niger	GU951769	China	Wang *et al*. (2011)
A. terreus	MK841490	India	Savaranan *et al*. (2019)
A. terreus	MN429223	China	An (2019)
A. tubingensis	KF747361	India	Amutha (2013)
A. tubingensis	HM753602	Saudi Arabia	Singh *et al*. (2010)
America clade			
A. terreus	EF669618	USA	Peterson (2008)
A. terreus	EF669579	USA	Peterson (2008)
A. terreus	EF669586	USA	Peterson (2008)
Outgroup			
Epicoccum sp.	MH824376	China	Xue (2018)
T. lixii	KY767642	South Africa	Jacobs (2017)

Table 4.4 ITS-based identification of some *Penicillium* species isolates analysed in this study in relation to Gen Bank

Species name	Accession No	Geographical location	Reference
Africa clade			
P. expansum	MN515068	Egypt	Abdel-Aziz (2019)
P. expansum	MN 634368	South Africa	Selvarajan *et al*. (2019)
P. expansum	MN634463	South Africa	Selvarajan *et al*. (2019)
	AAF1019	Ivory Coast	
	AAF1017	Ivory Coast	
	AAF106	Ivory Coast	
	AAF1014	Ivory Coast	
America clade			
P.brevicompactum	AF125943	USA	Peterson (2004)
P.brevicompactum	DQ123637	USA	Peterson (2004)
P.brevicompactum	KX667822	Colombia	Galeano-vanegas and Cendales (2016)
P.brevicompactum	KX965662	Colombia	Galeano-vanegas and Cendales (2016)
P.brevicompactum	KY401116	Chile	Vera *et al.* (2017)
P. griseofulvum	AF034452	USA	Haugland *et al*. (2004)
P. griseofulvum	AY373917	USA	Peterson (2004)
P. paneum	MT279261	Mexico	Camarena-Pozos *et al*. (2020)
P. paneum	KX664402	USA	Mayer *et al*. (2016)
P.panuem	KX664410	USA	Mayer *et al*. (2016)
P. paneum	KX664398	USA	Mayer *et al*. (2016)
Asia clade			
P.brevicompactum	MT558924	China	Zhu (2020)
P. chrysogenum	GU985086	China	Gao *et al*. (2011)
P.chrysogenum	JQ781768	Turkey	Ozdil *et al*. (2017)
P. chrysogenum	GQ121159	India	Anupama *et al*. (2009)
P. chrysogenum	JX156372	China	Zhang *et al*. (2013)
P. chrysogenum	MK630348	China	Jianwen *et al*. (2019)
P. crustosum	MT002724	India	Saravanan *et al.* (2019)
P. crustosum	MK761054	Italy	Quaglia *et al*. (2019)
P. crustosum	KT735861	China	Hou (2015)
P. expansum	MT294660	China	Salman *et al*. (2021)
Outgroup			
A. niger	MN429176	China	An (2019)
A. tubingensis	JN851045	China	Zhang *et al.* (2012)
T. pinophilis	Mk952202	China	Zhang (2019)

4.2 Mycotoxin Contamination

All the parameters needed for detection and quantification of mycotoxin were assessed.The performance of quantification of the mycotoxin showed linearity in the correlation with R^2 for all the levels of mycotoxins ranging from 0.9775 – 1.000 (Table 4.6). The retention time for all the analysed mycotoxins varied from 6.58 to 10.35 mins. The sensitivity of the method was assessed by the determined LOD and LOQ, in which LOQ ranged from (0.139 to 3.44 µg/kg) in cereals, between (0.076 – 5.36 µg/kg) for cassava and in vegetables, it ranged from (0.06 to 5.69 µg/kg) (Table 4.7).The LOD and LOQ were then used as a tool to confirm the quantification reliability of the mycotoxin data acquired in this study.

Table 4.5 Quantification parameters based on calibration curve

Compound	R^2	Calibration levels (µg/kg)	Retention time
3-ACDON	0.9957	10, 100, 250, 500, 1000	6.71
15-ACDON	0.9851	10, 100, 250, 500, 1000	6.58
AFB_1	0.9954	1, 10, 100, 500, 1000	8.29
AFB_2	0.9985	1, 10, 100, 500, 1000	8.03
AFG_1	0.9989	1, 10, 100, 500, 1000	7.55
AFG_2	0.9775	1, 10, 100, 500, 1000	7.31
OTA	0.9952	1, 10, 125, 250, 500	10.35
T-2	1	10, 50, 100, 250, 1000	9.76

R^2: Coefficient of determination. ppb: parts per billion. 3-ACDON:3-acetyldeoxynivalenol.15-ACDON: 15-acetyldeoxynivalenol. AFB_1: aflatoxin B_1.AFB_2: aflatoxin B_2. AFG_2: aflatoxin G_2. OTA: ochratoxin A.

Table 4.6 LOD and LOQ for the analysed mycotoxins in food commodities from Ivory Coast

Mycotoxin	Cereals n =30 (millet, rice, and maize)				
	N[a]>LOQ	% N[b]	LOD (µg/kg)	LOQ (µg/kg)	% Recovery
3-ACDON	7	37	7.60	25.20	75
15-ACDON	13	40	3.40	11.37	81
AFB_1	17	63	0.04	0.13	100
AFB_2	19	86	1.70	5.52	103
AFG_1	17	66	0.04	0.13	98
AFG_2	16	86	1.90	6.49	103
OTA	19	70	0.17	0.52	99
T-2	6	40	1.03	3.44	100
Mycotoxin	Cassava products n =13 (attieke and cassava flakes)				
	N[a]>LOQ	% N[b]	LOD (µg/kg)	LOQ (µg/kg)	% Recovery
3-ACDON	3	31	5.90	19.65	87
15-ACDON	1	31	14.20	47.33	83
AFB_1	1	54	0.24	0.81	90
AFB_2	7	62	0.024	0.076	96
AFG_1	3	54	0.041	0.14	89
AFG_2	6	54	0.16	0.54	90
OTA	5	46	0.37	1.12	98
T-2	3	23	1.60	5.36	97
Mycotoxin	Vegetables n = 27 (melon, okra, gnangnan, haricot and chilli)				
	N[a]>LOQ	% N[b]	LOD (µg/kg)	LOQ (µg/kg)	% Recovery
3-ACDON	9	38	1.10	3.69	85
15-ACDON	7	38	1.80	5.98	77
AFB_1	13	65	0.22	0.69	105
AFB_2	20	77	0.20	0.67	76
AFG_1	17	76	0.11	0.36	102
AFG_2	16	69	0.34	1.15	81
OTA	15	73	0.57	1.92	94
T-2	1	23	1.71	5.69	74

N[a] >LOQ: Number of samples greater than LOQ. %N[b]: Percentage of positive samples. LOD:Limit of detection. LOQ: Limit of quantification. 3-ACDON:3-acetyldeoxynivalenol.15-ACDON: 15-acetyldeoxynivalenol. AFB_1: aflatoxin B_1. AFB_2: aflatoxin B_2. AFG_2: aflatoxin G_2.OTA: ochratoxin A.

The incidence rates and levels of mycotoxins in food are presented in Table 4.8. Approximately 98 % of the samples analysed were contaminated with atleast one mycotoxin. High occurrences of total aflatoxins were found in millet and rice, with levels ranging from (0.11 – 66.1µg/kg) and (0.04 – 66.8 µg/kg), respectively.The incidence rate of AFB_1 was high in chilli, haricot, white maize and yellow

maize at 100% contamination rate followed by gnangnan and rice with an incidence rate of 83 % for each sample type. Total aflatoxins found in all the analysed food samples ranged from (2.7 – 24.76 µg/kg) for attieke, (1.04 – 21.76 µg/kg) for cassava flakes, chilli (16.12 – 89.24 µg/kg), haricot (9.45 – 37.45 µg/kg), gnangnan (12.06 – 29.66 µg/kg), melon (6.62 – 49.89 µg/kg), millet (7.66 – 87.04 µg/kg), okra (1.64 – 51.44 µg/kg), rice (4.99 – 84.71 µg/kg), white maize (20.08 – 46.38 µg/kg) and in yellow maize, levels varied from (6.96 – 101.55 µg/kg) (Table 4.8). Aflatoxin B_1 contamination in all analysed cereals was high. In maize, AFB_1 concentration found in the samples ranged from (0.04 – 5.31 µg/kg) with mean of 1.68 µg/kg. Thirty-three percent (n=4) of the analysed maize samples exceeded maximum level of 2 µg/kg for AFB_1 in unprocesses cereals stipulated by EU (Gbashi *et al.,* 2020). In millet and rice, 8 % and 33 % of the analysed samples were contaminated with AFB_1 above 2 µg/kg maximum limits set by EU respectively (Cheli *et al*., 2014). Chill had 20 % (n=1) contamination by AFB1 which is above EU regulatory standard of 5 µg/kg (EC, 2006).

High occurrence of OTA was recorded in white maize and rice, with 83 and 80% incidence rate was recorded in each chilli and melon, respectively. Ochratoxin A was present in the analysed maize samples at levels ranging from (0.17 – 7.33 µg/kg) of which 8 % of the positive sample exceeded 5 µg/kg EU regulatory standards (Gbashi *et al*., 2020). In millet and rice, 50 % of the samples were contaminated by OTA at concentrations above the maximum level of 5 µg/kg stipulated by the EU, while in chilli, 20 % sample had OTA above regulatory limit of 20 µg/kg set by EU for *Capsicum* species (EC, 2006).

Absence of T-2 was recorded in cassava flakes, chilli, melon and yellow maize, but high incidence of 67 and 66% were found in millet and white maize, respectively. T-2 was present in few samples at the levels ranging from <LOQ – 32.23 µg/kg. These contaminations are negligible as they were very low when compare with EU regulatory limits of 100 and 200 µg/kg in cereals and maize respectively (Cheli *et al*., 2014). Also, 83% of DON derivatives were found in rice and 50% in millet, which was the highest percentages recorded in this study. Cereals contamination by 3-ACDON and 15-ACDON were far below

the maximum limits stipulated by EU regulatory standard of 1250 and 1750 µg/kg for cereals and maize respectively (EC, 2006). However, there is no stipulated limit by EU for haricot, cassava prodcuts, okra and gnangnan. The result revealed as well that AFs and OTA were the main contaminants of cereals than other agricultural products analysed in this study.

Table 4.7 Occurrence of mycotoxins in food commodities from Ivory Coast

Sample	Description	Analytes							
		3-ACDON	15-ACDON	AFB_1	AFB_2	AFG_1	AFG_2	OTA	T.2
Attieke	% contamination	57	0.00	57	88	71	88	71	57
	Range (µg/kg)	<LOQ -64.20	<LOQ	0.43 - 1.12	2.1 -9.30	<LOQ - 0.75	<LOQ -3.50	<LOQ - 11.30	<LOQ -5.43
	Mean (µg/kg)	38.60	0.00	0.67	6.30	0.27	8.31	5.43	3.52
Cassava flakes	% contamination	0.00	0.00	50	33	33	16	16	0.00
	Range (µg/kg)	0.00	<LOQ	0.71 - 1.80	<LOQ - 1.98	<LOQ - 0.15	<LOQ - 5.11	<LOQ - 2.27	0.00
	Mean (µg/kg)	0.00	0.00	1.29	10.02	0.12	5.11	2.27	0.00
Chilli	% contamination	60	0.00	100	100	100	60	80	0.00
	Range (µg/kg)	39.3 - 64.30	<LOQ	<LOQ-17.50	6.3 -39.20	<LOQ - 3.82	11.5 - 28.70	9.27 - 25.49	0.00
	Mean (µg/kg)	53	0.00	4.59	20.70	2.95	18.20	14.73	0.00
Gnangnan	% contamination	50	67	83	67	100	67	67	33
	Range (µg/kg)	<LOQ -54.30	<LOQ - 31.60	<LOQ-2.20	1.11 - 11.10	0.81 - 3.85	6.6 - 16.70	2.13 - 17.12	3.56 - 8.00
	Mean (µg/kg)	36	16.55	1.26	6.38	2.80	11.35	8.41	5.78
Haricot	% contamination	25	75	100	75	25	25	75	0.00
	Range (µg/kg)	<LOQ -46.60	<LOQ - 36.30	0.95 - 1.30	8.5 - 30.70	<LOQ - 1.51	<LOQ - 3.90	<LOQ - 9.38	<LOQ
	Mean (µg/kg)	46.60	29.17	18.57	1.86	1.51	3.90	4.87	0.00
Melon	% contamination	20	40	0.00	100	80	100	80	0.00
	Range (µg/kg)	< LOQ – 34	35.1 – 44	0.00	6.1 - 27.70	<LOQ - 3.89	<LOQ -8.30	7.5 - 29.28	0.00
	Mean (µg/kg)	34	19.75	0.00	12.06	1.51	7.53	18.27	0.00
Millet	% contamination	50	50	25	92	83	92	67	67
	Range (µg/kg)	<LOQ -55.10	<LOQ - 40.40	0.75 - 2.51	4.9 - 14.90	0.11 - 3.78	<LOQ -6.10	0.38 - 24.68	1 - 7.39
	Mean (µg/kg)	29	23.10	1.61	14.83	1.95	7.53	10.97	3.37
Okra	% contamination	43	0.00	43	57	57	71	57	29

	Range (µg/kg)	<LOQ -36.70	0.00	0.78 - 2.43	<LOQ - 25.10	<LOQ - 3.31	<LOQ-20.60	<LOQ -17.29	<LOQ
	Mean (µg/kg)	10.10	0.00	1.41	9.55	2.08	7.48	5.15	0.00
Rice	% contamination	33	83	83	83	33	100	83	0.00
	Range(µg/kg)	33.5 - 49.50	16.6 - 39.90	0.25 - 3.57	<LOQ - 13.70	0.04 - 0.64	<LOQ-66.80	3.4 - 7.48	0.00
	Mean (µg/kg)	41.50	30.10	1.96	7.46	0.34	19.42	5.96	0.00
White maize	% contamination	33	50	100	100	50	50	83	66
	Range (µg/kg)	26.2 - 39.60	<LOQ - 37.90	0.27 - 5.31	<LOQ - 22.60	0.11- 0.25	<LOQ - 27	0.6 - 7.33	1.14 - 32.23
	Mean (µg/kg)	32.90	28.37	1.83	11.45	0.18	22.07	3.76	13.08
Yellow maize	% contamination	0.00	16	100	83	83	100	66	0.00
	Range (µg/kg)	<LOQ	<LOQ - 27.90	0.04 - 3.41	<LOQ - 38	0.52 - 1.75	<LOQ-33.60	0.17 - 4.96	0.00
	Mean (µg/kg)	0.00	27.9	1.52	26.26	1.14	10.36	2.69	0.00

Mean: average contamination of the samples including both positive (>LOQ) and (<LOQ) negative samples. % contamination: Percentage of the sample contaminated with each mycotoxin. LOQ - Limit of quantification. ND - not detected. 3-ACDON:3-acetyldeoxynivalenol. 15-ACDON: 15-acetyldeoxynivalenol. AFB_1: aflatoxin B_1. AFB_2: aflatoxin B_2. AFG_2: aflatoxin G_2. OTA: ochratoxin A.

Table 4.9 reveals the distribution of mycotoxins in all three markets where the samples were collected. In the Cocody market, there was high frequency (82.3 and 100 %) of AFB_1 And AFB_2, respectively, when compared to other markets. Also, high incidences of 3-ACDON and 15-ACDON were detected in Cocody with 57.1 and 47 %, respectively. OTA contamination in samples from Yopougon with 78.9 % frequency was relatively higher than that of Cocody (64.7 %) and Adjame (58.8 %). However, 41.2 % of the analysed samples from Adjame were contaminated with T-2, while in Cocody, 17.6 % incidence was noted.

Table 4.8 Distribution of mycotoxins in food commodities across three markets in Ivory Coast

	Adjame n=34			Yopougon n=19			Cocody n=17		
Analyte	Mean (µg/kg)	% F	Max.conc	Mean (µg/kg)	%F	Max.conc	Mean (µg/kg)	%F	Max.conc
3-ACDON	20.04	38.23	66.70	51.20	42.10	64.30	38.95	57.10	46.60
15-ACDON	Y29.55	38.24	44.00	22.18	47.30	40.40	24.21	47.00	36.30
AFB_1	1.67	55.90	5.31	1.08	57.89	3.41	2.71	82.30	17.52
AFB_2	14.41	70.60	65.00	11.99	84.20	38.00	11.79	100.00	39.20
AFG_1	1.28	64.70	3.89	2.32	68.40	3.85	1.52	70.50	3.83
AFG_2	11.99	73.50	66.10	15.79	89.40	66.80	15.31	52.90	33.60
OTA	6.58	58.82	19.28	10.37	78.90	29.28	8.20	64.70	17.12
T-2	7.19	41.18	32.23	6.46	21.00	17.06	2.84	17.60	4.28

%F: Percentage of frequency of mycotoxin. Max conc: Maximum concentration of the mycotoxin in the sample. 3-ACDON:3-acetyldeoxynivalenol. 15-ACDON: 15-acetyldeoxynivalenol. AFB_1: aflatoxin B_1. AFB_2: aflatoxin B_2. AFG_2: aflatoxin G_2.OTA: ochratoxin A.

The co-occurrence of mycotoxins was revealed in the analysed samples as noted in Table 4.9. Co-occurrence of AFs occurred at the highest level in yellow maize and rice than any other sample type (Appendix B – Table 2). The highest co-occurrence frequency of mycotoxins was that of AFs and OTA occur at the highest level in 19 of the 27 (70 %) of vegetable samplesas well as AFs and DON that co-occurred in 17/27 (63%) of the same sample type. AFs, DON and OTA occurred most frequently in cereals (13%) and vegetables (13%), meanwhile, cassava products (5%) were the least contaminated sample types analysed.

Table 4.9 Co-occurrence of mycotoxins in food commodities from Ivory Coast

Co-contaminants	Cereals	Cassava	Vegetables
AFB_1+AFB_2+AFG_1+AFG_2	29	10	26
AFs + DON	7	7	17
AFs + OTA	6	6	19
AFs + T.2	3	3	6
AFs+DON+OTA	13	5	13
AFs+DON+T-2	9	1	5
DON+OTA+T-2	6	2	5

3-ACDON: 3-acetyldeoxynivalenol.15-ACDON: 15-acetyldeoxynivalenol. AFB_1: aflatoxin B_1. AFB_2: aflatoxin B_2. AFG_2: aflatoxin G_2. OTA: ochratoxin A.

CHAPTER FIVE
DISCUSSION AND CONCLUSIONS

5.1 Discussion

Fungi and their metabolites are a threat to human health and the economy of any given country (Lacey *etal*., 2015) especially within the sub-Saharan Africa region, with prevailing favourable environmental conditions that encourage their proliferation. Their presence in agricultural products has a great impact on imperilling the quality of food.Therefore, there is a need to frequently examine and control this organism and to achieve this,their presence must be frequently evaluated in food commodities. Consequently, the present study analysed the food commodities from Ivory Coast to assess the degree to which these agricultural products are naturally contaminated with fungi and mycotoxins and it was observed that 69/70 of the analysed samples were contaminated with atleast one fungal species and mycotoxin.Various food commodities randomly selected and purchased from Adjame, Yopougon and Cocody markets with more samples from Adjame based on their availability during the sampling process and also, Adjame being more of an agricultural zone than the two other urban cities enabled the availability of these food commodities relatively easy.

5.2 Fungi isolation and identification

Mycological screening of the different food commodities showed a variation of filamentous fungi.Most of the samples analysedwere of low quality, as they contained more than one fungal species. In all the analysed samples, cereals have high levels of contamination (Table 4.1), while cassava products have the least contamination level. The low account of fungal contamination in cassava flakes may be due to the fermentation process occurring during the preparation of the product. According to Karlovsky *et al*. (2016) fermentation affects reducing the toxigenic microflora species in food samples, thus increase the food quality. A similar effect was observed in a survey carried out by Njobeh *et al*. (2009), it was reported that the cassava flakes and flour were found to have low contamination levels in Cameroun.

The total isolates recovered from this study majorly belong to three fungal genera,which include Aspergillus*, Penicillium* and *Fusarium.*Occurrences of these species have been reported in various agricultural and agricultural-based products (Tournas and Niazi, 2017; Olagunju *et al., 2018*).The co-occurrence of these fungal species had been recognized as causative agent responsible for the production of mycotoxins in maize and rice in Ivory Coast (Manizan *et al.,* 2018). Most of these fungi are widely recognized as producers of mycotoxin in the field and under storage conditions (Egbuta *et al*., 2015). 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 store houses are not well ventilated, thus improving the growth of these toxigenic fungi (Mohapatra *et al*., 2018). Also, climatic conditions are of paramount importance in fungal contamination of food commodities (Reddy *et al*., 2011).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 (Mohapatra *et al*., 2018). 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.*

The sample analysed were highly contaminated with *Aspergillus* species in comparison with other genera, this is in agreement with Olagunju *et al*. (2018) who presented a similar report on rice, spice (chilli), maize and Bambara groundnut, which are more contaminated by *Aspergillus* species (52.8%) followed by *Penicillium* species (31.8%). The high incidence of *Aspergillus* species may be as a result of their sporulating ability in the environment. In cereals (maize, rice and millet), the most prevalent fungi were *Aspergillus and Penicillium Fusarium. Aspergillus* species are the most prevalent genera in all the analysed cereals samples. The high occurrence rate of *Aspergillus* spp. was also recorded with 86 % incidence in yellow maize. This was also confirmed by several other pieces of research who reported the

high incidences of *Aspergillus* species ingrains and grains based products (Egbuta *et al*., 2015; Tournas and Niazi, 2017).

A survey conducted by Nyinawabali (2013) revealed the high incidence of *Aspergillus* species in the analysed grains samples. The total numbers of *Penicillium* species detected were 40 isolates, making them the most frequently isolated species next to *Aspergillus* species as shown in Table 4.2. Some of the isolated species are known to be a major producer of mycotoxins making their presence in food commodities a thing of concern. Various researches have reported their occurrence in food commodities especially in Africa (Boli *et al*., 2013; Koffi-Nevry *et al*., 2013; Egbuta *et al*., 2015; Tang *et al*., 2019).The occurrence of *Fusarium* species in food was confirmed in this study but at a low rate, about 20 out 70 analysed samples were contaminated with *Fusarium* this means most *Fusarium* species are more active in the field than the stores (Jedidi *et al*., 2018).

The incidence of fungal contamination in food commodities from the three markets as shown in Table 4.2 revealed the number of isolates from Adjame as 96, Yopougon (67) and Cocody (64). Literally speaking, the frequency of isolates from Adjame is higher than other markets but considering the number of samples, 49% of sample were selected from Adjame and recovered 42.3% of isolates from it. From Yopougon, 27% of sample and 29.5% of isolates were detected and from Cocody 24% of food samples were selected and discovered 28.2% isolates, which means fungi frequency in Cocody and Yopougon was higher than that of Adjame when considered the number of selected samples.

A sub-sample of isolates were screened to confirm the identity of the isolated fungi using ITS. Identities of *Aspergillus* species belong to the subgenus *circumati* and *fumigate* were revealed in Fig 4.2 and Table 4.3. Some of the isolated species from this study showed a strong relationship with their relative species from Gen Bank. The phylogeny showed a close genetic relationship between *Aspergillus* sect. *nigri* and *terrei* than *flavi* and *fumigati*. Also, Fig 4.3 and Table 4.4 revealed the identity of the isolated *Penicillium* species with most of the Africa clade used from Gen Bank were isolated by Selvarajan *et al*. (2019).

5.3 Mycotoxin contamination of food commodities from Ivory Coast

Since these fungi isolated from the analysed samples are capable of producing mycotoxin, therefore the mycotoxin analysis of the samples was performed.The efficiency of the method used for this analysis was tested and the method demonstrates good compliance with the guidelines of the European Commission, EC (EC, 2006). For all the mycotoxins evaluated in this analysis, the linearity of the coefficient of determination (R^2) ranged from 0.9775 to 1.00 as presented in Table 4.5. This is consistent with the acceptance range of (R^2) linearity equal to or greater than 0.95 by the International Harmonization Conference (ICH). To determine the sensitivity of the method, the LOD and LOQ of the method for each matrix were evaluated.The LOD and LOQ were relatively low, making it suitable for the quantification process as shown in Table 4.6. The recovery rates for all eight mycotoxins analysed were in line with European commission regulation for mycotoxin of 60 – 130 % recovery rate (EC, 2006).

The incidence rate and level of mycotoxin in 'attieke', cassava flakes (cassava products), chilli, 'gnangnan', haricot, melon, okra (vegetables), millet, rice, white and yellow maize (cereals) were presented in Table 4.7. In cereals, all the analysed samples were positive for atleast one of the eight tested mycotoxins. The same was observed in all vegetables analysed, while in cassava products 83% of analysed samples contain mycotoxins (Appendix II: Table 1).This study revealed the presence of AFs in the analysed sample. The high degree of aflatoxins in foodstuffs reveals that human populations were everyday exposed to these toxins. These exposures can aflatoxicosis or even liver cancer (Atanda *et al.,* 2013). Aflatoxins contamination was relatively high in the cereals compare to other samples. In cereals (maize and millet), 33 % of the each analysed samples have AFB_1 contamination level above 2 µg/kg acceptable by EC regulations (EC, 2006). This is similar to studies carried out in Ivory Coast by Manda *et al*. (2016) who reported a 27% of analysed sample being contaminated with AFB_1 maximum levels above EU regulations. Another research from Ivory Coast by Manizan *et al*. (2018) showed that 58% and 24% of the analysed maize and rice samples were contaminated with AFB_1 above EU regulations.

Among the cereals, yellow maize samples were found to have the highest contamination level of total aflatoxins while cassava products have the lowest concentration of aflatoxins. Similar to the situation in Tanzania, a surveycarried out by Sulyok *et al*. (2015) on cassava showed a low number of samples contaminated with aflatoxins and fumonisins. Cassava products, on the other hand, have the record of no or low levels of aflatoxin contamination, this was confirmed by the survey performed in Benin by Adjovi *et al*. (2014). In their report, AFB_1 been considered the most toxic aflatoxin was not detected in all the analysed cassava chips. Another research by Manjula *et al*. (2009) showed a low level of AFB_1 in cassava chips and flour with contamination levels varied from 0.3-44 µg/kg and 0.1 – 13 µg/kg respectively, which was close to what found in this survey. In chilli, 20% of the analysed samples have AFB_1 above 5 µg/kg acceptable limit of AFB_1 in spices by the EU. The record of high level of AFs indicates that there could be a possible risk in emerging AF-induced liver cancer as a result of frequent consumption of these commodities.

Ochratoxin A,(OTA), is among the most toxic member within the ochratoxin. OTA was present in 65% of the analysed sample. The positive samples in cereals have OTA contamination ranged from 0.17 – 24.68 µg/kg related to reports from Ivory Coast by Kouadio *et al*.(2014) and Manda *et al*. (2016) who found average concentration of 21 µg/kg OTA in maize flour and range of 57.48 – 174 µg/kg in chilli respectively. In the current study, total of 57% (17 samples) cereals (Appendice B: Table 1) have OTA the concentration above EU regulation limit of 5 µg/kg (FAO, 2004). OTA concentration was also low in cassava products as detected in AFB_1. However, high concentration of OTA contamination was recorded in melon, this was a bit differ from the survey carried out by Adedeji *et al.*(2017) in Nigeria where all analysed melons have the concentrations below the limit of detection. OTA has been classified as group 2B causative agent of human carcinogen by IARC (Arroyo-Manzanares*et al*., 2018) is of great concern as high concentration of OTA in melon may cause a serious health problem in Ivory Coast is one of their major foods consumed frequently.

The incidence rate of DON derivatives i.e 3-ACDON and 15-ACDON were analysed in this study. The highest level of 3-ACDON was found in chilli from Yopougon (Max conc: 66.7 µg/kg) and for 15-ACDON maximum concentration of 43.8 µg/kg was found in white maize from Yopougon. These metabolites are considered to be prevalent in the temperate region than the tropical region (Hickert *et al*., 2015), thus the relatively low frequency and concentration below regulationdetected in this study were expected. DON is known to be an immunosuppressant toxin, causing nausea and vomiting, especially in infants. Trichothecenes toxin (T-2) was not detected in over 70% of the analysed samples, which include all cassava flakes, chilli, melon, rice and yellow maize. The absence of T-2 was also reported by Bankole *et al*. (2010) in maize from Nigeria.

The distribution pattern of mycotoxin contamination in foods from all three markets as shown in Table 4.8 revealed the similarity in the type of mycotoxin found across the markets, although the incidence rates and contamination level of mycotoxin in Cocody and Youpougon were higher than Adjame. For instance, the mean of AFs for Adjame was 29.35µg/kg while Yopougon had 31.18 µg/kg and Cocody 31.33µg/kg. Similar trend was also detected in OTA. Adjame is known to be farming zone than Yopougon and Cocody.They (Yopougon and Cocody) are more reliant on other food places, that is food is transported and stored for longer periods, which can enhance the fungal growth and their metabolites (Jedidi *et al*., 2018). Adjame has the most important transport hub in the country serving the different regions of Ivory Coast as well as neighbouring countries. Food transportation in and out of Adjame is easy and more frequent than Yopougon and Cocody, thus food commodities are not remain in the stored for long period than expected to enhance the mycotoxin production. Transportation is one the major factor that increases the risk of microbial food contamination (Ackerley *et al*., 2010) that inadequate transport system can enhance the fungal growth and mycotoxin contamination. However, access to good transport system Adjame can actually contribute to low fungi count recorded compare to Youpougon and Cococy. Cocody is an upmarket area where food is not easily accesible and affordable for an average Ivorian. Longterm storage can contribute to high levels of fungal and mycotoxin contamination.

The co-occurrence of mycotoxins in cereals, cassava products and vegetables (Table 4.9) may be as a result of colonization of a single fungus that produces different mycotoxins or by the involvement of different fungi that produce different mycotoxin. This conforms with the various researches conducted in Ivory Coast where the co-occurrence of mycotoxins in agricultural products like cereals, chilli, spices, and peanut butter was reported (Kouadio *et al*., 2014; Manda *et al*., 2016; Manizan *et al*., 2018; Yapo *et al*., 2020). Most of this co-contaminant is potentially dangerous and it should not be neglected. The combined effects of these toxins can be synergistic and antagonistic effects on human and animals. Fungi have been recognized not only for their ability to cause systemic diseases in plant and animals but because they are toxigenic in producing significant mycotoxins. As the high frequency of co-occurrence of toxigenic species was recorded in this study, the co-occurrence of mycotoxins in many food samples may therefore increase the severity of the health problems caused by such contamination.

5.4 Conclusions

This study aimed to investigate the incidence of fungi and mycotoxin contamination with food commodities from Ivory Coast. Results obtained show that food commodities were contaminated with fungi and mycotoxin. Three major fungi genera: *Aspergillus, Penicillium* and *Fusarium* were responsible for producing these secondary metabolites. The high co-contamination of these toxigenic fungi and associated mycotoxins in food commodities considered in this study is of concern to Ivory Coast and the region at large. Although, some analysed foods were void of *Alternaria*, *Cladosporium, Chaetomium, Epicoccum, Emerica, Fusarium,Penicillium,* and *Tricoderm* spp., 98% of analysed food crops had at least one fungus and its metabolites. 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. Although all markets had food commodities contaminated with various fungi and their metabolites, the incidence rates in Cocody and Yopougon were higher than at the Adjame market. Furthermore, there is need for more research on fungi and mycotoxins, and the legislation to establish more regulations for mycotoxins in food commodities in Ivory Coast.

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APPENDIX
Appendice A

Table 1: Fungi isolated from the selected food commodities in Ivory Coast

	Adjame	Yopougon	Cocody
Rice 1	*A. aculeatus*	*A. niger*	*Alternaria alternate*
	A. flavus	*A. flavus*	*A. clavatus*
	F. graminearum	*A. fumigatus*	*A. fumigatus*
		C. brasilenses	*P. expansum*
		A. parasiticus	*F. solani*
		P. crustosum	*F. proliferatum*
		F. grminearum	
Rice 2	*A. niger*	*Alternaria alternate*	*A. niger*
	Alternaria alternate	*A. fumigatus*	*A. tubingensis*
	Emerica nidulans	*A. niger*	*F. solani*
	P. phirophilum	*A. aculeatus*	*P. oxalicum*
	P. crustosum	*F. culmorum*	*P. crustosum*
		Epicoccum nigrum	
White maize 1	*A. flavus*	*A. aculeatus*	*A. flavus*
	A.terreus	*A. terreus*	*A. ochraceus*
	Emerica nidulans	*F. graminearum*	*Epicoccum nigrum*
	P. oxalicum	*F. culmorum*	
	P. phirophilum		
White maize 2	*A. niger*	*A. niger*	*A. flavus*
	A. flavus	*Alternaria alternate*	*A. fumigatus*
	P. decumbens	*E. nidulans*	*F. oxysporum*
Yellow maize 1	*A. aculeatus*	*A. flavus*	*A. aculeatus*
	E. nigrum	*R. stolonifer*	*A. niger*
	P. restricum	*Alternaria alternate*	*A. tubingensis*
	R. stolonifer	*F. graminearum*	*Cladosporium*
Yellow maize 2	*A. flavus C. brasilenses*	*A. niger*	*A. aculeatus*
	F. oxysporum	*Epicoccum nigrum*	*A. fumigatus*
	P. phirophilum	*F. moniliforme*	*A. niger*
Haricot 1	*A. flavus*	*A. flavus*	*A. niger*

	A. fumigatus	*F. proliferatum*	*A. parasiticus*
	A. niger	*F. solani*	*C. brasilenses*
	P. decumbens	*P. phirophilum*	*F. solani*
	P. restricum	*P. restricum*	*F. proliferatum*
Haricot 2	*A. flavus*	NSS	NSS
	A. fumigatus		
	A. niger		
	P. phirophilum		
Attieke 1	*R. stolonifer*	*A. flavus*	*A. niger*
	A. flavus		*P. paneum*
			R. stolonifer
Attieke 2	*E. nidulans*	*A. niger*	*A. flavus*
		P. citrinum	
Attieke 3	*R. stolonifer*	NSS	NSS
Chilli pepper 1	*A. flavus*	*A. flavus*	*A. parasiticus*
	A. niger	*A. parasiticus*	*A. niger*
	A. terreus	*Alternaria alternate*	*C. brasilenses*
	C. brasilenses	*P. restricum*	*F. proliferatum*
	F. solani		*P. restricum*
	P. restricum		*R. stolonifer*
Chilli pepper 2	NSS	*A. flavus*	*A. clavatus*
		A. parasiticus	*A. fumigatus*
		P. phirophilum	*A. tubingensis*
			C. brasilenses
			P. restricum
Cassava flakes 1	*P. chrysogenum*	NSS	NSS
	A. niger		
Cassava flakes 2	*A. flavus*	NSS	NSS
	A. niger		
Cassava flakes 3	*A. clavatus*	NSS	NSS
Cassava flakes 4	ND	NSS	NSS
Cassava flakes 5	*A. niger*	NSS	NSS
Cassava flakes 6	*A. niger*	NSS	NSS
	P. griseofulvum		
Gnangnan 1	*A. niger*	*A. flavus*	*A. niger*
	P. citrinum	*A. terreus*	*Trichoderma lixii*
	C. brasilenses		
	P. crustosum		
Gnangnan 2	*A. flavus*	*A. fumigatus*	*A. flavus*
	A. fumigates	*A. parasiticus*	*A. fumigatus*
	A. niger	*C. brasilenses*	*P. phirophilum*
		P. oxalicum	
Millet 1	*A. tamari*	*A. terreus*	*A. candidus*
	Alternaria alternate	*P. oxalicum*	*A. parasiticus*
	A. terreus	*F. oxysporum*	*A. ochraceus*

	F. oxysporum		*Cladosporium*
Millet 2	*A. clavatus*	*A. flavus*	*A. clavatus*
	F. oxysporum	*A. clavatus*	*A. candidus*
	Epicoccum nigrum	*F. proliferatum*	*A. aculeatus*
Millet 3	*A. clavatus*	*A. terreus*	*A. flavus*
	F. poae	*F. proliferatum*	*P. expansum*
	P. decumbens		*P. restricum*
Millet 4	*A. parasiticus*	*A. clavatus*	NSS
	P. chrysogenum	*E. nidulans*	
	P. italicum	*F. graminearum*	
		A. ochraceus	
Millet 5	*A. aculeatus*	NSS	NSS
	A. parasiticus		
	F. proliferatum		
Melon 1	*A. niger*	*A. aculeatus*	*A. aculeatus*
	A. terreus	*A. niger*	*A. fumigatus*
	F. proliferatum	*E. nidulans*	*F. oxysporum*
		P. phiropilum	*P. brevicompactum*
			Trichoderma lixii
Melon 2	*A. clavatus*	*A. fumigatus*	*NSS*
	P. chrysogenum	*A. clavatus*	
Okra 1	*A. fumigatus*	NSS	NSS
	P. phirophilum		
Okra 2	*A. fumigatus*	NSS	NSS
	A. parasiticus		
	A. tamari		
	A. tubingensis		
	Cladosporium		
Okra 3	*A. candidus*	NSS	NSS
	A. niger		
	P. decumbens		
Okra 4	*A aculeatus*	NSS	NSS
	A. niger		
Okra 5	*A. niger*	NSS	NSS
	A. parasiticus		
Okra 6	*A. flavus*	NSS	NSS
Okra 7	*A. niger*	NSS	NSS

ND: Not detected, NSS: No Sample Selected, Numbers represent the amount of sample selected from each market.

Appendice B

Table 1: Mycotoxin concentration level in food commodities from Ivory Coast

Sample	Market	AFB_1	AFB_2	AFG_1	AFG_2	OTA	3-ACDON	T-2	15-ACDON
AT11	Adjame	0.491	8.9	0.05	10.6	8.65	5.9	ND	33.1
AT12	Adjame	ND	9.3	0.43	13.5	ND	ND	1.61	ND
AT13	Adjame	ND	2.1	ND	0.16	11.33	ND	5.43	31.9
AT21	Yopougon	0.43	ND	0.746	13	3.95	64.3	ND	ND
AT22	Yopougon	ND	9	0.07	1.6	0.36	ND	ND	ND
AT31	Cocody	0.63	3.6	ND	ND	2.86	ND	ND	23.8
AT32	Cocody	1.12	4.9	0.04	11	ND	45.6	ND	ND
CF11	Adjame	ND	0.24	ND	5.11	ND	ND	ND	14.2
CF12	Adjame	1.35	ND	ND	ND	ND	ND	ND	ND
CF13	Adjame	0.71	ND	0.09	ND	ND	ND	ND	ND
CF14	Adjame	ND	ND	ND	ND	ND	ND	ND	ND
CF15	Adjame	1.81	ND	0.15	ND	ND	ND	ND	ND
CF16	Adjame	ND	19.8	ND	ND	2.27	64.3	ND	37.7
CH11	Adjame	3.531	17	0.11	14.4	ND	ND	ND	ND
CH21	Yopougon	0.5	6.3	3.69	11.5	25.49	66.7	ND	1.8
CH22	Yopougon	0.21	31.9	3.68	28.7	9.27	ND	ND	ND
CH31	Cocody	1.18	9.1	3.42	ND	9.38	ND	ND	ND
CH32	Cocody	17.516	39.2	3.83	ND	14.77	39.3	ND	ND
GN11	Adjame	0.34	4.3	0.81	11.4	ND	23.1	ND	ND
GN12	Adjame	1.34	ND	2.93	ND	2.21	30.6	8	2.3
GN21	Yopougon	0.67	4.4	3.85	10.7	2.13	54.3	3.56	2.6
GN22	Yopougon	ND	11	3.72	6.6	ND	ND	ND	31.6
GN31	Cocody	2.21	1.11	3.76	16.7	12.18	ND	ND	29.7
GN32	Cocody	1.75	11.1	1.75	ND	17.12	ND	ND	ND
H11	Adjame	1.02	30.7	ND	3.9	4.65	ND	ND	14.9
H12	Adjame	0.95	ND	ND	ND	9.38	ND	2.45	36.3
H21	Yopougon	1.31	8.5	ND	ND	ND	ND	ND	ND
H31	Cocody	1.07	16.5	1.51	ND	0.58	46.6	1.71	36.3
M11	Adjame	ND	8.5	ND	11.2	17.16	ND	ND	4.4
M12	Adjame	ND	6.1	3.89	18.3	ND	ND	ND	35.1
M21	Yopougon	ND	10.5	0.18	0.34	19.12	34	ND	ND
M22	Yopougon	ND	27.7	0.54	3.4	29.28	ND	ND	ND
M31	Cocody	ND	7.5	1.427	4.4	7.5	ND	ND	ND
MT11	Adjame	ND	9.4	1.408	1.9	19.28	ND	1.39	ND
MT12	Adjame	1.57	5.2	ND	14.1	6.05	ND	1.59	ND
MT13	Adjame	ND	14.9	3.78	14.4	ND	19.5	4.69	ND
MT14	Adjame	0.75	7	0.43	66.1	ND	14.6	7.39	ND
MT15	Adjame	ND	65	0.25	4.8	4.13	25.5	1	10.2
MT21	Yopougon	ND	7.3	2.56	26.1	24.68	ND	4.08	ND

MT22	Yopougon	ND	24	3.53	5.8	0.38	ND	ND	34.1
MT23	Yopougon	ND	10.7	3.38	18.9	10.47	35	ND	22.2
MT24	Yopougon	ND	ND	3.65	15.6	14.74	55.1	ND	40.4
MT31	Cocody	2.51	8.9	0.11	18	ND	ND	ND	ND
MT32	Cocody	ND	4.9	ND	ND	8.03	24.3	4.28	14.9
MT33	Cocody	ND	5.8	0.36	2.7	ND	ND	2.54	16.6
O11	Adjame	0.78	ND	2.66	20.6	17.29	36.7	4.08	ND
O12	Adjame	1.02	0.4	3.31	ND	1.22	ND	2.39	ND
O13	Adjame	2.43	ND	ND	ND	0.75	1.1	ND	ND
O14	Adjame	ND	25.1	2.178	0.51	ND	ND	ND	ND
O15	Adjame	ND	12.5	ND	5.1	1.34	ND	ND	ND
O16	Adjame	ND	ND	0.15	4.1	ND	ND	ND	ND
O17	Adjame	ND	0.2	ND	7.1	ND	10.1	ND	ND
R11	Adjame	ND	5.9	0.639	3	7.48	ND	ND	16.6
R12	Adjame	0.61	10.5	0.04	9.8	3.4	ND	ND	39.9
R21	Yopougon	3.41	ND	ND	3.6	7.43	49.5	ND	ND
R22	Yopougon	0.25	5.5	ND	66.8	ND	33.5	ND	39.7
R31	Cocody	3.57	1.7	ND	29.1	ND	ND	ND	21.5
R32	Cocody	1.94	13.7	ND	4.2	5.52	ND	ND	32.6
WM11	Adjame	5.31	17.3	0.19	18	2.96	26.2	1.9	ND
WM12	Adjame	0.27	1.7	0.11	27	3.04	39.6	32.23	37.9
WM21	Yopougon	0.31	4.1	ND	21.2	0.6	ND	17.06	43.8
WM22	Yopougon	0.87	3.3	ND	ND	4.87	ND	1.14	3.4
WM31	Cocody	1.94	22.6	0.25	ND	7.33	ND	ND	ND
WM32	Cocody	2.33	19.7	ND	ND	ND	ND	ND	ND
YM11	Adjame	3.41	63.9	1.525	8.3	2.71	ND	ND	ND
YM12	Adjame	1.72	ND	1.75	6.33	0.17	7.6	ND	ND
YM21	Yopougon	1.33	3.8	0.597	4.1	ND	ND	ND	ND
YM22	Yopougon	2.53	38	ND	2.6	2.91	ND	ND	ND
YM31	Cocody	0.11	6.4	1.286	33.6	ND	ND	ND	ND
YM32	Cocody	0.04	19.2	0.52	7.2	4.96	ND	ND	27.9
	Mean	1.798818	13.12018	1.602894	13.08137	8.073478	34.12	5.426	25.9
	Max	17.52	65	3.89	66.8	29.28	66.7	32.23	44
	Min	0.04	0.2	0.04	0.16	0.17	1.1	1.0	1.8

3-ACDON: 3-acetyldeoxynivalenol. 15-ACDON: 15-acetyldeoxynivalenol. AFB_1: aflatoxin B_1. AFB_2: aflatoxin B_2. AFG_2: aflatoxin G_2. OTA: ochratoxin A.

Table 2 Occurrence and incidence rate (%) of mycotoxins in food commodities from Ivory Coast

Food sample	3-ACDON	15-ACDON	AFB_1	AFB_2	AFG_1	AFG_2	OTA	T-2
Attieke	9	9	12	17	15	17	15	6
Cassava flakes	8	17	25	17	17	8	8	-
Chilli	8	4	20	20	20	12	16	-
Gnangnan	7	13	16	13	19	13	13	6
Haricot	5	17	22	17	5	6	17	11
Melon	5	9	-	24	19	24	19	-
Millet	9	10	5	17	16	17	13	13
Okra	12	-	12	16	16	20	16	8
Rice	7	7	17	17	17	21	14	-
White maize	6	9	19	19	9	9	16	13
Yellow maize	4	4	22	18	18	19	15	-

3-ACDON: 3-acetyldeoxynivalenol. 15-ACDON: 15-acetyldeoxynivalenol. AFB_1: aflatoxin B_1. AFB_2: aflatoxin B_2. AFG_2: aflatoxin G_2. OTA: ochratoxin A.