FUSARIUM AND MYCOTOXINS PROFILE OF MAIZE (*Zea Mays*) AND SORGHUM (*Sorghum bicolor*) GROWN IN NIGER STATE, NIGERIA

BY

BADMOS, Fatimah Omolola
MTECH/2017/SLS/6841

DEPARTMENT OF BIOCHEMISTRY
FEDERAL UNIVERSITY OF TECHNOLOGY MINNA,
NIGER STATE

APRIL, 2021

FUSARIUM AND MYCOTOXINS PROFILE OF MAIZE (*Zea Mays*) AND SORGHUM (*Sorghum bicolor*) GROWN IN NIGER STATE, NIGERIA

BY

BADMOS, Fatimah Omolola
MTECH/SLS/2017/6841

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL,
FEDERAL UNIVERSITY OF TECHNOLOGY MINNA, NIGER STATE, NIGERIA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF
MASTER OF TECHNOLOGY IN BIOCHEMISTRY

APRIL, 2021

ABSTRACT

Fusarium is a filamentous fungal found to contaminate agricultural produce and is capable of causing diseases in humans and animals. Sixty-four composite samples of different varieties of maize and sorghum were formed from 480 samples collected from both stores and market from the four micro-climatic zones of Niger State. *Fusarium* species isolation from the composite samples were carried out using plate dilution method and identification was done using identification keys and atlas. Deoxynivalenol (DON) and Nivalenol (NIV) were identified and quantified using High Performance Liquid Chromatography (HPLC) while fumonisin (FUM) and zearalenone (ZEN) were quantified using Enzyme Linked Immunosorbent Assay (ELISA). Occurrence data obtained were used to estimate exposure level and risk associated with the studied mycotoxins. *Fusarium* species isolated in maize and sorghum are *Fusarium verticilloides, F. sporotrichioides, F. graminerium, F. equiseti F. subglutinans F. nygamai, F. semitectum, F. solani and F. oxysporum*. *Fusarium verticilloides* was the leading species found in maize and sorghum with percentage frequency of 59.38 %, while *F.oxysporum* was the least species found with percentage frequency of 7.8. Deoxynivalenol was detected in 37.5 % and 31.25 % of maize and sorghum samples at concentration range of (0-342.62) µg/kg and (0-40.84) µg/kg, whereas NIV concentration was found in range of (0-28.80) µg/kg and (0-12.85) µg/kg, for maize and sorghum, with incidence rate of 28.13 % and 25.00 % respectively. Approximately 91% and 56 % of maize and sorghum samples were contaminated with FUM in concentration range of 0-4889.3 µg/kg and 0-3269.81 µg/kg respectively. ZEN contamination of maize and sorghum is in the range of (0-235.79) µg/kg and (0-277.37) µg/kg. Fifty percent of the analysed samples were co-contaminated with deoxynivalenol, nivalenol, fumonisin and zearalenone. Based on the dietary intake calculated for average consumers of maize and sorghum across the zones, exposure to FUM was found to be the highest (9125.83 µg/kg bw/day and 4604.77 µg/kg bw/day), accounting for 456.29 % (maize) and 230.24 % (sorghum) of the tolerable daily intake (TDI). The demonstrated high prevalence to this harmful and carcinogenic compound raises public health and trade concerns.

TABLE OF CONTENTS

APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AFs- Aflatoxins

AFB_1- Aflatoxin B_1

AAL- *Alternaria Alternata*

CAST- Council for Agriculture Science and Technology

DON- Deoxynivalenol

EFSA- European Food Safety Authority

FBs- Fumonisin Bs

FB_1- Fumonisin B_1

FB_2- Fumonisin B_2

FB_3-Fumonisin B_3

FB_4- Fumonisin B_4

FUM- Fumonisins

NIV- Nivalenol

ZEN- Zearalenone

IARC- International Agency for Research on Cancer

FAO- Food and Agricultural Organization of the United State

WHO- World Health Organization

OTA- Ochratoxin A

THs- Trichothecenes

DAS and MAS - Diacetoxy- and monoacetoxy-scirpenol

NEO- Neosolaniol

DNA- Deoxynivalenol

RNA- Ribonucleic acid

3-Ac-DON- 3-acetyl-deoxynivalenol

DON3G- Deoxynivalenol-3-Glucoside

ENN B- Enniatin B

MON- Moniliformin

FUS- Fusaproliferin

APS- American Phytopathological Society

FHB- Fusarium Head Blight

FR- Foot rot

RR- Root rot

CR- Crown root

ZEL- Zearalenol

ZEN-14G - Zearalenol-14- glucoside

DON-3-G – Deoxynivalenol-3-glucoside

N.D- Non-Detectable

UDP- Uridine diphosphate

ATP- Adenosine triphosphate

Z14S- Zearalenone-14-sulphate

Z14G- Zearalenone-14-glucoside

HRMS- High Resolution Mass Spectroscopy

SCF- Scientific Committee on Food

GI- Gastro-intestinal Tract

TDI- Tolerable Dialy Intake

EDI- Estimated Daily Intake

LOD- Limit of Detection

LOQ- Limit of Quatification

UV- Ultraviolet

DCM: Dichloromethane

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Fusarium is a ubiquitous filamentous pathogenic fungal species widely distributed in the soil, on subterranean and aerial plant parts, plant debris, organic substrates and air capable of causing diseases in a wide range of agricultural crops as well as animals (Piacenti *et al*., 2019). Over the decades, *Fusarium* species have become important as pathogens of human patients with compromised immune systems (Ma *et al*., 2013). They are common in tropical and temperate regions and are also found in desert, alpine, and arctic areas, where harsh climatic conditions prevail (Manshor *et al*., 2012). *Fusarium* genus is capable of producing several mycotoxins, including trichothecenes, zearalenone (ZEN), fumonisins (FUMs), which are the most economically significant mycotoxins found in maize, sorghum, rice and wheat (FAO, 2016). Consequently, seed grain infected with mycotoxins is of poorer quality and has lower yields thereby affecting productivity and trade (Pinotti *et al*., 2016).

Trichothecenes (THs) comprises large group of (about 150 related compounds) structurally related fungal metabolites that inhibit protein synthesis with consequent weight loss, abortions, feed refusals, vomiting, haemorrhaging of internal organs, immunosuppression, nervous system disturbance and blood disorder in humans and animals (Kumar *et al.,* 2008). The economically important trichothecenes include T-2, HT-2, deoxynivalenol (DON) and nivalenol (NIV) and this is so because they occur frequently in foods and feed and are capable of causing severe damage to human health (Abbas *et al.,* 2013). Fumonisins were first discovered through its association with equine leukoencephalomalacia outbreak and further investigations also found its

association with porcine pulmonary oedema in pigs (Marasas, 2001). Of major concern is the extent of association of fumonisin B_1 (FB_1) with human oesophageal cancer in part of South-Africa, liver cancers in Northern Italy (Marasas *et al.*, 2004; Sun *et al.*, 2007, Sun *et al.*, 2011) and neural tube defects in human babies (Hendricks 1999; Marasas *et al.*, 2004). Also, it has been demonstrated that fumonisin induce apoptosis in cultured human cells and in rat kidneys (Tollenson *et al.*, 1996) and is now recognised as possible human carcinogens (Group 2B) as classified by International Agency for Research on Cancer (IARC, 2002). Zearalenone being a non-steroidal oestrogenic mycotoxin adversely affect humans and animals and its metabolite has been found to bind competitively to oestrogen receptor in various species resulting in hyper-estrogenism and infertility in livestock (Cortinovis *et al.*, 2013). It has also been suggested to have a possible involvement in human cervical cancer (Zinedine *et al*. 2017).

Maize production in Africa was about 75 million tons in 2018, therefore contributing about 7.5 percent of total world maize production (Dowswell, 2019). Nigeria is the second and twelfth largest producer of maize in Africa and the world respectively (Adejuwon, 2018). The country produces about 33 million tons of the food crop and the production rate is still on the rise especially in the northern part of Nigeria due to increase in demand for food and feed, thereby making it a principal source of mycotoxins. On the contrary, sorghum is a drought resistant crop indigenous to Africa that is staple for almost a billion people in Africa, Asia and Latin America (FAOSTAT, 2018), According to same FAO document, sorghum is the 15^{th} food commodity that supplies the highest energy in terms of kcal/capita/day to the world and is the fourth most consumed cereal in Africa after maize, rice and wheat. The report also revealed that it has the 5^{th} highest food supply energy (kcal/capita/day) value in Nigeria after

cassava, yam, maize and rice. However, Nigeria was ranked the second largest producer of sorghum in the world (Edia, 2018) after USA, making it the highest annual producer of sorghum among all African countries with almost 7 million tons (Ssepuuya *et al.*, 2018), and its usefulness to both human and livestock has increased the nation's economy. Niger state grows the largest quantity of maize and it is the second largest producer of sorghum throughout the country for commercial purpose (Merem *et al*., 2017). The state experiences hot and humid climate for most part of the year, especially between fifth and tenth month of every year (29.5 ℃ and 73.1 %) which is favourable for fungal and mycotoxin growth.

There are several researches that have shown the susceptibility of maize and sorghum to fungi and mycotoxins in Nigeria (Makun *et al*., 2009, Atanda *et al.*, 2013, Adetunji *et al*., 2014; Anjorin *et al*., 2016; Chilaka *et al*., 2016; Hertveldt, 2016, Garba *et al*., 2017, Muhammad *et al*., 2019 and Onyedum *et al*., 2020). In all these works, there is still little or no available information on the level of NIV contamination in different varieties of maize and sorghum from Niger state and Nigeria at large. Except for the work of (Onyedum *et al*., 2020), there is no information on the dietary intake and risk characterization of mycotoxins in maize and sorghum from Niger State, Nigeria. Furthermore, surveillance studies need to be carried out annually due to climate change. It is on this note; that the present study was, therefore, conducted to ascertain the presence and distribution of *Fusarium* fungi and the level of contamination of fumonisins, deoxynivalenol, nivalenol, and zearalenone in this highly cultivated and consumed maize and sorghum from Niger state. This study is also designed to elucidate the possible risk associated with the ingestion of these toxins with a view to knowing the health impact associated with the consumption of the grains.

1.2 Statement of the Research Problem

The tropical condition in most Africa countries (particularly in Nigeria) together with inadequate storage facilities for foods promotes fungal growth and subsequent mycotoxin production (Kemboi *et al*., 2020). Contamination of food and food commodities with these toxins negatively affect household food safety, livelihood, animal productivity and income, leads to significant costs and huge economic losses for producing countries (Ayelign and De Saeger, 2020). Among various fungal metabolites present in food crops, *Fusarium* toxins are considered to be widespread on crops planted in the field and even during storage (Udomkun *et al*., 2017a), and their occurrence have been linked to health related problems of various magnitudes including anorexia, diarrhoea, cancer and immunosuppression (Chilaka, 2016).

Uncertainty of the occurrence estimates resulting from different threshold limits (such as lower and upper regulatory limit of European Union legislation and Codex Alimentarios standards) greatly affect the availability of toxicity data to access the likely hazard of mycotoxins (Eskola *et al*., 2020). There is also lack of awareness on the occurrence and risk of mycotoxins, poor agricultural and post-harvest practices, poor legislation and regulations which expose different populations to dietary intakes of mycotoxins (Ayelign and De Saeger, 2020). Furthermore, most African countries lack effective strategies to control the level of mycotoxins in foods and thus, this has contributed to the increase in the level of mycotoxins in Africa which surpassed the maximum limit set by the European Union.

1.3 Justification of the Study

The overarching challenges of mycotoxin contamination in food crops necessitates the development of strategies such as good agricultural practices and improved storage

facilities to be implemented to combat their effects on humans and animals. Be that as it may, there is still need for the identification and determination of mycotoxins in order to assess possible effects on consumers. To meet the needs of detecting mycotoxin contamination and/or mycotoxin concentrations in food crops at low level, an improved analytical technique involving the use of "High Performance Liquid Chromatography techniques (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA)" have received considerable attention owing to their advantages such as high selectivity, high sensitivity, better accuracy and ease of operation. Their application in the determination of these mycotoxins can help to evaluate food safety related risk, thereby serving as a guide for regulatory bodies, food and animal feed producers to safeguards consumer's health. Therefore, this study makes it more vital for the regulatory agencies such as Standard Organization of Nigeria (SON), National Agency for Food Drug Administration and Control (NAFDAC), Non-Governmental Organizations (NGO's) and Mycotoxicology Society of Nigeria (MSN) to foster on strong campaign to enlighten the farmers on unsatisfactory ways of storing their produce. Further, this research may help the regulatory agencies to come up with the method of reducing fungal and mycotoxin contamination as a short term major.While in the long-term major, it may include a combined effort between the farmers, food industries, mycotoxicologist, building engineers and policy makers on how to build up on strategies and manufacture storage facilities that is efficacious, economical, available and accessible to local farmers.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this study was to evaluate the occurrence and health impact of *Fusarium* fungi and some of their selected mycotoxins in maize and sorghum grown in Niger State, Nigeria.

1.4.2 Objectives

The above aim was achieved through the following objectives; which were to:

i. isolate and identify *Fusarium* species in maize and sorghum grains using their morphological characteristics.
ii. analyse for fumonisins, deoxynivalenol, nivalenol and zearalenone in maize and sorghum using High Performance Liquid Chromatography and Enzyme Linked Immunosorbent Assay
iii. estimate human exposure and characterise the risk from the consumption of maize and sorghum from the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fungi

Fungi are the second largest group of eukaryotic organism on earth, ranging from 1.5 to 5.1million species (Bahadur *et al*., 2015). Members of this group include yeast and moulds as well as more familiar mushrooms. These organisms are classified as kingdom fungi which are separate from plants, animals and bacteria. The major difference is that fungi produces mycelia and contain a cell wall mainly composed of chitin and chitosan. Furthermore, they have no photosynthetic function and reproduce via sporulation or through fragmenting hyphae (Lene, 2010). Fungi are usually classified into four divisions: Ascomycota (yeast or sac fungi), Zygomycota (bread molds), chytridiomycota (chytrids) and Basidiomycota (club fungi). Deuteromycota and lichens are two conventional groups, which are not recognized as formal taxonomic group. Members of the fungi kingdom play a significant role in human life and have the ability to occupy a wide variety of natural and artificial niches (Raja *et al*., 2017).

Fungi produce a wealth of natural products that have many industrial applications (Hofrichter, 2010) and are well known for their ability to produce secondary metabolite with biological activities that can be used for drug discovery. However, taxonomic identification of fungi is necessary if industrial, agrochemical or pharmaceutical products are to be derived from a fungal strain (Smith and Ryan, 2009; Aly, *et al*., 2011).

The infection of plants by various fungi not only cause reduction in crop yield and quality, but also contaminates grains with poisonous fungal secondary metabolites. Many fungi produce mycotoxins that have profound influence on agriculture, humans

and animals life. Yet, global health and socio-economic impact of diseases caused by fungi or food spoilage are under recognized and increasing (Brown *et al*., 2012a). The most important and well studied fungal genera includes *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Phoma*, *Cladosporium*, *Mucor*, *Trichoderma*, *Rhizopus* and others.

2.1.1 *Aspergillus* species

Aspergillus is a large genus of anamorphic fungi, which consist of about 300 identified species of mould. They are regarded as one of the oldest genera of fungi as described by Micheli in 1729 (Afzal *et al*., 2013). The commonest species that fall under the genus *Aspergillus* includes, *A. niger*, *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. nidulans*, *A. glaucus*, *A. ustus* and *A. terrus* (Bennett, 2010). *Aspergillus* produces a range of mycelia that are vesicle in the shape of a circle, with filamentous extensions growing out from it (Khalil *et al*., 2018). Most *Aspergilli* reproduce asexually by forming conidiosphere through a process called conidiogenis (Bennett, 2010). Basically, the conidia and conidial head structure are the most essential morphological features that are used by mycologist to identify and classify *Aspergillus* species (Khalil *et al*., 2018). The colonies are typically blue-green with a suede-like surface consisting of a dense felt of conidiospheres. Conidial heads are typically columnar (Up to 400 by 50 µm but often much shorter and smaller) and uniseriate. This class of species can be found in a variety of environments throughout the world.

Aspergillus species are widely distributed in the tropics and desert environment. It is found on decaying leaves and compost, plants, trees and grain crops (such as corn, wheat, millet, sorghum, rice and others) (Schoustra *et al*., 2019). Species of highly diverse fungal genus *Aspergillus* are well-known agricultural pests, and most importantly, producers of various mycotoxins threatening food safety worldwide. The

major mycotoxins produced by the *Aspergilli* are ochratoxin A (OTA), aflatoxins (AFs), as well as less predominant toxins such as patulin (Keller, 2015). Optimum production of AFs by *A. parasiticus, A. flavus*, and certain strains of and *A. nomius and A. termarri* is at temperature between 25-30 ℃ and kernel moisture content of about 18 % (Li *et al*., 2016). These toxins are found in different food commodities and are commonly regulated with different threshold limits depending on the matrix (Taniwaki *et al*., 2018).

2.1.2 *Alternaria* species

The genus *Alternaria* are characterized as *Alternariod hyphomycetes*, which comprise a biologically, ecologically and morphologically rich group of fungi that has suffered from taxonomic flux for many years (Lawrence *et al*., 2014). The taxonomy of these fungi has been dominantly based on conidial characters which include shapes, colour, septation and pattern of secondary sporulation, and to lesser extent on host association, biochemistry and metabolites. The morphological classification of *Alternariod hyphomycetes* has no less than 14 genera which typically produce phaeodictyospores and phaeophragmospores. Most of the alternariod species are considered to be cosmopolitan saprobes that are ubiquitous through natural and man-made environment (Woudenberg *et al*., 2015). *Alternaria* species are characterized as alternariod hyphomycetes that produce darkly pigmented multi-celled conidia that are typically dictyosporous, some phragmosporous, while some borne-singly (Inderbitzin *et al*., 2006; Lawrence *et al*., 2014).

Alternaria is a type of mould that requires very little moisture to grow. It is commonly found on basement, clothing, carpets, window and doors. Most harmful indoor mould growth starts with dust, dander and moisture. Many species of *Alternaria* are saprotrophs or plant pathogens, affecting crops in the field, causing stem and leaf spot

diseases, or spoiling the plant fruits or kernels during pre-harvest stages and reducing crop yields (Patriarca *et al*., 2019). Also, this fungus has been found to be responsible for different disease during the post-harvest shelf-life of many different horticultural products. The distribution of *Alternaria* species in a wide range of agricultural products such as cereals, fruits and vegetables is well known and it has been reported globally (Patriarca *et al*., 2019). However, there are several noteworthy examples of *Alternaria* species as major plant pathogens, but not limited to *A. bassicicola* and *A. solani* (Rodriguez *et al*., 2010).

These species (*Alternaria*) are well known for their ability to produce diverse secondary metabolites, particularly, toxins (Gill and Vasundhara, 2019). More than 70 small molecule compounds have been reported from *Alternaria*. Among them, only a few are thought to pose a risk to human health. The tetramic acid derivatives which include tenuazonic acid (TeA), perylene derivatives, altertoxins (ATXs) and the dibenzene derivatives, alternariol (AoH), altenariol monomethyl ether (AME) are considered the primary Alternaria mycotoxins because of their known toxicity and their frequent presence as natural contaminants in food.

2.1.3 *Penicillium* species

Fungi belonging to the genus *Penicillium* are ubiquitous in nature, which contains 35 accepted species (Visagie *et al*., 2014). Several of these species are common contaminants on various substrates and they are known as potential mycotoxin producers. In respect to their morphological characteristics, there colonies are usually fast growing in shades of green or sometimes white, mostly consisting of a dense felt of conidiospheres (Perrone and Susca, 2017). The chains of single-celled conidia are produced in basipetal succession from a specialized conidiogenous cell called a phialides. Such chain of conidia where the youngest conidium is at the basal end of the

chain is often regarded as “basocatenate”. In the genus *Penicillium*, phialides may be produced singly, in groups or from branched metulate, giving a brush-like appearance (Visagie *et al*., 2014).

Several novel species have been documented such as *P. isariaeforme*, *P. krugerri*, *P. verrucosum*, *P. cyclopium*, *P. parvulum*, *P. griseofulvum*, *P. buchwaldii*, *P. corvianum* (Frisvad *et al*., 2013; Visagie *et al*., 2016) and others. A quite number of *Penicillium* has been reported from saline habitats (such as saline soil, hyper saline region and saline lakes) such as *P. chrysogenum*, *P. citrinum*, *P. islandicum*, *P. italicum adametzioides* while some favour acidic or alkaline habitats. In this case, most *Penicillium* species prefer low pH and *P. chrysogenum*, *P. citrinum*, *P. oxalicum*, *P. digitatum*, and *P. flavigenum* have all been reported in acidic soil.

Further, the genus *Penicillium* has been reported from plants as phyllospheric, rhizospheric, endophytic and from different decaying fruits. The species has also been reported from different plants such as wheat, banana, giant dogwood, rice and orchid tree (Mwajita *et al*., 2014). The species of *Penicillium* are frequently encountered at post-harvest, processing and handling. The most important aspect of food spoilage caused by these organism is, however the formation of mycotoxins, which may have toxic effects on human and animal health. Toxins produced by *Penicillium* species includes, brevianamid A, citreoviridin, citrinin, cyclopiazonic acid, griseofulvin, fumitremorgin B, ochratoxin A (Waing *et al*., 2015). Studies conducted by Rao *et al*. (2013) indicated that the temperature range for the growth of *Aspergillus* ochraceus and *P. viridicatum* (prominent producers of OTA) was 8-37 ℃ and 0-31 ℃ respectively.

2.1.4 *Fusarium* species

Members of the genus *Fusarium* produce a wide array of mycelia that are cottony in nature with shades of pink, yellow and purple (Abdel-Azeem *et al*., 2019). However, this characteristic is not enough to distinguish the member of *Fusarium* genus. The main approach for the classification of *Fusarium* species is perhaps the morphology, and the primary trait for species to be placed in *Fusarium* genus is the occurrence of the asexual spores, the distinctive banana-shaped macroconidia (Moretti, 2009).

Fusarium species produces three types of spores. Some produces macroconidial, or microconidial as asexual reproductive structures, while others produces chlamydospores (Ohara and Tsuge, 2004). Septated macroconidia is mainly produced on monophialides and polyphialidees in the aerial mycelium, but also on short monophialides in specialized structures called sporodochia (Santos *et al*., 2019). Microconidia varied in shapes and sizes, and are produced in the aerial mycelium in clumps or chains, both on monophialides and polyphialides. The chlamydospores are resistance structures with thickened walls and high lipid contents. They are usually formed in the middle of the hyphae or at their termini. Hence, the variability in the shapes of microconidia remains the most crucial features in differentiating the species (Santos *et al*., 2019).

Members of the genus *Fusarium* are numerous and can be isolated from plants and soil as pathogens, endophytes and saprobes (Summerell *et al*., 2010). Some of these species includes *F. graminearium, F. verticillioides, F. equiseti, F. semitectum, F. solani, F. proliferatum, F. nygamai* and others. *Fusarium* species produces secondary metabolites (such as the trichothecenes, fumonisin, zearalenone) which vary widely in chemical form (Santos *et al*., 2019). Other secondary metabolites of potential importance include; beauvericin, culmorin, enniatins, fusaric acid, fusarin, moniliformin and others. These species are capable of colonizing small grain cereals (such as wheat, sorghum, barley

and oat), and maize. The dominant species may vary according to the type of crop species involved, the geographical region and environmental conditions (Pfordt *et al*., 2020). In general, this species prefer humid condition, that is, water activity higher than 0.86 and grow well at temperature of around 0-37 ℃. However, no *Fusarium* species is thermophilic in nature.

2.2 Mycotoxins

Mycotoxins are poisonous low molecular weight secondary metabolite that can contaminate a wide array of food or feed that are produced under appropriate environmental conditions by filamentous fungi (Binder 2007). Mycotoxin producing fungi can be categorized into either field or storage fungi. Field fungi such as *Fusarium spp*. are capable of producing mycotoxins on crops in the field, while storage fungi such as *Aspergillus* and *Penicillium* species produces mycotoxins on the crop after harvesting (Filtenborg *et al*., 1996). However, there are hundreds of fungi mycotoxins but those whose content in food is legally regulated include three major groups of toxins: aflatoxins (produced by *Aspergillus*), ochratoxins A (produced by *Aspergillus* and *Penicillium*), zearalenone, deoxynivalenol and fumonisns (produced by *Fusarium*) (Bryla *et al*., 2018).

2.2.1 History of mycotoxins

From a global perspective, it is of belief that all natural food products are safe for human consumption (Bhat *et al*., 2010). Contaminations via agriculturally important fungal toxin might result in outbreak of several diseases. Before now, these groups of toxins were first recognized as animal disease and often there was a long journey before their significance in humans was adequately understood. The colonizing fungi are capable of producing toxins and can cause serious adverse health issues in human and/or livestocks consuming the contaminated food products. As such, cases of fungal

poisoning have resulted to death of animals, but are rarely fatal to humans (Pfohl-Leszkowicz, 2002).

Even though several researchers from different part of the world have confirmed that fungal toxins are present in various food commodities, issues that are beyond the realm of normal expectations and the psychological biases still make people individually and collectively blind to uncertainty. As a consequence, the idea that fungal toxins were important was discounted. Ever since then, mycotoxins have always been "black swans". In this context, the Romans presume that the black swans did not exist, that is, it is hard-to-predict (Moore, 2014).

In the first two decades of the 21st century, it is relatively easy to identify the major mycotoxins that affect food and feed. This idea prompted the early researchers to make use of next generation sequencing combined with alpha taxonomy and reliable sequence database which inform their perspective on the presence of fungi on crops. Hence, their presence can be rapidly assessed in new species (Miller, 2016). Nowadays, the focus of modern researchers is primarily on the management of known mycotoxins emerging or re-merging due to different climatic conditions and changes in agronomic practices. Besides, genetic variability in the principal toxigenic fungi including *F.graminearum* and *A.flavus* is of great challenge (Moore, 2014).

Small grains such as barley, wheat, and rye came up from Northern part of Africa and the near east. This enabled the appearance of the first agricultural settlements around 9000 BC along the Fertile Crescent between the Euphrates and Tigris Rivers (Hillman, 1978). The need to store crops and the movement of crops outside their areas of adaptation began the issue of mycotoxins problems. The storage of cereals initiated the transition by human from hunter gatherer to cultivars, at the same time providing a large

number of new ecological niches for fungi pathogens on grain crops or saprophytes on harvested grains. All these process produces mycotoxins. For instance, climate in sites of origin of crops is dry but as the crops were moved east and north, the season became shorter, cooler and/or damper which may increases the susceptibility of the crops to fungal attack and mycotoxin production (Salamini *et al*., 2002).

In the British Isles, the predominant crops in the Neolithic age were emmer, naked barley, spelt and wheat. By Roman times, this had changed to hulled barley, oats, emmers, wheat and rye. So, rye was an important crop from the seventh century (McKerracher, 2016) such that by the middle ages, rye became dominants and way was opened for ergot of rye to become a serious problem. Even though ergot is mentioned in the Old Testament and during Roman times, the epidermics of ergotism were not reported until about 800 AD (Van Dongen and de Groot, 1995). However, the toxicity associated with ergot sclerotia in bread was not proven until 1630 (Miller, 2016) after which efforts were made to promote the sieving of sclerotia from grain used for bread making. The first pure ergot alkaloid was not reported for another 250 years (Barger, 1937). Thereafter, wheat became more important throughout the 17^{th} and 18^{th} centuries.

Cullen (1789) observed that "wheat is the farinaceous food most generally used by the better sort of people over the whole of Europe, excepting the very northern parts in which it cannot be produced, but even there, it is imported for the use of persons of condition. In the second half of the 18^{th} century, mortality rates declined in England. Matossian (1981) has suggested that this decline coincided with the change from a rye-based diet to a wheat-based diet. The disease referred to as "slow nervous fever" exhibiting the symptoms of ergotism, declined in importance as the 18^{th} century progressed. The sharp rise in human population which began at about 1750, was due to reduced mortality, and may have resulted from a change in diet from rye to wheat. This

is not because of nutritional factor, but because of a reduction in the ingestion of a potent mycotoxin.

2.2.2 Biotransformation of mycotoxins

Ingestion of contaminated food is considered as a major route for exposure to many mycotoxins. Upon ingestion, mycotoxins may induce local toxicity or cross the intestinal barrier to enter the bloodstream and reach target organs (Smith *et al*., 2018). Mycotoxins biotransformation is a complex modification which alters the structure of mycotoxins by chemical reactions within the body (Loi *et al*., 2017). It is often referred to detoxification, but biotransformation enzymes can also convert certain chemicals into highly toxic metabolites in a process known as bioactivation (See Figure 2. 1) (Beyerle *et al*., 2015). Biotransformation of mycotoxins involves two distinct stages, namely phase I and phase II. The process allows metabolites created during phase I to enter conjugation processes (phase II), but in some cases, the substances may be eliminated directly after phase I (Gajecka *et al*., 2009).

In phase 1, the mycotoxin could be oxidized, reduced, or hydrolysed based on their chemical structure. The enzymes involved in detoxification belong to the cytochrome P (CYP) superfamily. The CYP superfamily comprises of enzymes involved in oxidative metabolism, such as monooxygenases, prostaglandin synthases, amine oxidases and alcohol dehydrogenases; and reductive metabolism mainly governed by epoxide hydrolases, and aldehyde or ketone reductases (Gajecka *et al*., 2009). CYP450 enzymes play an important role in the oxidative and reductive metabolism of many endogenous or exogenous chemical compounds (Wen *et al*., 2016), including most mycotoxins. In mammals, CYPs are present in the endoplasmic reticulum and mitochondria of most cells (Beyerle *et al*., 2015). Among CYPs, CYP3A with an average content from 50–70

% of total enteric CYPs is the major subfamily expressed in the human small intestine (Sergent *et al*., 2008).

Phase II reactions are known as conjugation reactions, which usually refer to covalent binding of endogenous hydrophilic substances such as glucuronic acid and sulfate. The reactions provide more hydrophilic compounds, which are quickly eliminated. In general, phase II reactions decrease the toxicity (Beyerle *et al*., 2015). Uridine 5′-diphospho-glucuronosyltransferase (UDP-glucuronosyltransferase-UGT) and glutathione S-transferase (GST) enzymes play an important role in the phase II metabolism.

Although the liver is the main detoxification organ, extrahepatic tissues in the gastrointestinal tract (GI tract), kidney, and bladder also show metabolic activity. The GI tract is a first physical barrier for mycotoxins but it also influences the biotransformation process and bioavailability of mycotoxins in other ways. Microorganisms from guts have been reported to possess the ability to degrade mycotoxins (Du *et al*., 2017). Moreover, P-glycoprotein (P-gp) and multidrug resistance protein (MRP), members of the ATP–binding cassette (ABC) superfamily of transport proteins, are able to pump mycotoxins out of the intestinal cells, leading to limit bioavailability of the substrates (Antonissen *et al*., 2017). Both CYP450 and P-gp in the gut play a crucial role in defense mechanisms against mycotoxins that reach the intestinal mucosa.

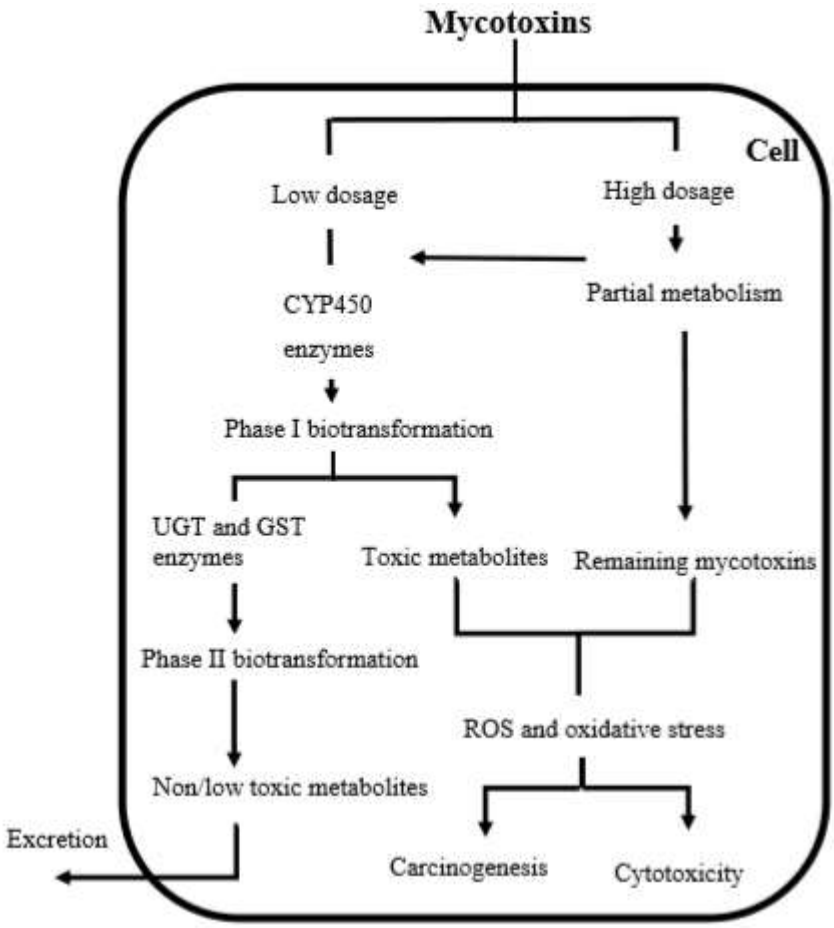


Figure 2.1: Major Biotransformation and Adverse Cellular Effects of Mycotoxins
Source: Tran *et al*. (2020)

2.2.3 General toxic effects of mycotoxins

Mycotoxins are secondary metabolites of moulds that exert toxic effect on humans and animals. The effect of these toxins on human and animal health is known as mycotoxicosis. However, the severity of each toxin depends on the toxicity of the compound, the extent of exposure, age and nutritional status of the individual and possible combined effects of other chemicals to which each person is exposed (Ulger *et al*., 2020). Therefore, involvement of mycotoxins in disease causation should be considered with urgency when several people are diagnosed with no peculiar connectivity to a known ecological agent such as micro-organism (Claeys *et al*., 2020).

Individual get exposed through ingestion of contaminated foods, but alternate route include inhalation and dermal absorption of toxicogenic moulds containing mycotoxins (IARC, 2012). It is still difficult to link the former to disease other than upper and lower respiratory tract infections, but it represent a feasible risk since growth of some trichothecene-producing fungi like stachybotrys has been documented in water damaged constructions and deleterious health effects seen in humans in contact with damp damaged buildings. Toxic effects on human and animals health are caused by important mycotoxins such as ochratoxins, aflatoxins, fumonisins and zearalenone among others (Pitt *et al*., 2012). Acute exposure to high levels of AFs causes diseases (aflatoxicosis) and death in humans. Signs of aflatoxicosis include acute hepatic necrosis, bile duct proliferation-lethargy and edema (Jin *et al*., 2021).

Just like Aflatoxins, trichothecenes have been implicated in human diseases and linked to cases of food poisoning (Pestka and Smolinski, 2005). DON has been reported as a causative agent of gastro-intestinal poisonings (Schelstraete *et al*., 2020), as well as suspected etiologic agent of gastroenteritis in children (Centre for Disease Control and Prevention (CDC), 1999). As regards the chronic effect of mycotoxins, the most critical instance is the case of AF exposure. Liu and Wu. (2010) reported that 25,200-155,000 of new hepatocellular carcinoma (HCC) cases out of 550,000-600,000 annual cases worldwide (4.6-28.2 %) maybe related to AFs exposure.

Besides, AFs and FBs have been recognized as contributors to young children's growth suppression and development of natural tube defects, respectively. Other mycotoxins such as OTA, DON, NIV, ZEN and others have also contributed to adverse health effect in human populations. Hence, strict control of food and feed, and effective public health measures should be of significant importance in reducing the risks to human and animal health.

2.3 Types of Mycotoxins

Mycotoxins consist of highly diverse molecular structures characterized by a variety of heteroatom containing functional groups. A good number of these potent chemicals can enter the food chain through contaminated cereals (such as maize, sorghum, millet) and foods obtained from animals fed mycotoxin-contaminated feeds (Tian *et al*., 2020). While several authors have extensively covered mycotoxins chemical nature (Pitt *et al*., 2012), this section briefly explain the important types of mycotoxins. Based on literature search, the most notorious and extensively researched mycotoxins includes: aflatoxins, ochratoxins, fumonisins, zearalenone, deoxynivalenol, nivalenol and T-2 toxins (IARC, 2012).

2.3.1 Aflatoxins

Aflatoxins are highly toxic, tetratogenic, mutagenic and carcinogenic compounds that are produced as secondary metabolites by fungi belonging to the genus *Aspergillus* species. Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* (O'Riordan and Wilkinson, 2008). There are various naturally occurring aflatoxins (AFs) and their metabolites (such as aflatoxin B_1, B_2, G_1, G_2, M_1 and M_2) that are capable of producing human disease (Cast, 2003). These toxins are highly present in tropical and subtropical region where humidity and temperature conditions are optimal for fungi growth and toxin production. The name aflatoxin is derived from the combination of "a" from the *Aspergillus* genus and "fla" from the species *flavus*, and toxin being regarded as poison (Fouche *et al*., 2020). Depending on the colour produced from fluorescene spectroscopy or other analytical characterization, AFs are categorized as blue (AFB_1, AFB_2) or green (AFG_1, AFG_2).

Earlier reports have confirmed that the modern era of mycotoxicology dates back to the 1960s in England, with severe outbreak of turkey "X" disease which resulted to the

deaths of more than 100,000 turkey and other farm animals (Fung and Clark, 2004). The great loss was attributable to animal feeds containing aflatoxin contaminated peanut meal. Subsequent studies affirmed that aflatoxins are potent liver toxins and liver carcinogens in a wide variety of animals, thereby causing hepatocellular carcinomas in some species at dietary levels below 1.0 µg/kg of feed. Human exposure can also results from consumption of wide range of important agricultural commodities including cereals (maize, sorghum, millet, rice) oil seeds (ground nut, soybean, cottonseed) spices (black pepper, turmeric, ginger), butter, meat, egg and milk from animals (Fung and Clark, 2004).

Successfully, invitro studies revealed the following metabolic reactions for AFB_1; reduction produces aflatoxicol (AFL), hydroxylation gives AFM_1, hydration produces AFB_2 and epoxidation produces AFB_1-2, 3-epoxide. However, the most predominant and potent hepatocarcinogen among the class of AFs is AFB_1. They have been classified as group 1 carcinogen. In general, exposure to AFs is typically by ingestion of contaminated food stuff. Dermal exposure results in slow and insignificant absorption (Afshar *et al*., 2020). Inhalation exposure in humans has not been studied due to lack of relevancy in food toxicology.

2.3.2 Ochratoxin A

Ochratoxin A (OTA) is a pentaketide derived dihydroisocoumarin moiety linked through the 12- carboxy group by a peptide bound to L-phenylalanine. Though there are other analogous of OTA which includes ochratoxin B, C and alkyl esters of ochratoxins with similar structures but are less toxic. Just like aflatoxins, OTA is the second most important mycotoxin. They are produced by the fungi *Aspergillus ochraceous* and *Penicillium verrucosum*. Some other researchers reported that isolates of *A. niger* and *A. carbonanus* are capable of producing OTA (Abarca *et al*., 2019). Ochratoxins have

been isolated from foods in both warm and cool climates and are common contaminants of grains (corn, barley, wheat) and to some extent beans (coffee, soy and cocoa), wine, beer and food from animal source particularly pork. However, the levels of OTA contamination are typically less than 200 µg/kg (da Silva *et al*., 2020).

OTA is a potent nephrotoxic compound and based on animal studies, it has been categorize as a group 2B carcinogen (IARC, 1993). There are no evident studies on skin or inhalational absorption of ochratoxins. However it has been reported that when OTA is absorbed, it has a high binding affinity for plasma protein. This was found in decreasing order of exposure to kidney, liver, fat and muscle tissue. The toxicity of OTA involve several mechanistic pathway related to the formation of DNA adducts (Haighton *et al*., 2012). OTA also distrupts hepatic microsomal calcium homeostasis by impairing the endoplasmic reticulum membrane via lipid oxidation (Tao *et al*., 2018). Remarkably, OTA is the major ochratoxin component and it is the most toxic among all other analogues. However, it has been reported that an infant could eat up to 10 kg of food contaminated with 20 µg/kg without significant adverse health effects (Freire *et al*., 2019).

2.4 *Fusarium* Mycotoxins

The most common *Fusarium* mycotoxin groups are trichothecenes, zearalenones and fumonisins; however, other mycotoxins (enniatins, moniliformin, beauvericin and fusaproliferin) can be identified in combination with the above-mentioned toxins (Jestoi, 2008). The distribution of mycotoxins in different regions is determined not only by the environmental conditions that affect *Fusarium* populations but also by endogenous and exogenous factors that can affect mycotoxin production.

2.4.1 Nature, chemistry and distribution of *Fusarium* mycotoxin

Unlike aflatoxigenic *Aspergillus* species, *Fusarium* species have been linked with temperate cereals, because these fungi require lower temperature for their growth and mycotoxin production (Mannaa and Kim, 2017). Generally speaking, these species are recognised based on morphological species concept, biological species concept, phylogenic species concept or their combinations. Recently, these classifications majorly focussed on the morphological species concept and identification of the species exclusively involves the use of morphological characterizations (Hyde *et al*., 2017). *Fusarium* species synthesize a wide array of mycotoxins of diverse structure and chemistry. The most important from the perspective of animal health and productivity are the trichothecenes, zearalenone, fumonisins and the moniliformin (Birr *et al*., 2021).

The trichothecenes are divided into four different sub-groups, with type A and B representing the most important members. The synthesis of the two types of trichothecenes appears to be specific for each *Fusarium* species. For example, production of type A trichothecenes occurs mainly in *F. culmorum* and *F. graminearum*. Besides, chemotypes are discernable within-species. Crippin *et al*. (2019) identified two chemotypes of *F. graminearum*, one producing NIV and DON, while the other synthesizes a mixture of DON and its acetylated derivatives. However, a common feature of many *Fusarium* species is that, they have the ability to synthesize zearalenone, and its co-existence with certain trichothecenes raises alarm because of its additives and synergistic effect in the aetiology of mycotoxicoses in humans and animals.

In relation to the co-presence of *Fusarium* mycotoxins, the secondary metabolism of *F. moniliforme* is relevant because it has been linked with the synthesis of at least three mycotoxins namely; moniliformin, fumonisins and fusarin C. The fumonisins (FB_1, FB_2

and FB_3) are unique in that, they are also structural congeners of the host-specific AAL-toxins produced by *Alternaria alternata* (the causative agent of alternaria stem canker of tomato) (Placinta *et al*., 1999). Specifically, *F. moniliformin* and *F. proliferatum* have recently been linked with the neural co-contamination of maize with fumonisin B_1 and two other novel mycotoxins fusproliferin and beauvericin (Ritieni *et al*., 1997). The toxin (moniliformin) is also produced by *F. oxysporum* which in addition, it is a recognised source of the mycotoxins fusaric acid and wortmannin (D'Mello and Mac Donald, 1997). Nonetheless, the diversity of *Fusarium* mycotoxin is further explained in which production of fusarochromanones (TDP-1, TDP-2 and TDP-6) by *F. equiseti* was illustrated. This species was able to synthesize some trichothecenes and zearalenone as well.

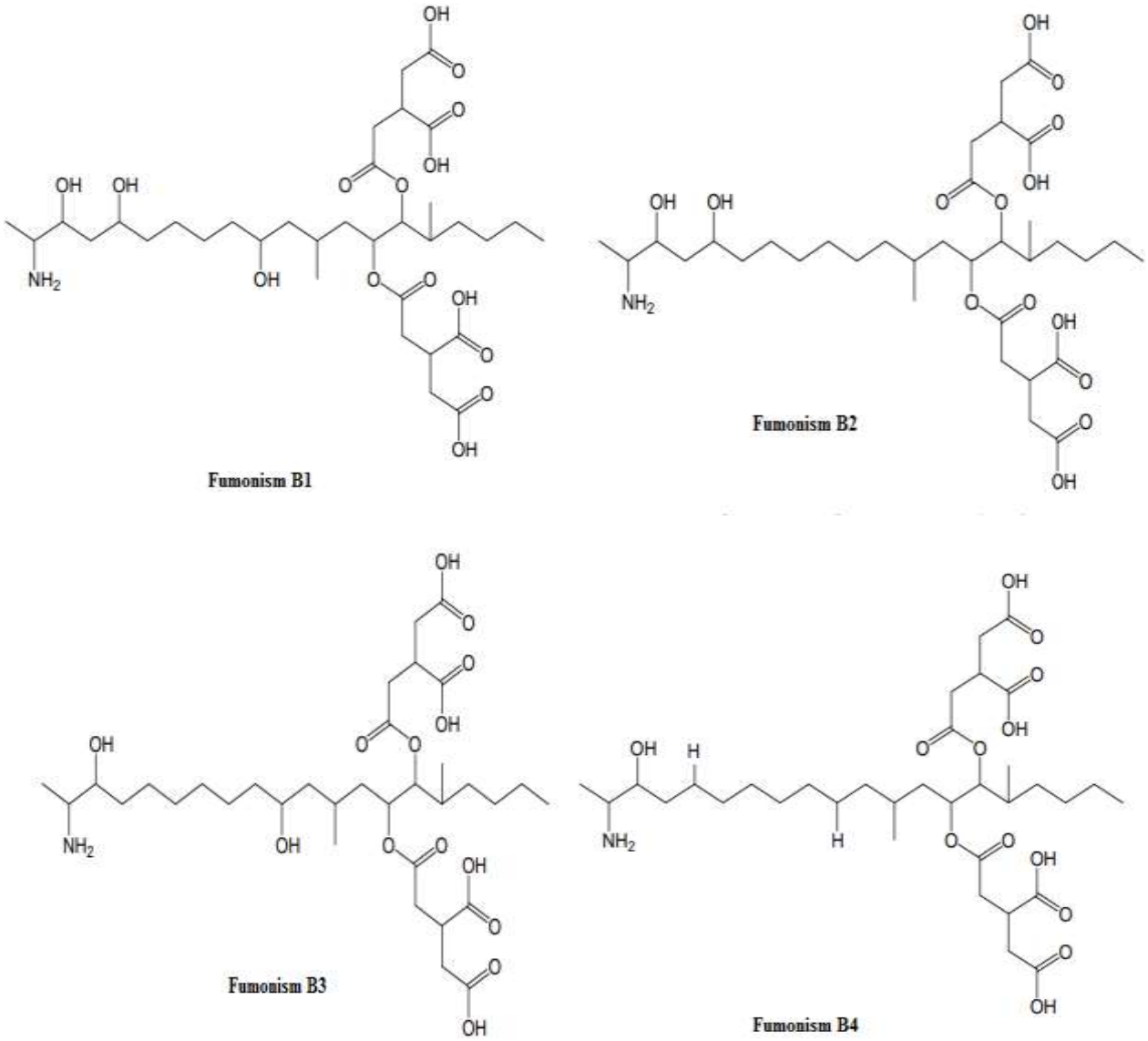
2.4.2 Fumonisins

Fumonisins were firstly described by Bezuidenhout *et al*. 1988 in South Africa. About 28 fumonisins analogous have been described and divided into four types, A, B, C and P series, with those belonging to B series FB_1, FB_2, FB_3 and FB_4 being the most abundant and of toxicological importance (Bartók *et al*., 2010). These fumonisin Bs occur naturally in contaminated maize and fumonisin B_1 predominates at highest concentration (Marasas, 1996). FB_1 accounts for 70–80 % of total fumonisins compared with 15–25 % (FB_2), 3–8 % (FB_3) and 1–2 % (FB_4) (Szécsi *et al*., 2010). They are produced by *F. verticillioides* and *F. proliferatum. Aspergillus niger* has also been found to produce fumonisin B_2 (Mogensen *et al*., 2010). The occurrence of FBs has been reported in several cereals, legume crops, spices, and food products all over the world. Maize and its products remain the most contaminated because of the susceptibility of the maize crop to FB-producing fungi. Fumonisins are heat stable and do not dissolve in organic solvent but are water soluble making them difficult to study

(Bennett and Klich, 2003). As indicated below, Figure 2.2 represents the different chemical structure of fumonisins B series.

Figure 2.2: Chemical Structures of Fumonisin Bs

Source: (Zain, 2011)

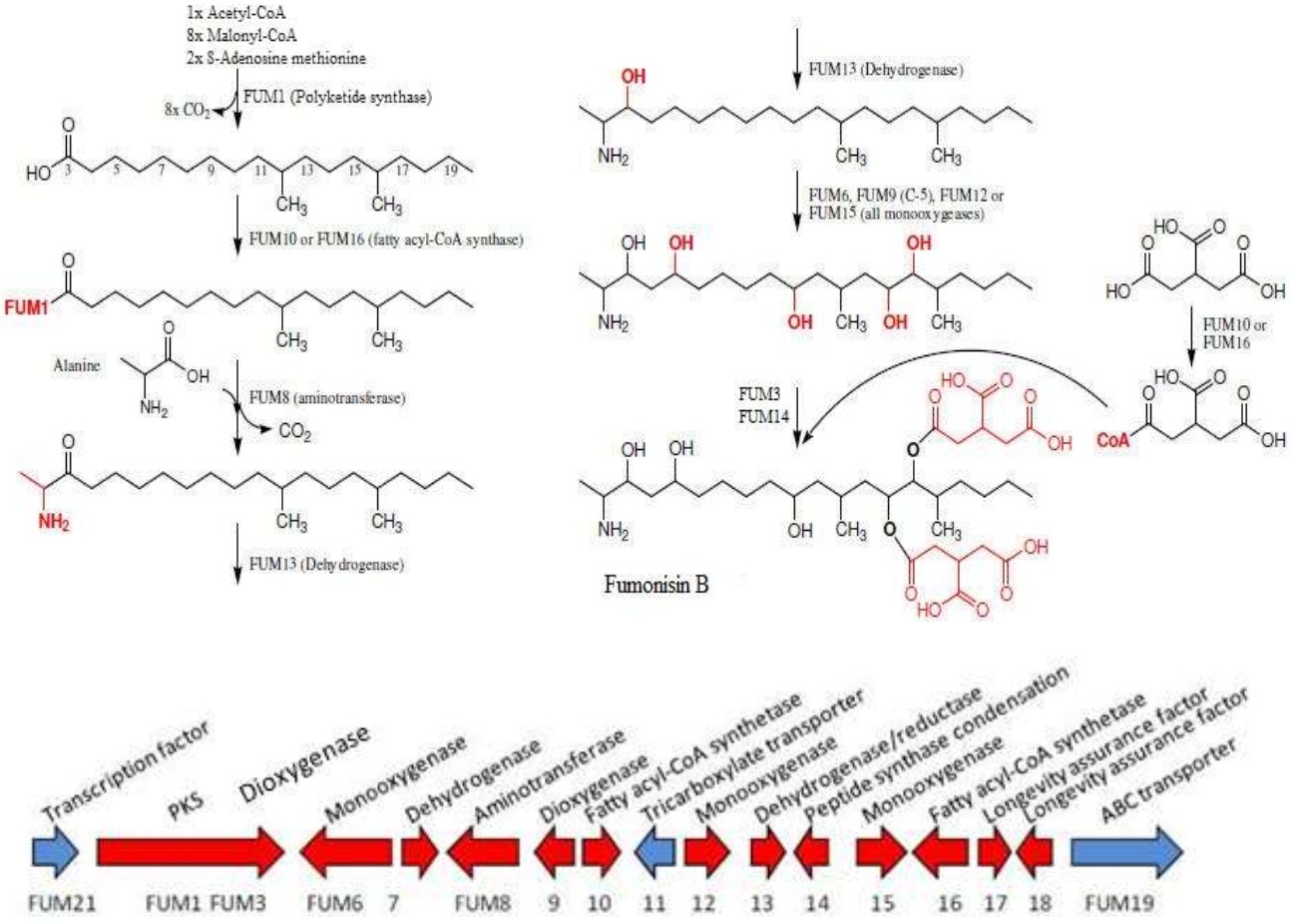


2.4.2.1 Biosynthesis of fumonisins

Fumonisins are polyketide-derived mycotoxins produced by some filamentous fungi in the genus *Fusarium*. Up till now, all fumonisins biosynthetic enzymes are encoded at one locus, the 17-gene FUM cluster. The FUM cluster also encodes a protein that control expression of the cluster genes and proteins involved in transport of fumonisins or biosynthetic precursors across membranes (Butchko *et al*., 2003). The biosynthesis starts with the Fum1p-catalyzed carbon chain assembly from one molecule of acetyl CoA, eight molecules of malonyl CoA, and two molecules of methionine (in *S*-adenosyl form). The C18 polyketide chain is released from the enzyme by a nucleophilic attack of a carbanion, which is derived from R-carbon of alanine by decarboxylation, on the carbonyl carbon of polyketide acyl chain. This step is catalyzed by a pyridoxal 5′-phosphate- (PLP-) dependent aminoacyl transferase Fum8p (Seo *et al*., 2001). The resultant 3-keto intermediate is then stereospecifically reduced to a 3-hydroxyl product by reductase Fum13p (Butchko *et al*., 2003).

Subsequent oxidations at C-14 and C-15 positions by cytochrome P450- dependent oxygenases Fum6p, Fum12p, or Fum15p, tricarballylic esterification of the hydroxyl groups on C-14 and C-15 by acyltransferase Fum14p, and C-5 hydroxylation by 2-ketoglutarate- dependent dioxygenase Fum3p furnish the biosynthesis of fumonisins as shown in Figure 2.3. The tricarballylic moieties are most likely derived from the citric acid cycle (e.g., aconitic acid) (Blackwell *et al*., 1996), and their addition to the carbon backbone may involve Fum7p, Fum10p, Fum11p, and Fum14p. A reduction of aconitate by Fum7p followed by a thioesterification with CoA as cosubstrate, catalyzed by Fum10p, would produce an activated acyl-*S*-CoA of tricarballylic acid. Fum14p then

catalyzes the transfer of tricarballylic acyl-CoA to the diols on C-14 and C-15 to form the diesters of fumonisins. The validation of the sequence of latter reactions in the



fumonisin biosynthetic pathway will require further studies with both genetic and biochemical approaches (Bojja *et al*., 2004).

Figure 2.3: Biosynthetic Pathway for Fumonisin B_1 Production
Source: Alexander *et al*. (2009)

2.4.2.2 Mechanism of action

Fumonisin toxin resembles the structure of cellular sphingolipids and thus inhibits ceramide synthase (Figure 2.4) which catalyzes the formation of ceramide from sphingoid base (sphiganine or sphingosine) and palmitate or another fatty acid. The immediate consequence of ceramide synthase inhibition are increased cellular sphinganine (and to a lesser degree sphingosine) concentration, increase in sphingoid

base break down products such as sphingosine 1-phosphate and decrease in cellular complex sphingolipids (Voss *et al*., 2007).

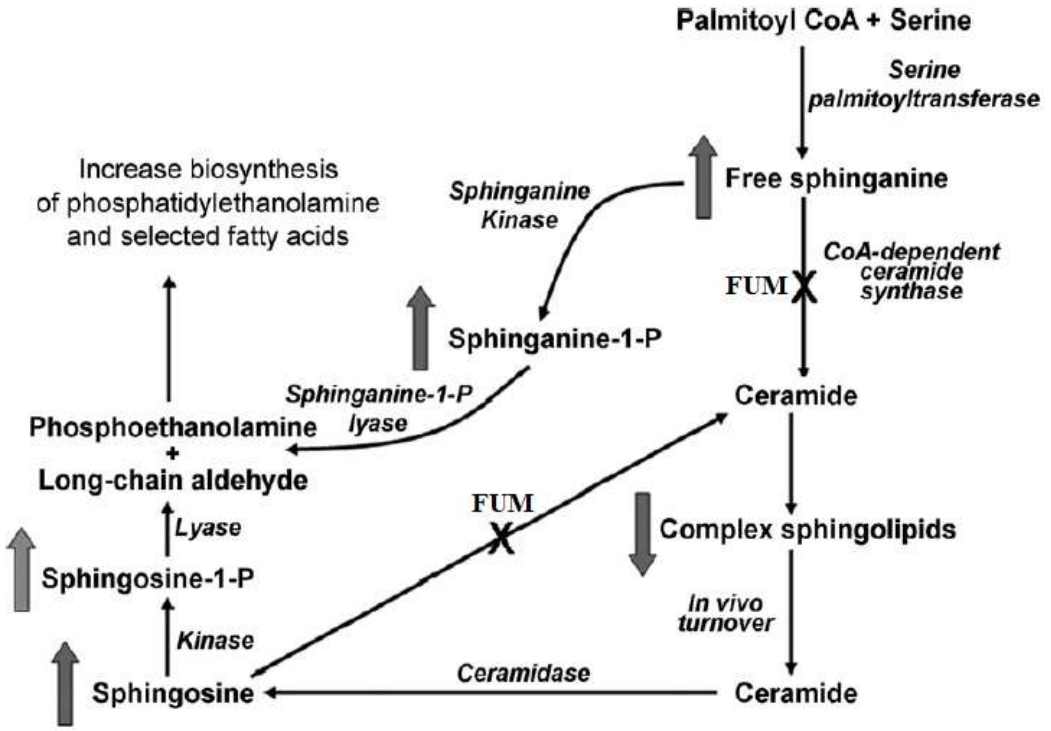


Figure 2.4: Mechanism of Fumonisin Inhibition of Ceramide Synthases and Global Disruption of Sphingolipid Metabolism
Source: Bulder *et al*. (2012)

2.4.3 Zearalenone

Zearalenone (ZEN) is produced by several *Fusarium* fungi species such as *F. culmorum, F. verticillioides, F. cerealis, F. semitectum, F. equiseti, F. graminearum,* and *F. crookwellense* (Chilaka *et al*., 2017). Chemically, zeralenone is known as 6-(10-hydroxy-6-oxo- trans -1-undecenyl)-β-resorcylic acid μ-lactone. The keto group at C-8 is reduced to α- and β-isomers in mammalian tissues, and these metabolites can be produced at low concentrations by the fungi. *Fusarium* fungi contaminate crops prior to harvest and even during storage (Chilaka *et al*., 2017). Contamination often exist together with DON, 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), NIV, and fusarenon-X (FX) as a result of the ability of *Fusarium* to produce

many mycotoxins resulting in synergestic effects on host organism (Grenier and Oswald, 2011).

The concentration of zeralenone is low in feeds contaminated on the field. Under storage condition, when the moisture content is greater than 30-40 %, its concentration increases.The occurrence of ZEN in foods and feeds in Nigeria has also been described by Makun *et al*. (2011). As depicted in Figure 2.5, zearalenone also known as F2 is commonly found in cereals such as maize, wheat, barley, rye, soybean products, cheese snacks, vegetable and dried fruit (EFSA panel on Contaminants in the Food Chain, 2011).

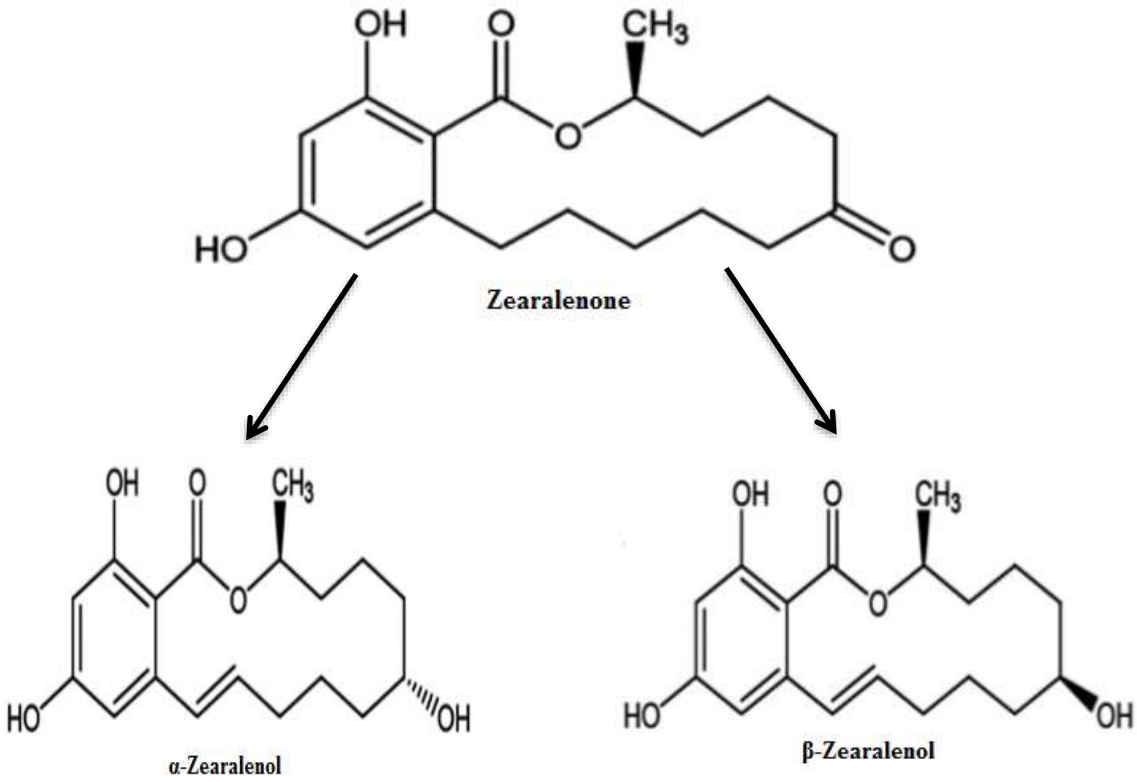


Figure 2.5: Chemical Structures of Zearalenone
Source: Ouanes *et al*. (2005)

2.4.3.1 Zearalenone biosynthesis pathway

ZEN is a polyketide metabolite, synthesized by sequential reactions catalyzed by large multienzyme protein complexes that contain polyketide synthases (PKSs). Genome

sequencing has revealed that *F. graminearum* has 15 PKSs, but the function of only 8 PKSs has been identified (Hansen *et al*., 2012). Among them, one reducing (PKS4) and one non-reducing (PKS13) PKSs are essential for ZEN production (Gaffoor and Trail, 2006). Fungal PKS genes generally reside in clusters of genes that encode transcription factors, metabolic enzymes, and transporters. Four neighboring genes (PKS4, PKS13, ZEB1, and ZEB2) are required for ZEN biosynthesis (Figure2.6) and constitute the ZEN biosynthetic gene cluster (Brown *et al*., 2012b).

ZEB1 encodes an isoamyl alcohol oxidase that is required for the conversion of β-zearalenol into ZEN. ZEB2 is a transcription factor that carries a basic leucine zipper (bZIP) DNA-binding domain and regulates the activity of other ZEN biosynthetic cluster genes.

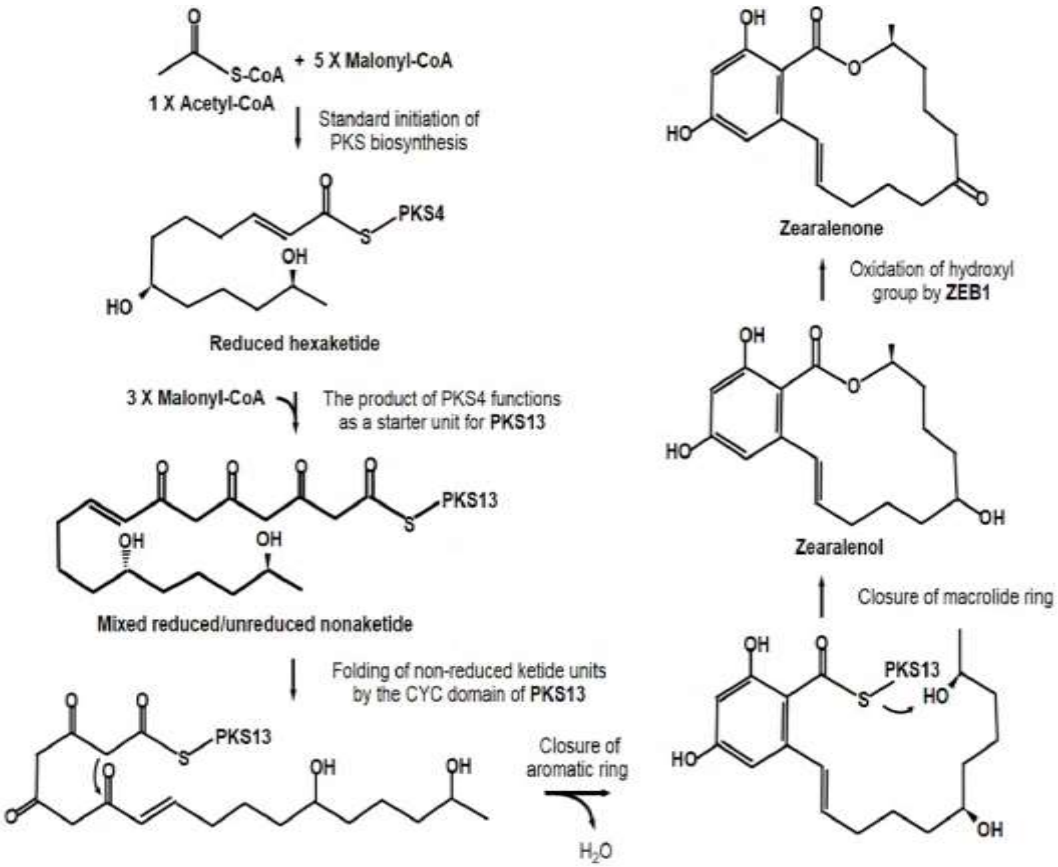


Figure 2.6: Zearalenone Biosynthetic Pathway
Source: Kim *et al*. (2018)

Based on the results of molecular genetic analyses of these genes, it is proposed that the ZEN biosynthetic pathway is initiated by PKS4, which catalyzes the condensation of carbons from a single acetyl-CoA and five malonyl-CoA molecules, resulting in the formation of a hexaketide (Kim *et al*., 2005). In the next step, PKS13 completes three iterations extending the ZEN chain by adding three malonyl-CoA molecules, resulting in a nonaketide. Then, the unreduced ketones undergo two rounds of intramolecular aromatic reactions, causing the formation of an aromatic ring and a macrolide ring structure containing a lactone bond. The final conversion of zearalenol to ZEN is catalyzed by ZEB1. ZEN biosynthetic genes are not essential for other traits of *F. graminearum* such as hyphal growth, conidiation, pigmentation, trichothecene production, virulence or sexual reproduction (Brown *et al*., 2012b)

2.4.3.2 Mechanism of action

ZEN and its metabolites are structurally related to oestrogen (Figure 2.7), thus they can bind to estrogen receptors and exert the estrogen-like effects (Tatay, *et al*., 2018). Estrogen regulates the biological processes through estrogen receptors which can activate cell signalling pathways and the extracellular-signal-regulated kinase 1/2 (ERK1/2) mediates the estrogen-like signal for cell proliferation (Kunishige *et al*., 2017). After binding to oestrogen, ZEN activates mitogen-activated protein kinases (MAPKs) and stimulates cell proliferation by changing the expression of estrogen-responsive genes (Darbre *et al*., 2002).

Many studies have revealed that ZEN could disrupt the function of gap junctional intercellular communication (GJIC) (Ouanes-Ben Othmen *et al*., 2008). It was reported that the dysfunctional GJIC was a prerequisite of oncogenes and tumor. It seems to have a significant effect on the process of tumors especially for solid tumors. Also, ZEN has carcinogenic property which may be another reason for stimulating cell proliferation

(Mesnil *et al.*, 2005). ZEN-induced DNA lesions may cause DNA fragmentation and disturb the progression of cell cycle in indifferent cells including Vero, Caco-2 and DOK. ZEN could cause DNA damage and induce chromosome aberrations, which might be an important reason for inducing cancer (Zinedine *et al.*, 2007).

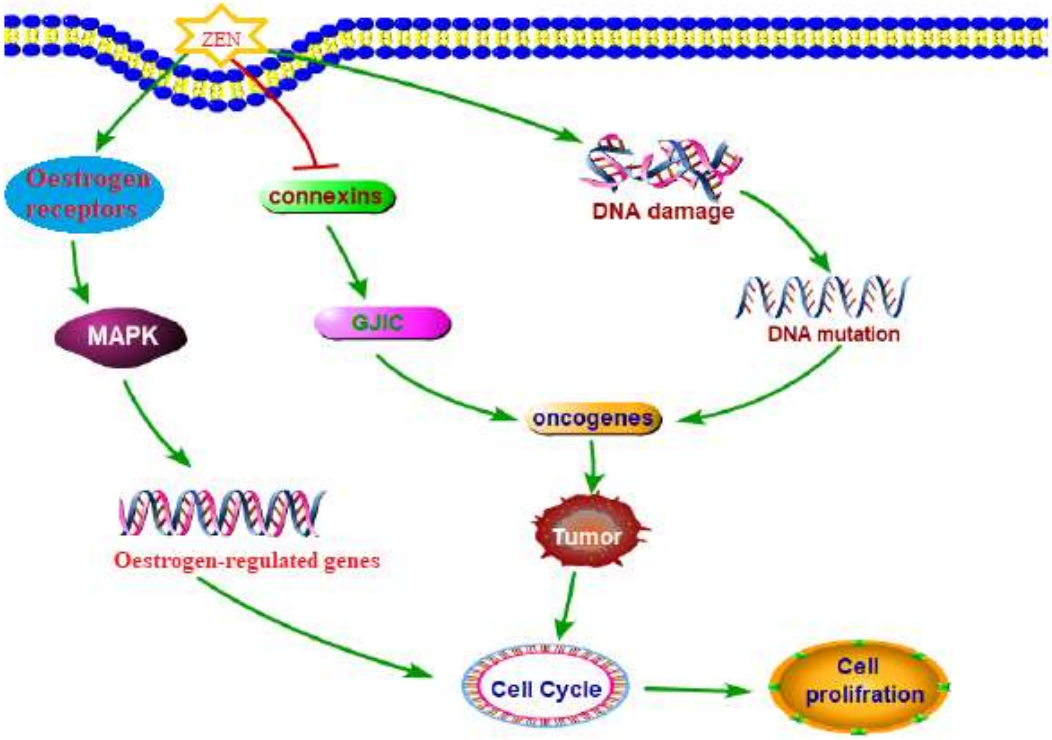


Figure 2.7: Mechanisms of ZEN stimulated cell proliferation
Source: Zheng *et al*. (2018)

2.4.4 Trichothecenes

Trichothecenes (THs) comprises a large group of structurally related fungal metabolites containing an epoxide which is responsible for their toxicological activity. THs have tetracyclic 12, 13-epoxytrichothecene sesquiterpenoids in common. Trichothecenes produced by *Fusarium* species are widespread in all cereal-growing areas of the world and they are divided into four groups based on their chemical properties which are A, B, C and D (Shank *et al*., 2011). The groups important economically are group A which includes T-2 and HT-2 toxins, diacetoxy- and monoacetoxy-scirpenol (DAS and MAS) and neosolaniol (NEO) and group B which includes deoxynivalenol (DON), nivalenol

(NIV), 3-AcetylDON, 15-AcetylDON and fusarenone X because they occur frequently in foods and feeds and have toxic effects (Abbas *et al*., 2013). THs are found in cereals like wheat, maize, oat, rice, rye, barley and other cereal based foods mainly consumed by humans and animals, hence generating a global concern.

2.4.4.1 Biosynthesis of trichothecenes

Biochemical synthesis of trichothecenes starts with the cyclization of farnesyl pyrophosphate, a major metabolic intermediate, to form trichodiene. The terpene cyclase trichodiene synthase (Tri5) that catalyzes this reaction and the gene that encodes it (TRI5) were first characterized in a T-2 toxin (Type A trichothecene) producing strain of *Fusarium sporotrichioides* (McCormick *et al*., 2006). Trichodiene undergoes a series of oxygenations catalyzed by a cytochrome P450 monooxygenase encoded by TRI4. TRI4 controls the addition of four oxygens at C-2, C-3, C-11, and the C-12, C-13-epoxide to form the intermediate isotrichotriol.

Isotrichotriol undergoes a non-enzymatic isomerization and cyclization to form isotrichodermol. In the course of this reaction, the oxygen at the C-2 position becomes the pyran ring oxygen and the hydroxyl group at C-11 is lost. More complex Type A trichothecenes are built by modifying isotrichodermol through a series of paired hydroxylation (–OH) and cetylation or acylation steps. Isotrichodermol is converted to isotrichodermin (Figure 2.8) by an acetyltransferase encoded by TRI101 (McCormick *et al*., 1999). A second hydroxyl group is added to C-15 (controlled by TRI11), which is subsequently acetylated under the control of TRI3 (Garvey *et al*., 2009). A third hydroxyl group is added at C-4 (controlled by TRI13), which is subsequently acetylated under the control of TRI7. In *F. sporotrichioides*, a fourth hydroxyl group is added to C-8 (controlled by TRI1), followed by the addition of an isovaleryl moiety controlled

by TRI16. Lastly, the acetyl group is removed from the C-3 position by an esterase (controlled by TRI8) to produce T-2 toxin (Lee *et al*., 2002).

Biosynthesis of Type B trichothecenes, follows a similar pathway to that of Type A trichothecenes, with paired hydroxylations and acetylations at C-3 and C-15. Rather than TRI1 controlling the final hydroxylation at C-8 as in Type A-producing strains, TRI1 in Type B-producing strains controls the addition of hydroxyl groups at both the C-7 and C-8 positions (McCormick *et al*., 2006).

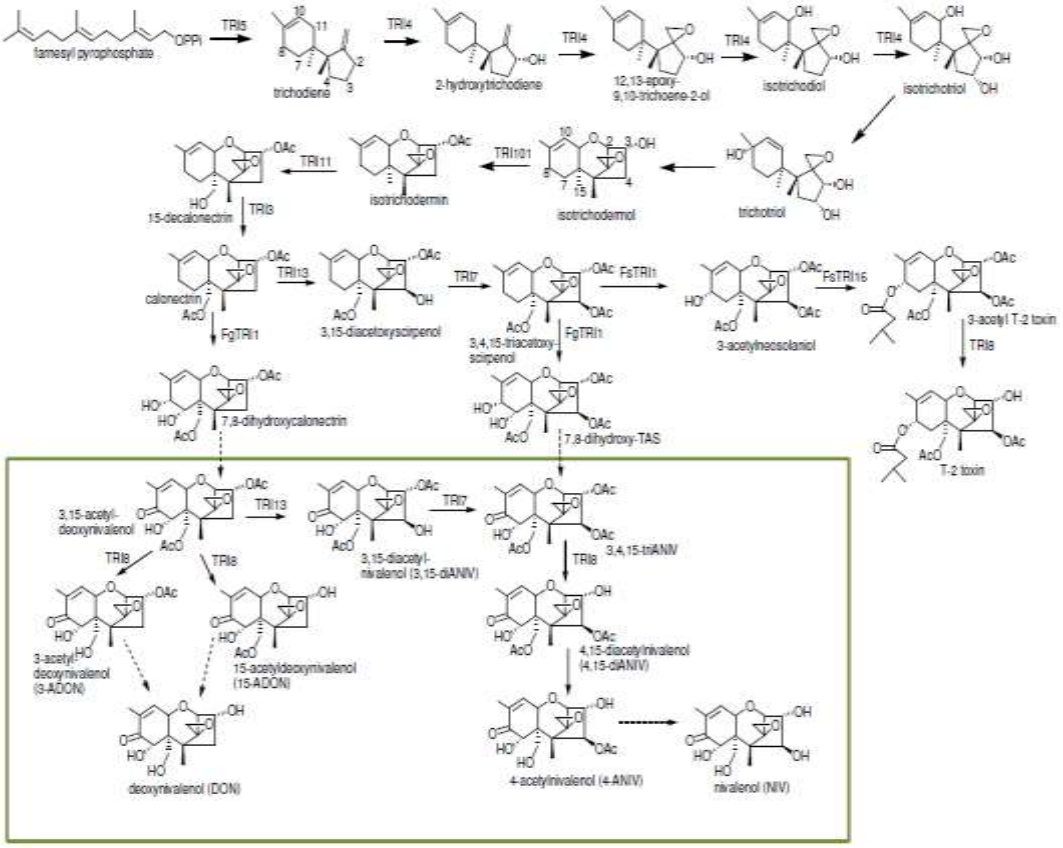
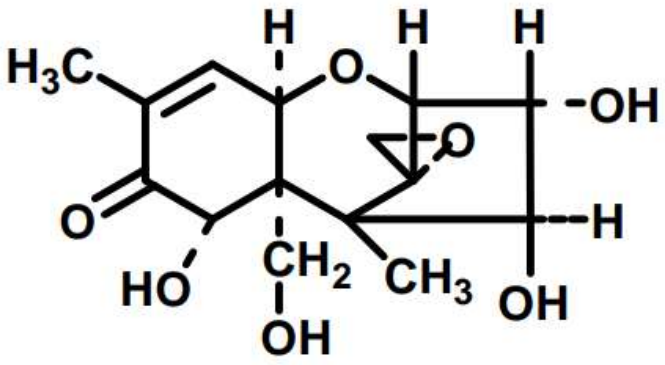


Figure 2.8: *Fusarium* Trichothecene Biosynthetic Pathways
Source: McCormick *et al*. (2011)

2.4.4.2 Nivalenol

Nivalenol (NIV) is a well-known type B trichothecenes co-occuring with DAS, T2 and DON (Covarelli *et al*., 2015). With respect to toxicity, nivalenol is more toxic in animals than DON (Wu *et al*., 2012) while DON is more toxic in plant (Abbas *et al*., 2013). It inhibits RNA, DNA and proteins synthesis resulting in cell necrosis (Alassane-

Kpembi *et al*., 2013). Nivalenol are produced by *F. graminearum*, *F. cerealis*, *F. poae* and *F. culmorum* (Haratian *et al*., 2008). The contamination of cereals by nivalenol appears to be lower than that of DON (Lindblad *et al*., 2013). However, the low exposure to nivalenol made EFSA consider it not a health concern based on available



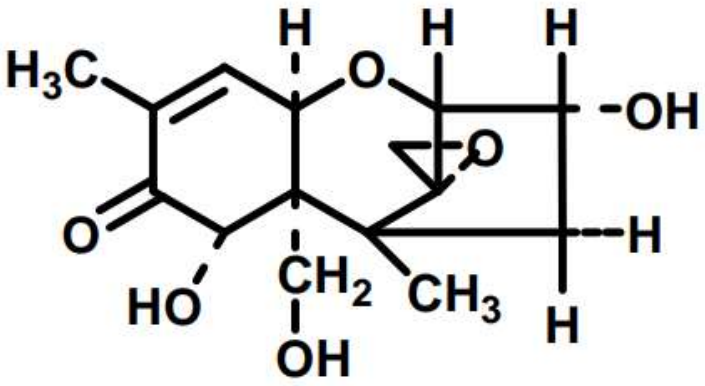
Nivalenol (NIV)

data (European Commission, 2007). Below is the chemical structure of nivalenol (Figure 2.9).

Figure 2.9: Chemical Structure of Nivalenol
Source: Hossen *et al*. (2012)

2.4.4.3 Deoxynivalenol

Deoxynivalenol (DON) also referred to as vomitoxin is a contaminant of wheat and wheat product (Sobrova, *et al*., 2010). It is produced mainly by *F. graminearum*, and *F. culmorum*, and to a lesser extent, by *F. cerealis* and *F. pseudograminearum*. DON as represented by chemical structure in Figure 2.10 has been classified by IARC in group III meaning not carcinogenic in human (European Food Safety Authority (EFSA), 2017). It also occurs in acetylated forms; 3-acetyl-DON (3-Ac-DON), 15-acetyl-DON (15-Ac-DON) and DON-3-glucoside (DON3G). DON3G is the masked form of DON in plants representing the major plant metabolite of DON (Ostry *et al*., 2017). Accumulation of DON in plant can result in *Fusarium* Head Blight affecting the overall wheat kernels yield and quality (Somma *et al*., 2014).



Deoxynivalenol (DON)

Figure 2.10: Chemical Structure of Deoxynivalenol

Source: Sobrova *et al*. (2010)

2.4.4.4 Mechanism of action of deoxynivalenol

Deoxynivalenol enters the cell and binds to active ribosomes, which transduce a signal to RNA-activated protein kinase (PKR) and hemoitopoeitic cell kinase Hck (Figure 2.11). It is well known that trichothecenes and other translational inhibitors which bind to ribosomes can also rapidly activate mitogen-activated protein kinases (MAPKs) and induce apoptosis in a process generally referred to as the "ribotoxic stress response" (Laskin *et al*., 2002). MAPKs modulate physiological processes including cell growth, differentiation, and apoptosis and are critical for signal transduction in the immune response (Dong *et al*., 2002). Subsequent phosphorylation of mitogen-activated protein kinases drives transcription factor (TF), activate apoptosis that results to chronic and immunotoxic effects (Pestka *et al*., 2004).

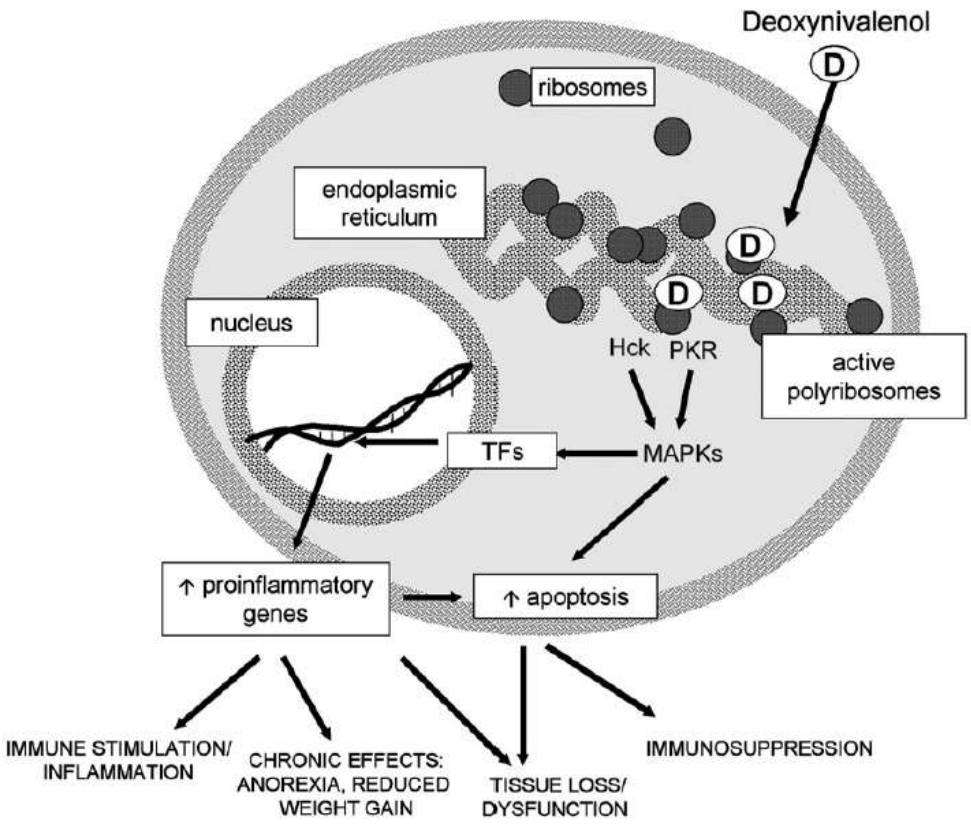
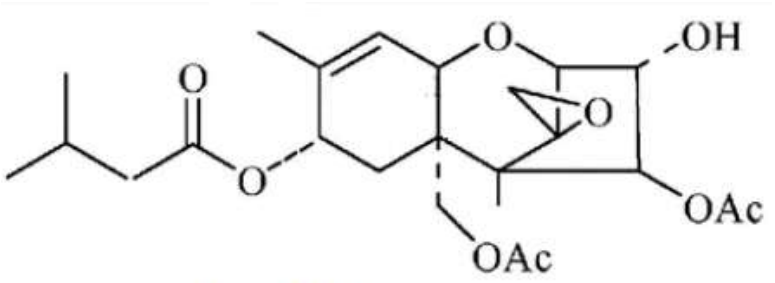


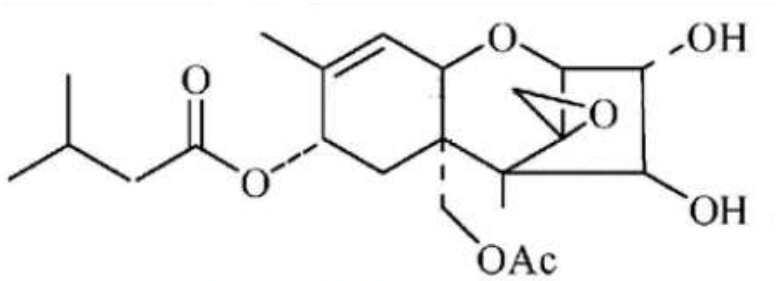
Figure 2.11: Molecular Mechanism of action of Deoxynivalenol and other Trichothecenes.
Source: Pestka, (2007)

2.4.4.5 T-2 and HT-2

Type A trichothecenes (As illustrated in Figure 2.12) are produced mainly by *Fusarium sporotrichioides*, and *Fusarium langsethiae* in grains and mostly present in oat. T-2 and HT-2 can co-occur with diacetoxyscirpenol because it is synthesized at the side chain of the T-2 toxin pathway (Lattanzio *et al*., 2013).



Type A: T-2 toxin



Type A: HT-2 toxin

Figure 2.12: Chemical Structures of T-2 and HT-2 toxins

Source: Canady *et al*. (2001)

These *Fusarium species* infect crops on the field and even during storage. HT-2 toxins are the major contaminant in cereals but its active metabolite T-2 is responsible for its toxic effect. T-2 toxicity is influenced by factors such as physical condition of the exposed animals, age, sex, and co-occurrence with other toxins, dosage and route of administration (Stoev *et al*., 2010).

2.5 Emerging *Fusarium* Toxins

Aside the commonly occurring *Fusarium* mycotoxins, other mycotoxins have been reported and are referred to as emerging mycotoxins. These include beauvercins, enniatins, fusaproliferin, and morniliformin (Santini *et al*., 2012). Evidence on their occurrence in different food products have been reported (Sebastia *et al*., 2012) thus posing a great concern. Limited research has been done on the occurrence of this mycotoxin in foods and feeds. Current research using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) for structure determination gives clue on this newly discovered metabolite (Santini *et al*., 2012).

2.5.1 Beauvericin

Beauvericin is produced by a wide range of *Fusarium* species and it is regarded as being cytotoxic. It also has anticancer (Heilos *et al*., 2017), insecticidal and nematocidal

actions. Beauvericin, as shown in Figure 2.13 possess antimicrobial activity and does not distinguish between gram positive and gram negative bacteria. They also act on cell membrane thereby affecting physiological ion balance and disrupting the cellular homeostasis (EFSA Panel on Contaminants in Food Chain (CONTAM), 2014). It has been reported to inhibit cell proliferation, modify cell cycle phases, induces apoptosis and mitochondrial damage (Mallebrera *et al*., 2016). The most sensitive animal species to beauvericin are birds and minks.

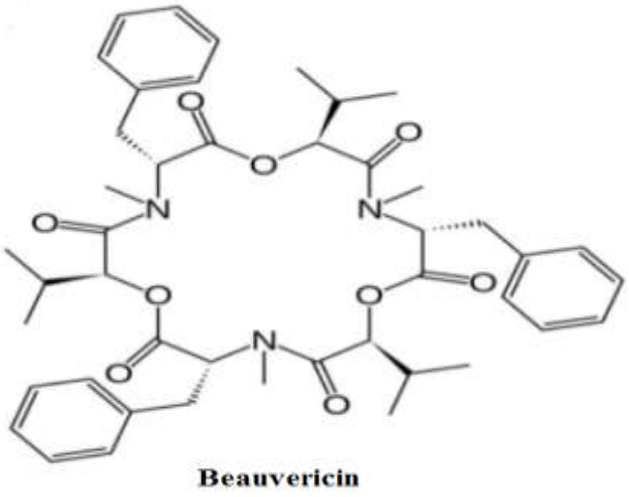


Figure 2.13: Chemical Structure of Beauvericin

Source: Logrieco *et al*. (2002)

2.5.2 Enniatins

Enniatins represented in Figure 2.14, with different substituted alkyl (R) groups in table 2.1, are produced by various *Fusarium* species and are structurally known as cyclohexadepsipeptides. They are usually found in cereals and cereal based products, fish, dried fruits, nuts, spices, cocoa and coffee products (Tolosa *et al*., 2017; Zinedine *et al*., 2017). Sy-Cordero *et al*. (2012), reported that several enniatin analogue (A, A_1, B, B_1, B_2, B_3, B_4, D, E, F, and G) have been identified but, the most important in terms of incidence and the most studied is the ENN B. Enniatins are lipophilic in nature,

hence incorporated into the lipid bilayer of the cell membrane thereby creating cation selective pores that increases the permeability for cations, resulting in disturbances of the physiological cation level in the cell (Tonshin *et al*., 2010).

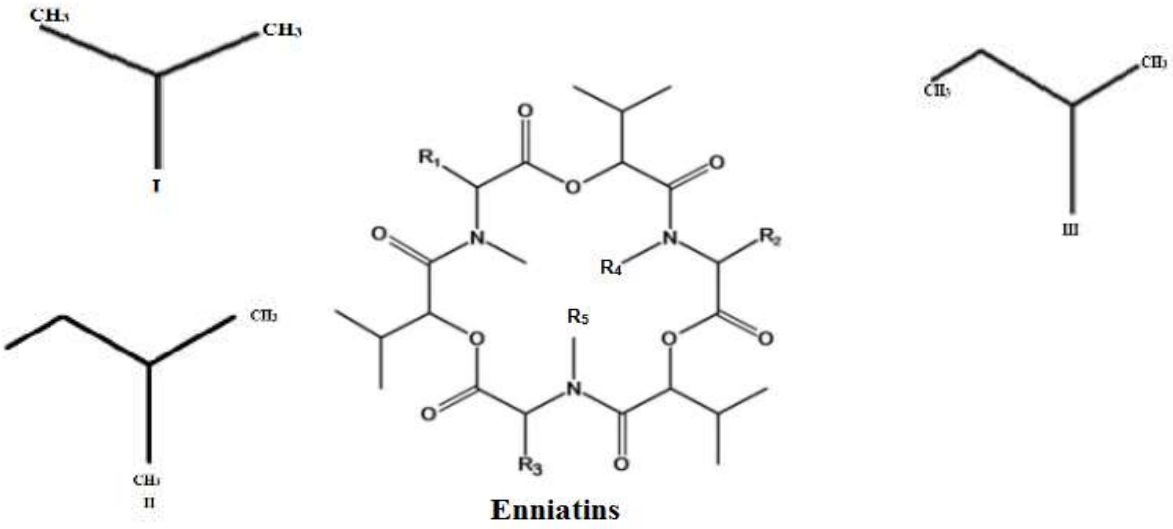


Figure 2.14: Chemical Structure of Enniatins

Source: Wu *et al*. (2018)

Table 2.1: Alkyl group substituting Enniatins Chemical Structure.

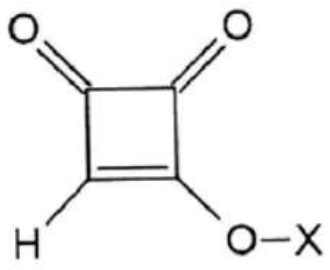
Mycotoxin	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**
ENNA	III	III	III	$-CH_3$	$-CH_3$
$ENNA_1$	I	III	III	$-CH_3$	$-CH_3$
ENNB	I	I	I	$-CH_3$	$-CH_3$
$ENNB_1$	I	I	III	$-CH_3$	$-CH_3$
$ENNB_2$	I	I	I	-H	$-CH_3$
$ENNB_3$	I	I	I	-H	-H
$ENNB_4$	I	I	II	$-CH_3$	$-CH_3$

Source: Wu *et al*. (2018)

Enniatins showed insecticidal, antifungal, antibacterial, and antihelmintic properties (Jestoi, 2008; Prosperini *et al*., 2017). It also shows cytotoxic effect on humans and animals cell lines at very low micromolar range (Jonsson *et al*., 2016). EFSA concluded that acute exposure to enniatin does not indicate concern for human health while the assessment of chronic exposure in diet could not be drawn as a result of limited toxicological data (EFSA Panel on Contaminants in Food Chain (CONTAM), 2014).

2.5.3 Moniliformin

Moniliformin (MON) is produced mainly by *F. proliferatum* and *F. subglutinans*. It was first reported in 1982 from mouldy maize obtained from Transkei-South Africa at levels of 16-25 ppm (Thiel *et al*., 1982). Worldwide, it has been found as a natural contaminant in cereals such as maize, rice, rye, wheat and barley (Zain, 2011). It is an alkali salt of cyclobutane 1-hydroxycyclobut-3, 4-dione. Nevertheless, much attention has not been drawn to moniliformin since it does not appear to be carcinogenic and relatively high amounts appear to be necessary to cause significant toxicological effects (Sanhueza and Degrossi, 2004). In humans, MON affects metabolic pathways involving pyruvate and inhibits kreb cycle intermediate thereby, causing respiratory stress, cardiotoxicity, immunosuppression, muscular weakness, and intestinal problems. This is as a results of it structural similarity to pyruvate (Li *et al*., 2000). Below is the chemical structure of moniliformin (Figure 2.15)



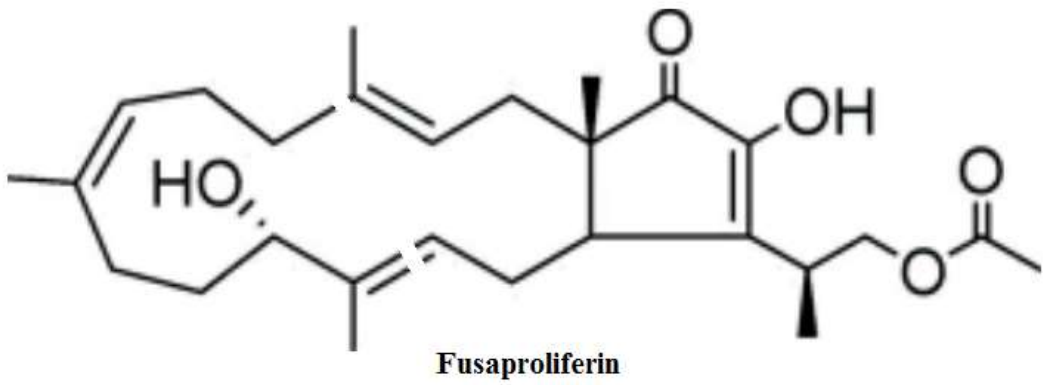
Moniliformin

X = H, Na, K

Figure 2.15: Chemical Structure of Moniliformin
Source: Morgan *et al*. (1999)

2.5.4 Fusaproliferin

Fusaproliferin (FUS) is a bicyclic sesterterpene produced by several *Fusarium* species (Fumero *et al*., 2015). FUS occur in deacylated form which shows limited toxicological activity when compared with the non-deacylated form (Ritieni *et al*., 1997). It has been found to be toxic to insect cell, mammalian cells and brine shrimp (*Artemia salina L*.). FUS causes tetratogenic effects in chicken embryos and pathogenic effects on human B-lymphocyte cells (Prosperini *et al*., 2012). The production of FUS (Figure 2.16) by *F. proliferatum* strains and the natural co-occurrence of these mycotoxins in maize samples contaminated by *Fusarium* species have been reported in Italy, USA and South Africa (Ritieni *et al*., 1997; Shephard *et al*., 2013; Munkvold *et al*., 1998)



Fusaproliferin

Figure 2.16: Chemical Structure of Fusaproliferin

Source: Santini *et al*. (2012)

2.6 Masked Mycotoxins

Mycotoxins have attracted increasing attention around the world, as they are among the most hazardous compound in terms of high toxicity and stability in food and feedstuffs (Fromme *et al*., 2016). Over the decades, plant derived mycotoxins have emerged as important contaminants, because in most cases, the degree of mycotoxicosis in animals is significantly greater than what would be expected from the contamination levels of mycotoxins in feedstuff (Gareis *et al*., 1990). The unforeseen high toxicity has been attributed to undetected conjugated forms of mycotoxins that were possibly hydrolysed into the parent toxins in the digestive tract of animals.

The term 'Masked mycotoxins' was first introduced by Gareis *et al*., (1990) who revealed that ZEN-14-glucoside (ZEN-14G) could be hydrolysed into free ZEN in the intestine during digestion in swine. Furthermore mycotoxin derivatives that are undetectable by conventional analytical techniques because their structure has been changed in the plant are referred to as masked mycotoxins. Masked toxins are either bound to carbohydrate, proteins or sulphate and are therefore not extractable with existing protocols aimed at the extraction of the toxins (Berthiller *et al*., 2009)

Recently, increased awareness has led to the study of masked mycotoxins. The most frequently detected are glucose conjugates of deoxynivalenol-3-glucoside (DON-3G), T-2-toxin-3-glucoside (T2-3G), HT-2-toxin-3-glucoside (HT2-3G), nivalenol-3-glucoside (NIV-3G), zeralenol-14-glucoside (ZEN-14G), α- zeralenol-14-glucoside (α-ZEL-14G) and β- zeralenol-14-glucoside (β- ZEL-14G) (Zhang *et al*., 2019). However, the conversion of a masked mycotoxin to its free form may lead to increased bioavailability of mycotoxin and may pose a potential risk to human and animal health (Berthiller *et al*., 2013).

2.6.1 Occurrence of masked mycotoxins

Masked mycotoxins frequently co-occur with free mycotoxins in various cereals and cereal based foods. However, the frequent co-occurrence of masked mycotoxins with their free forms in various cereals and cereal based foods is of great concern because of its toxic effect in humans and animals. Conversely, it is of concern that of all the masked mycotoxins existing DON-3-G is the most extensively studied masked mycotoxin and it was first identified as the primary DON metabolite in *Zea mays* suspension cultures (Sewald *et al*., 1992). Simsek *et al*., (2013) reported that DON-3-G co-exist with DON in crops such as wheat, maize, barley, oats and other cereal based foods at high proportions and at concentrations ranging from 2-1700 µg/kg.

In addition, the presence of ZEN-14-G was first reported in naturally contaminated wheat in 2002 (Schneweis *et al*., 2002). Thereafter, only a limited number of studies investigated the occurrence of masked ZEN (Zhang *et al*., 2019). It has been proven that ZEN can be converted into ZEN-14G and α and β-ZEL-14-G in grains and cereal based foods at levels ranging from non-detectable (n.d) to 3.2-42 % (Nathanail *et al*., 2015). With reference to other masked mycotoxins, Yoshinari *et al*. (2014) reported that NIV-3G has been found in wheat, barley and oats with the incidence of 10.6-61.8 %. T2-3G and HT2-3G have also been discovered in wheat and oats. However, country specific occurrence data on masked mycotoxins on a global scale are very limited and these renders risk assessment impossible

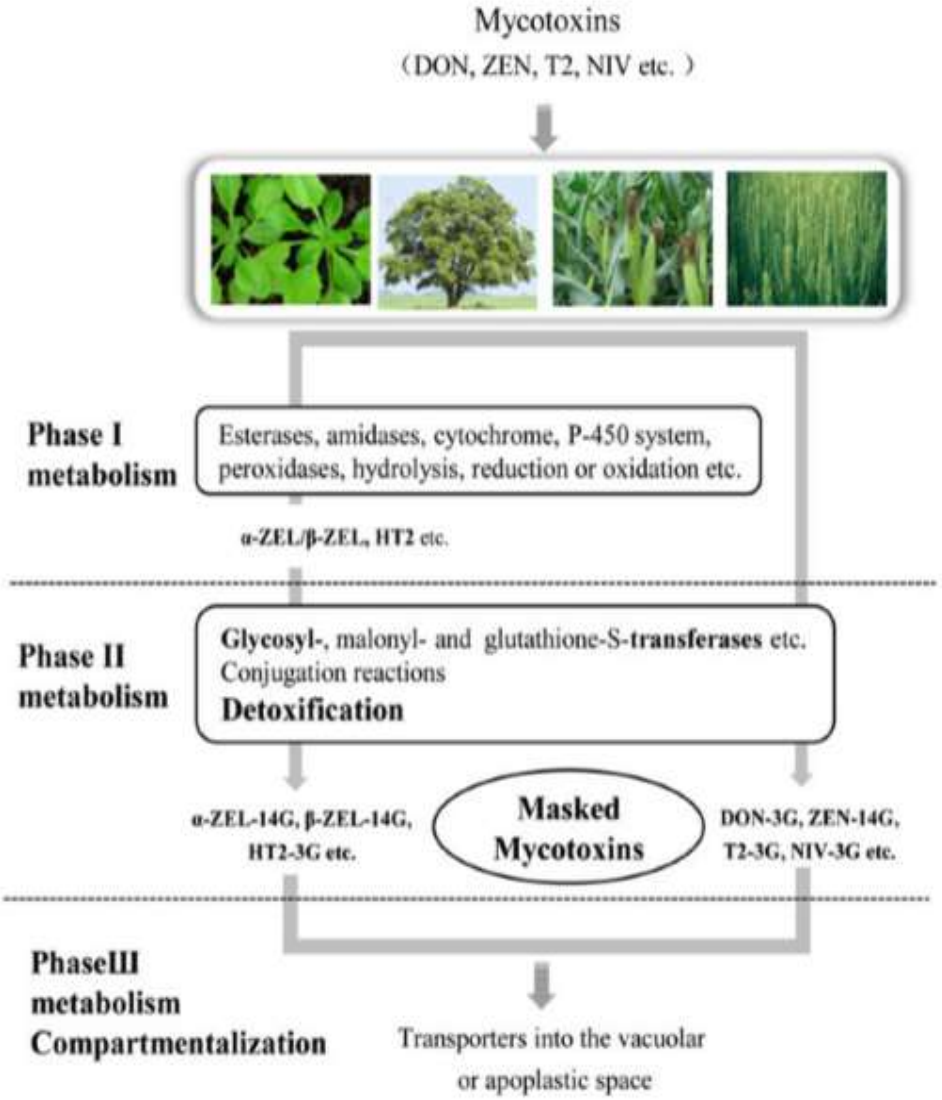
2.6.2 Formation of masked mycotoxins

In foods and feeds, mycotoxins can be subjected to biological modification through their defence mechanisms; plants can alter the structures of mycotoxins via three main phases, i.e. chemical modification (phase I and phase II metabolism) and compartmentalization (phase III) (Broekaert *et al*., 2015; Zhang *et al*., 2019). These modified mycotoxins can contribute to the degree of contamination and may escape

detection methods causing an under estimation of the mycotoxin load and leading to increased exposure and risk. Mechanisms of masked mycotoxins formation includes:

2.6.2.1 Plant conjugates

Mycotoxins are readily absorbed by plants and could accumulate to toxic levels without been detoxified efficiently (Broekaert *et al*., 2015). Plants as living organisms have the ability to defend themselves against the potentially toxic effects of mycotoxins, besides; the defence mechanism (Figure 2.17) includes three phases of biosynthetic pathway (Coleman *et al*., 1997). In phase I (transformation and activation phase), mycotoxins are



subjected to hydrolysis, reduction or oxidation, resulting in the production of reactive groups in their chemical structure (Broekaert *et al*., 2015).

Figure 2.17: Mycotoxins metabolic pathway
Source: Broekaert *et al*. (2015)

However, this phase typically affect lipophilic compounds by making them more polar and rendering them a suitable substrate for phase II metabolic reaction. If a mycotoxin already has a reactive or functional group suitable for phase II, then detoxification can omit phase I, which is often the case for more hydrophilic compounds (Sandermann, 1992, Sandermann, 1994). During phase II (solubilisation or conjugation phase), the activated mycotoxins are conjugated with endogeneous hydrophilic molecules such as glutathione, sugars, sulphate or amino acids by the enzymes glutathione-S-transferases, UDP glucosyltransferase (UGT) among others. The resulting conjugated mycotoxins have an increased polarity and are therefore more water soluble. This facilitates their transport mediated by ATP-dependent-glutathione-conjugate transporters to the vacuole or apoplastic space outside the cell. This is called compartmentalization (phase III) (Bryla *et al*., 2018). These conjugated mycotoxins are refrained from interacting with plant components and are consequently unable to exert harmful effects to the plants. In contrary to phase I and phase II biotransformation pathways in mammals, residues may persist in plants for considerable periods and can have important toxicological consequences on their consumers (Broekaert *et al*., 2015).

2.6.2.2 Fungal conjugates/precursors

Some mycotoxin conjugates can be excreted directly by fungi; although the numbers of examples are rear. Among the fungal conjugates, the most well known (modified) mycotoxins in this respect are 3-acetyl deoxynivalenol (3ADON) and 15- acetyl deoxynivalenol (15ADON), which can be found in *Fusarium* contaminated cereals. Both compounds arise from common precursors of 3, 15-diacetyl deoxynivalenol and

are biosynthetic precursors of DON (Berthiller *et al*., 2009). The final step in the biosynthesis of ADONs is the removal of an acetyl group from 3, 15-diacetyl DON at either the C-3 or C-15 position, mediated by an esterase encoded by TRI8. Differential activities of this esterase determines the production of either 3ADON or 15ADON chemotypes in *Fusarium graminearum* (Alexander *et al*., 2011).

Zeralenone-14-sulphate (Z14S) was found to be a natural *Fusarium* metabolite, and it is produced in fungal cultures in molar ratios from 1:12 to 1:2 of ZEN, depending on the strain and growth conditions used. Also, Z14S was found in maize samples in concentrations from 0.1 to 50 µg/kg by LC-MS/MS (Sulyok *et al*., 2006). Z14S can be easily converted back to ZEN during extraction either by sulfatase or through chemical hydrolysis. Glucosylation of ZEN by fungi has also been described. *Rhizopus species, Mucor bainieri* and *Thamnidium elegans* are all able to convert ZEN into zearalenone-14-β-D-glucopyranoside (Z14G), and the latter is also able to convert ZEN into zearalenone 2, 4-O-β-diglucoside (Berthiller *et al*., 2009).

Nevertheless, fungi can also metabolise existing mycotoxins apart from fungi precursors. When fungi capable of producing mycotoxins (mycotoxigenic fungi) co-occur with fungi which do not have that capability (non- mycotoxigenic fungi), they actively compete for space and nutrients from the host plant. Consequently, there is reduction in the growth and metabolism of mycotoxigenic fungi, thereby resulting to a diminished production of mycotoxins (Bacon *et al*., 2001). Next to this mycotoxigenic fungi growth antagonism, the non-mycotoxigenic fungus may also metabolise mycotoxins produced by toxigenic fungus. Such metabolism has already been described for zearalenone to zearalenone-14-glucoside and zearalenone-14-sulphate with the saprobic *Rhizopus* fungus. This fungus has frequently been found on plants, fruits and vegetables (El-Sharkaway *et al*., 1991).

Over the decades, few cases in which fungus itself produces a glucoside conjugate was reported. For instance, a strain of *Fusarium sambucinum* that produced monoacetoxyscirpenol-4-α-glucopyranoside was reported by Gorst-Allman *et al*., (1985). A decreased toxicity was exhibited compared to the free mycotoxin. However, this compound has been detected in corn together with the free mycotoxin monoacetoxyscirpenol using High Resolution Mass Spectroscopy (HRMS) (Nakagawa *et al*, 2013).

2.6.2.3 Food processing conjugates

Food processing such as heating or fermentation can alter mycotoxin. The thermo stability of trichothecenes allows them to withstand most food and feed process (Malachova *et al*., 2010). In trichothecenes, deoxynivalenol-3-glucoside (D3G) was reported to occur in malt and beer made from barley contaminated naturally by *Fusarium* toxins (Lancova *et al*., 2008; Kostelanska *et al*., 2009). The researchers suggested that the daily tolerable amount of DON (1 µg/kg body weight) established by scientific committee on food (Scientific Committee on Food, 1999) could be exceeded by average beer consumption when D3G and acetylated forms of DON are considered. Other researchers reported a significant increase of DON in malt as compared to DON and D3G levels increase in the grain used. The nature of this increase has not been fully elucidated. However it is obvious that during the initial step of the malting process of *Fusarium* infected barley, the fungus could produce additional mycotoxin that is conjugated efficiently by the metabolically highly active germling. It is also possible that bound mycotoxins originally present in the cell wall polymer fraction might be released enzymatically during malting (Sandermann, 2004).

Another food commodity frequently contaminated with modified mycotoxin is bread. During processing of wheat to bread, it has been described that milling had little

influence on the ratio of deoxynivalenol-3-glucoside (DON3G) to DON. Due to fractionation, milling decreased the D3G and DON content in white flour by 40 % compared to initial unprocessed wheat (Kostelanska *et al*., 2011). These findings are supported by a previous study that investigated the fractionation of 16 *Fusarium* mycotoxins during dry milling of maize. It was observed that bran, the hard outer layer of the cereal grain which is discarded for the production of white flour, contained the highest concentration for all tested mycotoxins (Schollenberger *et al*., 2008). During kneading, fermenting and proofing, no significant changes occurred for DON, DON3G and 3ADON. However when barley improvers such as enzyme mixtures were employed as dough ingredient, a distinct increase up to 145 % of DON3G were noticed in fermented dough (Kostelanska *et al*., 2011; Vidal *et al*., 2014).

It is hypothesized that this increase in DON3G is due to a release from starch based matrix – bounded forms. According to Kostelanska *et al*., 2011 a decrease of 10 % and 13 % of both DON3G and DON, respectively, took place during baking (240 °C 14 min) when compared to fermented dough. Valle-Algarra *et al*. 2009 baked at 240 °C for 30 min and observed a reduction inside the bread of 46–83 % and 43–64 % for 3ADON and DON respectively, whereas in the crust an even higher reduction took place. The influence of food processing on T2-glucoside, HT2-glucoside and FUS-X has also been investigated but to a lesser extent (De Angelis *et al*., 2014; Lancova *et al*., 2008).

2.7 *Fusarium* Toxins induced Animal Mycotoxicoses

Fusarium mycotoxins have been linked with *Fusarium* head blight in grains such as corn, wheat, barley, rice and other small grains utilized in the production of animal feeds (Palacios *et al*., 2021). These mycotoxins are transformed from the contaminated feeds to animals and eventually to humans. They are able to induce both acute and chronic toxicities in animals. Clinical signs of acute mycotoxicoses in high doses for

these groups of toxins include abdominal distress, diarrhoa and emesis in pigs for DON, alimentary toxic aleukia for T-2 toxins, pulmonary edema in pigs and equine leukoencephalomalacia in horses for fumonisins (Munkvold, 2017). Chronic exposure to low doses of *Fusarium* toxins which is common in practice can damage the gastrointestinal epithelial cell layer. The damage is escalated by the combine effects owing to the co-existence of *Fusarium* mycotoxins in animals (Huang *et al*., 2019).

T-2 was investigated for its toxicological properties and it was found to be highly cytotoxic causing lessions of the mucosa of the gastrointestinal tract resulting in extensive haemorrhage and a general inflammatory response (Jaćević *et al*., 2020). The obvious clinical signs of intoxification with DON such as feed refusal and vomiting occur only at concentration exceeding 12.5 ppm. It is worth mentioning that very recently these high contaminations have been noticed in food and feed commodities for the first time after many years of low level contaminations. The potential of deoxynivalenol (DON) to act as endocrine disruptor has been investigated in pigs, ruminants, poultry and horses (Schelstraete *et al*., 2020). However pigs have been shown to be the most susceptible animal while ruminant and poultry has been regarded as less sensitive. Also, male animals are more susceptible than female (Fremy *et al*., 2019).

Zearalenone being a non-steroidal oestrogenic mycotoxin adversely affect animals and its metabolite has been found to bind competitively to oestrogen receptor in various species resulting in hyper-estrogenism and infertility in livestock (Cortinovis *et al*., 2013). Studies have also shown that ZEN stimulate the growth of estrogen-responsive positive cells, modulate the estrous cycle, increase uterine weight and compete with estradiol for estrogen-responsive binding (Chilaka *et al*., 2017). The estrogenic activity

of ZEN has been implicated in numerous mycotoxicoses in farm animals, especially pigs and cattle (EFSA panel on Contaminants in the Food Chain, 2011).

ZEN causes an enlargement of mammae and nipples in both sexes, vulva swelling and vulvovaginitis progressing to virginal prolapse particularly in young piglets. In adult animals, fertility is impaired (cystic ovaria). Fumonisin B_1 has been implicated in animal disease because of their nephrotoxicity, immune stimulation, hepatotoxicity, immune suppression, liver and kidney problems (Howard *et al*., 2001). The clinical symptoms resulting from exposure to FB_1 in feed shows significant variation across species. The most sensitive animals to fumonisin B_1 are horses. FB_1 causes encephalomalacia in horses, a disease characterized by the degradation of neurons. In pigs, the main symptoms of FB_1 exposure is pleural edema (PPE-porcine pulmonary edema), thereby impairing respiratory and heart function (Schertz *et al*., 2018). Fumonisin B_1 has also been found to cause hepatic cancer in rats (Marasas, 2001).

2.8 *Fusarium* Toxins Induced Human Mycotoxicosis

Fusarium species causes superficial, locally invasive and diffuse infections in humans (Askun, 2018). Conventionally, *Fusarium* has been more of an agronomic threat than a medical one, but over the last decades, a variety of contributing factors has undergone a radical change. Although, *Fusarium verticillioides* including *Fusarium moniliforme* and *Fusarium fujikuroi* species complex are opportunistic pathogens, the species in the *F. solani* complex include pathogenic species (Austen *et al*., 2001). In addition, infections caused by *Fusarium species* are generally referred to as fusariosis which is dependent on the immune status of the host and the route of entry of the infection (Nucci and Anaissie, 2007). Nevertheless, diseases caused by *Fusarium* human pathogens have increased considerably, although they remain underestimated (Brown *et al*., 2012a).

Consumption of fumonisin B_1 (toxin produced by *Fusarium verticillioides*) in contaminated maize have been linked to oesophageal cancer in Southeast of United State, South Africa, North Eastern Italy and china (Bennett and Klich, 2003). In India, diarrhoea and abdominal pain have been found to occur following consumption of mouldy maize with high level of fumonisin B (Bhat *et al*., 1997). Exposure to fumonisin B_1 at early stage of pregnancy is associated with high incidence of neural tube defects (Missmer *et al*., 2006). Chronic intake of fumonisin mycotoxins has also been associated with stunted growth in children (Kimanya *et al*., 2014). The International Agency for Research on Cancer (IARC) has reported FB_1 in Group II B as possibly carcinogenic to humans (IARC, 2002).

In humans, trichothecenes causes haemorrhaging of internal organs, immunosuppression, nervous system disturbance, blood disorder, abdominal discomfort, diarrhoea, vomiting, weight loss and anorexia (Pestka, 2007). Hematotoxicity, myelotoxicity, growth retardation, and necrotic lesion manifest as a result of group A THs while gastro intestinal disorders and reduction in weight are caused by group B THs (Vanderfels-Klerx and Stratakou, 2010). THs inhibit protein synthesis, DNA and RNA synthesis, affect cell division and inhibit mitochondrial function (Yazar and Omurtag, 2008). DON and NIV inhibits protein synthesis by interacting with peptidyl transferase enzyme that binds the 60S ribosomal subunit, thus inhibiting translation (Sudakin, 2003). T-2 and HT-2 toxins inhibits DNA, RNA, protein synthesis and mitochondrial function, immunoglobulin synthesis, alters lipid peroxidation and also induces oxidative stress (Yang *et al*., 2016). Dietary exposure to T-2 and HT-2 causes apoptosis of proliferating cells and cells of the immune system (Stoev *et al*., 2010).

Also, Ahamed *et al*., (2001) reported that ZEN has the potential of increasing the risk of breast cancer cells in human having oestrogen response receptors. However, the biological potency of ZEN is relatively high but its toxicity is low and has been placed in Group III (IARC, 1993) meaning that it is not classifiable with regard to their carcinogenicity to humans. In some part of the world, researchers have also reported a possible link between ZEN and other mycotoxins such as trichothecenes and fumonisns resulting in higher incidence of oesophageal cancer. Consumption of ZEN contaminated grains or cereals can lead to symptoms such as enlargement of mammary glands, vaginal and rectal prolapses, atrophy of the testes, weakened libido in males, vaginal swelling, infertility in adults, abortion, stillbirth and birth of malformed offspring (CAST, 2003). However, the daily recommended dose of ZEN for human consumption has been approximated to be 0.05 mg/kg body weight.

2.9 Economic Impacts of *Fusarium* Mycotoxins

Global consumption of fungi and mycotoxin contaminated foods has drawn the attention of many researchers to a clear food security threats (Gbashi *et al*., 2018). The risk of exposure to these toxins has not diminished despite their debilitating effect. However, direct consequence of a mycotoxin contaminated diet results to significant losses, which negatively affects human and animal health, productivity, livelihood, security, household, income and consequently national economic implications (Enyiukwu *et al*., 2014). Economic losses arising from mycotoxicoses in Africa are shocking. Losses experienced by developed countries are usually trade related, whereas Africa tends to experience both economic losses and additional cost related to health challenges (Gbashi, 2018).

In general, economic losses resulting from fungi and mycotoxin contamination are group into three stages, that is, crop production, livestock production and human health

levels. At the level of crop production, millions tonnes of crops are destroyed annually by fungi. Based on this fact, the Food and Agricultural Organization (FAO) of United Nation estimated that between 25 % and 50 % of the world agricultural produce are lost each year due to mycotoxins (Wagacha and Muthomi, 2008). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) estimated the volume of food grains wasted by mycotoxins each year globally: rice 12 million tons, 16 million tons of maize, 1.8 million tons of sorghum, 3.7 million tons of copra, 378,000 of millet and 2.3 million tons of groundnuts. Worldwide, more than USD 100 billion of exported commodities are susceptible to mycotoxin contamination (Wild and Gong, 2010).

In the United State, most trade-related losses due to excessive *Fusarium* toxin contamination in animal feed would be due to failure to meet domestic standards set by the US Food and Drug Administration (FDA), rather than through export market losses. A case study regarding fumonisin in US maize intended for animal feed is presented. In a normal year (without a significant *Fusarium* ear rot outbreak), it is overwhelming that an annual cost of US$ 1–20 million is accrued to fumonisins (FUMs) in animal feed. In a year with a significant *Fusarium* ear rot outbreak, losses would total US$ 31–46 million (Wu, 2007). Compared with the case for another mycotoxin, aflatoxin, far fewer nations worldwide have standards on acceptable levels of *Fusarium* toxins in human and animal feed. It is worthwhile to note that overall trade-related losses are expected to be much lower for these toxins than, for example, aflatoxin. The first report implicating fumonisins in human disease was in connection with high human oesophageal cancer rates in Transkei, South Africa in 1988. The following year, interest in these mycotoxins increased dramatically after unusually high horse and pig death rates in the US linked to contaminated feed (Marasas, 1996).

In sub-saharan African, estimates suggests that approximately 250,000 deaths are caused by hepatocellular carcinoma (HCC) annually and this can be related to risk factors such as AFs exposure and high prevalence of hepatitis B (Zain, 2011). Also, AFs contamination in maize and groundnut in Nigeria contributed to 7761 liver cancer cases, which results in a total burden of 100,965 disability-adjusted life years (DALYs) (Atanda *et al*., 2015). In 2014, due to AFs contamination about 3334 cases of HCC was calculated in Tanzania, 95 % of which ended as death resulting to a loss of 96,686 DALYs. Worldwide, chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV), accounts for 350 million and 170 million people that are at major risk factors, the fraction of HCC cases attributable to HBV and HCV has been estimated to be 23 and 20 % in developed countries and 59 and 33 % in developing countries (Parkin, 2006).

2.10 Maize Production in Niger State

Maize is an important cereal that is highly cultivated and consumed in tropical regions of the world (Mateo *et al*., 2018) particularly in Africa, where it serves as source of livelihood to the citizenry. Maize production in Africa was about 75 million tons in 2018, therefore contributing about 7.5 percent of total world maize production (Dowswell, 2019). Nigeria is the second and twelfth largest producer of maize in Africa and the world respectively (Adejuwon, 2018). The country produces about 33 million tons of the food crop and the production rate is still on the rise especially in the northern part of Nigeria. The general increase in annual demand for this cereal in foods and feeds is an indication of a farm produce that is highly cultivated, consumed and marketed in Nigeria, thereby making it a principal source of mycotoxins. Niger State grows the largest quantity of maize in the country followed by Taraba, Kaduna, Adamawa and Plateau States (Merem *et al*., 2017).

Maize is highly consumed by middle and low income earners in the state, and it is often prepared alone or with other cereals such as millets, sorghum or wheat by mothers for infants less than 5 years old as traditional weaning food (Rodriguez-Carrasco *et al*., 2013; Adetunji *et al*., 2014; Kimanya *et al*., 2014). Considering the fact that maize is commercially lucrative in the state, there are still risks associated with its production. For instance, maize is very sensitive to drought and drought in Nigeria particularly that of Niger State could lead to crop's failure which may results to increase in percentage loss of productivity in the crop. Also, the grains are prone to fungal infection and maybe contaminated with different mycotoxins (Stanciu *et al*., 2017). Nonetheless, contamination of maize grains by various species of fungi could pose a major health challenge in humans and animals. Distribution of *Fusarium* fungi in maize samples across the globe is presented in Table 2.2.

2.11 Sorghum Production in Niger State

According to United State Department of Agriculture (USDA Foreign Agricultural Service, 2014), Nigeria remains the second highest producer of sorghum throughout the world and presently accounts for 65-70 % of the total sorghum produced in West Africa (Akinseye *et al*., 2020). Sorghum production rate sum up to 63.08 million metric tons in 2016 while a total area of 5.35 million hectares was cropped with an output of 6.55 million metric tons in the 2017/2018 cropping season. The Nigeria's sorghum consumption data for the year 2017/2018 stood at 6.45 million and 150,000 metric tons for food and feed respectively. With respect to sorghum cultivation and its production rate in Nigeria, Niger State was ranked second among the leading states producing the staple.

Sorghum is the third most important staple food crops in Niger State. They are grown predominantly in the state due to increasing demand from processing industries, as well

as the position it occupies as the most dependable source of food/ livelihood for a significant number of people across the entire region (De Morais *et al*., 2015). Notwithstanding, sorghum is a very valuable staple for human consumption and it is very useful in diverse industries such as malting industries (for making beverages), flour mills (substitute for wheat to produce composite flours as well as high energy foods), and among others (Ajeigbe *et al*., 2018). Also, the high presence of micronutrient (Fe and Zn) in sorghum makes it a healthy food than any other cereals except for millet (Ajeigbe *et al*., 2018). This justifies the high production rate of the staple by many rural communities. However, the pest and disease infestation, poor storage facilities, inefficiency of resource utilization, as well as the proven presence of toxigenic fungi in sorghum makes it a major source of mycotoxins exposure to animals and human being (Sadiq *et al*., 2013), and thus, it is of great public health concern as it can influence percentage loss of productivity in sorghum, in Niger State and Nigeria as a whole. Table 2.3 shows the summary of global distribution of *Fusarium* fungi in sorghum.

Table 2.2: Global Incidence of *Fusarium* Mycotoxins in Maize

Region	Country	Toxic Fungi Species	Potential Mycotoxins	References
Africa	Burkina Faso	*F. verticillioides, F. graminearium, F. culmorum, F. proliferatum*	DON, FB_1, FB_2, BEA, ZEN, MON, $ENNB_1$,	Nikiema *et al*., 2004; Warth *et al*., 2012
	Cameroon	*F. verticillioides, F. Poae, F. graminearium, F. culmorum*	DON, FB, FB_1, FB_2, FB_3, NIV, ZEN, DON-3G, ENNB, ENNA, BEA	Njobeh *et al*., 2010; Ediage *et al*., 2015; Abia *et al*., 2013
	Cote d' Ivore	*F. morniliforme, F. graminearium*	FB, ZEN	Sangare-Tigori, *et al*., 2006
	DR Congo	*F. verticillioides, F. graminearium*	FB, ZEN	Mulunda *et al*., 2013
	Ethiopia	*F. verticillioides, F.graminearium*	FB, DON, NIV	Ayalew, 2010
	Ghana	*F. morniliforme*	FB	Kpodo, 2001
	Malawi	*F. verticillioides, F. Poae, F. graminearium, F. culmorum F. cerealis*	DON, FB, NIV, DAS, MON, FX, ZEN, BEA, ENNA, FUS, α-ZEl, β-ZEL, ZEL-4S, FUS, T-2	Matumba *et al*., 2015a
	Mosambique	*F. moniliforme, F. graminearium,*	FB_1, FB_2, FB_3, DON, NIV, ZEN, ENNA, BEA	Warth *et al*., 2012
	Nigeria	*F. verticillioides, F. graminearium, F. culmorum, F. poae, F. sporotrichioides, F. solani*	FB_1, FB_2, FB_3, DON, NIV, DAS, HT-2, ZEN, FX, FUS, DON-3G, 3-ADON, ZEN-14-G, T-2, α-ZEL, β-ZEL	Adetunji *et al*., 2014, Bankole and Mabekoje, 2004, Bankole *et al*., 2003, Chilaka *et al*., 2016, Gratz *et al*., 2017, Adejumo *et al*., 2007.
	Republic of Benin	*F. moniliforme*	FB_1, FB_2, FB_3	Fandohan *et al*., 2005, Fandohan *et al*., 2006,
	South Africa	*F. verticillioides, F. graminearium*	FB_1, FB_2, FB_3, DON, ZEN	Shephard *et al*., 2013; Chilaka *et al*., 2012, Phoku *et al*., 2012
	Tanzania	*F. moniliforme, F. graminearium, F. solani*	FB_1+FB_2, FB_1, FB_2, FB_3, DON, HT-2, ZEN	Kimanya *et al*., 2008; Kamala *et al*., 2015
	Zambia	*F. verticillioides*	FB	Mukanga *et al*., 2010

	Zimbabwe	*F. verticillioides, F. sporotrichioides, F. graminearium*	FB_1, FB_2, FB_3, DAS, DON, NIV, ZEN, 15-ADON	Hove *et al*., 2016, Gamanya and Sibanda, 2001
America	Argentine	*F. graminearium, F. moniliforme*	ZEN, ∑FB, FB_1	Scudamore and Patel, 2000
	US	*F. verticillioides*	FB_1, FB_2	Al-Taher *et al*., 2017
	Spain	*F. graminearium*	DON, T-2, HT-2	Cano-Sancho *et al*., 2011
	Albania	*F. graminearium, F. verticillioides, F. solani*	DON, ZEN, T-2, FB_1, FB_1+FB_2	Van Der Fels-Klerx *et al*., 2012
Europe	France	*F. graminearium, F. verticillioides*	ZEN, ∑FB, FB_1,	Scudamore and Patel, 2000
	Serbia	*F. crookwellense, F. equiseti, F. poae*	FUS, ZEN, ENN	Kos *et al*., 2014, Jajic *et al*., 2019
	Hungary	*F. graminearium, F. solani*	DON, T-2, ZEN	Tima *et al*., 2016
	Slovenia	*F. graminearium, F. verticillioides, F. solani*	DON, ZEN, FB_1+FB_2, T-2, HT-2	Jakovac-Strajn *et al*., 2010, Kirinčič *et al*., 2015
	Croatia	*F. graminearium, F. verticillioides, F. solani*	DON, ZEN, FB, T-2	Pleadin *et al*., 2013
	Bulgeria	*F. graminearium, F. verticillioides*	ZEN, FB_1+FB_2	Manova and Mladenova, 2009
	Norway	*F. graminearium, F. morniliforme*	DON, ZEN, FB, T-2	Binder *et al*., 2007
	Sweden	*F. verticillioides, F. graminearium*	FB, DON, ZEN	Binder *et al*., 2007
	Netherlands	*F. graminearium*	DON, ZEN	Van Der Fels-Klerx *et al*., 2012
Asia	China	*F. verticillioides, F. graminearium*	FB_1, ZEN	Xing *et al*., 2017; Yilmaz, 2017
	Malaysia	*F. verticillioides*	FB	Binder *et al*., 2007
	Japan	*F. proliferatum*	BEA	Yoshinari *et al*., 2016
	Korea	*F. graminearium, F. morniliformine*	DON, T-2, ZEN, FB	Binder *et al*., 2007
	Philippines	*F. graminearium, F. verticillioides*	DON, T-2, ZEN, FB	Binder *et al*., 2007

Table 2.3: Global Incidence of *Fusarium* Mycotoxins in Sorghum

Region	Country	Toxic Fungi Species	Potential mycotoxins	References
Africa	Ethiopia	*F. semitectum, F. verticillioides, F. graminearium,*	DON, ZEN, FB_1, FB_2, FB_3, NIV, DAS, DON-3-G, ZEN-4S, α-ZEL, β-ZEL, FA, ENB, MON, ENNA, BEA	Ayalew *et al*., 2006, Chala *et al*., 2014
	Nigeria	*F. semitectum, F. moniliforme, F. equiseti, F. graminearium, F. nygamai*	ZEN, DON, HT-2, DAS, 15-ADON, DON-3G, ZEN-14-G, MON, NIV, FUS, α-ZEL, β-ZEL,	Bankole and Mabekoje, 2004
	Burkina Faso	*F. thapsinum, F. verticillioides*	MON, FUM	Glenn (2007), Somda *et al*., 2007
	Sudan	*F. moniliformine*	Trichothecenes, FUM	Ahmed *et al*., 2008
	Egypt	*F. graminearium, F. verticillioides, F. solani*	Sterigmatocystin, ZEN, DON, NIV, FUS, FB, MON, FUS	Soliman (2003)
	Lesotho	*F. semitectum*	FB, MON, BEA	
	Zinbabwe	*F. compactum, F. dimerum, F. proliferatum, F. oxysporum*	ZEA, MON, Neosolaniol, trichothecens (DAS), HT-2, T-2, DAS, BEA, FUS	Ratnadass *et al*., 2003
	Tanzania	*Fusarium*	FB, DON, NIV, DAS, T-2	Bandyopadhyay *et al*., 1998
America	Brazil	*F. verticilloides, F. proliferatum*	FB, MON, FUS, FBs, MON, FP	Pitt and Hocking, 2009
	Argentina	F. napiforme	FB, MON, FUS	Glenn (2007)
Asia	India	*F. verticillioides, F. proliferatum, F. semitectum*	Fumonisins, DON, NIV, DAS, NIV, FUS-X, ZEA, MON	Lincy *et al*., (2011), Sharma *et al*., 2011
Europe	Australia	*F. nygamai*	FB, MON, BEA	Glenn (2007)

2.12 Factors Influencing Fungal and Mycotoxins Contamination of Cereals

Numerous classifications are used in categorizing the factors that influence the occurrence or presence of mycotoxins in the food chain. Some classifications categorize these factors as intrinsic and extrinsic (Mannaa and Kim, 2017), physical, chemical and biological factors while others classified them as ecological, environmental and storage factors (Zain, 2011). Based on physical, chemical and biological factors affecting the production of mycotoxins, the physical factors includes the environmental conditions that are conducive for fungal colonization and mycotoxin production such as temperature, relative humidity and insect infestation. Chemical factor includes the use of fungicides and/or fertilizers. Increase temperature, drought and an increase in relative humidity may selectively alter colonization and mycotoxigenic fungi metabolism and thus alter mycotoxin production (Russell *et al*., 1991). The biological factors are based on the interaction between the colonizing toxigenic fungal species and the substrate (D'mello and Marc-Donald, 1997). Regardless of the form of classification, the key determinant in the type and amount of mycotoxin produced is always dependent on the fungi, substrate and environmental factors (Lacey, 1986). However, without necessarily adhering to any classification systems in Nigeria, the factors are outlined below.

2.12.1 Climatic conditions

Climate is among the most important factors affecting the occurrence and distributions of *Fusarium* mycotoxins. The two most important environmental components favouring mould growth and incidence of mycotoxin are hot and humid conditions. However, this fungi exist in tropical climate such as those in Nigeria which is conducive for the growth of mould and production of mycotoxins. Mycotoxigenic fungi are most abundant in the tropics and as such, they are major food spoilage agents in this warmer climate (McLean and Berjak, 1987). Though, the most effective temperature and

moisture content for growth and toxin production for numerous toxigenic fungi vary, many of them achieve best growth and toxin synthesis between 24 ℃ and 28 ℃ and seed moisture content of at least 17.5 % (Ominski *et al*., 1994). Researchers also indicated that drought conditions constitute stress factors to plants rendering them vulnerable to mycotoxin production. Higher mycotoxigenic fungal contamination was recorded during rainy season than in the dry harmattan season among agricultural produce in Nigeria.

2.12.2 Pest infestation

Insects are one of the several factors that can affect the occurrence of mycotoxins in commodities such as maize, peanuts, cottonseed and consequently causing deterioration and loss of grains and seeds. Their invasion on grains reduces the quality, grade and market values of these products which in most cases are rendered unhealthy for human and animal consumption (Atanda *et al*., 2013). For instance, an important source of *Fusarium* inoculum is related to the activity of insects and *Fusarium* species have been isolated from a wide range of insects (Miller *et al*., 1998). In small cereals, aphids are important insect pests often correlated with *Fusarium* head blight (FHB) severity, but the efficacy of insecticide application seems strongly related to pest pressure. However, prophylactic sprays with insecticides will not enhance wheat yields in the absence of high pest pressure (Chen *et al*., 2015). In maize, phytophagous insects represent one of the most important infection pathways for *F. verticillioides* and consequently fumonisin contamination. Furthermore, Avantaggiato *et al*. (2003) found that insect damage of maize is good predictor of *Fusarium* mycotoxin contamination. On this note, considerable variability in the importance of insects in increasing mycotoxins has been reported. The relative importance of insects and other factors need to be considered in the context of the complex environment in which they interact (Dowd, 2003).

2.12.3 Soil types and soil conditions

Soil composition such as clay, loam or sand, pH of soil, mineral salts and trace elements are natural factors that exert a powerful impact on the production of fungi. Hence, crops grown on various type of soil may have different significant levels of mycotoxin contamination. For example, peanuts grown on light sandy soils support fast growth of fungi specifically under dry conditions while heavier soils result in lower contamination of peanuts due to their high water holding capability which helps the plants to prevent drought stress (Codex Alimentarius Commission, 2002).

2.12.4 Farming system and agricultural techniques

Numerous farming techniques have been shown in various reports as factors influencing mould growth in agricultural produce (Atanda *et al*., 2013). For instance, produce harvested from land on which groundnut has been cultivated the previous year were infested more by *Aspergillus flavus* and contained more aflatoxin than crops grown on land previously planted with rye, oats, melon or potatoes. However, this contrasting result suggests the importance of agronomic and environmental factors indicating that crop rotation influences mycotoxigenic mould growth.

Good agricultural practices in cereals also offers adoption measures to combat infections of *Fusarium* species and toxins accumulation in all phases of crop production (Ferrigo *et al*., 2016). Majorly, infected cereals debris which is sources of inoculum for *Fusarium* infection (Rossi *et al*., 2009; Keller *et al*., 2014) decomposes slowly (Nicolardot *et al*., 2007) and can therefore be present in subsequent crops for at least two years (Khonga and Sutton, 1988; Cotton and Munkvold, 1998). With respect to tillage, general agricultural practices include to plough the soil and bury the remains of previous crops and weeds while in minimum or no tillage practices, seeds are drilled into the previous crop stubbles directly. However, it is clear that conventional tillage

systems alter the physico-chemical property of soil influencing the nutrient distribution and the organism environment, thereby changing microbial population, complexity and layer distribution at different degrees for a limited time. These changes are likely influenced by soil structure and environment (Sengupta and Dick, 2015). Nevertheless, no tillage practices avoid soil disturbance but increases the organic matter and modify the microbiota components, which in turn favour fungi as primary decomposers with respect to the bacteria (Sengupta and Dick, 2015).

Some studies evidenced the effect of tillage system for deoxynivalenol contamination level in wheat (Peigné *et al*., 2013), and also a lack of effect with respect to fumonisin in maize. Other authors reported that minimally prepared soil after a *Fusarium*-host crop was conducive to a high incidence of *Fusarium* disease and mycotoxin contamination of wheat and maize (Ono *et al*., 2011; Blandino *et al*., 2012) whereas no *Fusarium* effect was detected when the previous crop was not a *Fusarium*-host plant (Dill-Macky and Jones, 2000).

2.12.5 Pre-harvest conditions

Genotypes, drought, soil type, plant density, fertilization level and insect activities are essential components in examining the likelihood of pre-harvest contaminations (Cole *et al*., 1995). However, high night time temperatures appears to be the most significant factor, which favour fungal growth and toxin production at a time when the plant is deprived of its usual source of energy and thus least able to resist fungal attack (Abbas *et al*., 2002, Abbas *et al*., 2007).

2.12.6 Time of harvesting

Harvest is the first stage in the production chain where moisture content or water activity becomes the most essential parameter in terms of the management and

protection of the crop (Kiaya, 2014). It also marks a shift from problems caused by plant pathogenic fungi like *Fusarium,* to problem caused by storage fungi such as *Penicillium verrucosum* (Choudhary and Kumari, 2010). In an ideal condition, grains will always be harvested after a spell of dry weather when it is at "safe" moisture content so that immediate drying is not necessary. However, this is not always possible hence inappropriate harvest time is a risk factor in Nigeria. Visual examination of the grain for symptoms of disease, and segregation of diseased batches from healthy grain is an important control measures at harvest period. Early harvesting also reduces fungal infection of crops in the field and subsequent contamination of harvested produce (Lavkor and Var, 2017). Although majority of farmers in Africa are well aware of the need for early harvesting, lack of storage space, unpredictable weather, labour constraint, need for cash, threat of thieves, rodents and other animals compel the farmers to harvest at inappropriate time (Bankole and Adebanjo, 2003). Thus, if produce are harvested at early stage, they have to be dried to safe levels to stop fungal growth.

2.12.7 Post harvest handling

The post-harvest phases are those phases following harvest and leading up to primary processing such as milling (Choudhary and Kumari, 2010). This will typically involve drying (if necessary), storage and transportation steps. Post-harvest movement of food/feeds commodities can be complex, as it may pass through a number of intermediaries such as traders and intermediate processors, who may be located at various geographical zones (Atanda *et al*., 2013). In mere simpler case, produce may remain on farm in store or buffer storage for a short period before being passed directly onto the processor. At all times the produce can become vulnerable to fungal contamination and mycotoxin production if the storage conditions are not strictly

controlled. Nevertheless, even if the contamination occurs or persist after this phase, the hazard associated with toxin must still be managed through post-harvest handling, if the products are to be used for food and/or consumption purpose (Choudhary and Kumari, 2010).

2.12.8 Storage factors

Mycotoxin contamination of cereals may results from improper storage facilities, uncontrollable ambient temperature, relative humidity and inadequate handling of harvested products (Liu *et al*., 2016). In order to preserve quality of cereals in storage, prevention of biological activity through adequate drying to less than 10 % moisture is necessary. Also, elimination of insect activity that can intensity moisture content through condensation of moisture resulting from respiration, low temperatures and inert atmospheres is essential in preserving the quality of cereals (Lanyasunya *et al*., 2005). Several studies on the occurrence of field and storage fungi have also been reported in Nigeria and the post-harvest contamination is normally characterized by the activity of the "storage" fungi typically species that are able to grow in relatively dry conditions.

2.13 *Fusarium* Mycotoxins Control Strategies

The control of mycotoxin contamination in food and feeds commodities has been the major problem in many countries globally (Agriopoulou *et al*., 2020). These toxins can be controlled to a certain extent by controlling the factors that affect mycotoxins production (Bullerman *et al*., 1984). Pre-harvest (field) and post-harvest control of mycotoxin production represent the most effective approach to reduce mycotoxins in foods and feeds (Haque *et al*., 2020) compared with physical and chemical approaches. The use of biological control is an up-to-date pre-harvest concept to control mycotoxin production (Nesic *et al*., 2021). This method involves biotransformation of mycotoxins into less toxic metabolites, which are generally more unique, productive and eco-

friendly. In line with this biological detoxification method, genetic improvement and application of nanotechnology showed tremendous potential in reducing mycotoxin production thereby improving food safety and food quality for extended shelf-life (Haque *et al*., 2020). Thus, this section explains the various detoxification methods and innovations for control and mitigation of mycotoxins problem.

2.13.1 Chemical control measures

Different chemicals such as bases (hydrated oxide, ammonia), chitosan and ozone have been employed in treatment of food and feed ingredient to reduce a number of mycotoxins to undetectable levels (Agriopoulou *et al*., 2020). However, the use of bases is prohibited in the European Union for food intended for human consumption. The application of bases such as KOH and NaOH has been documented in the degradation of mycotoxins in contaminated oil. However, this can lead to secondary contamination thereby contributing to the harmful effect on the nutritional value of the product. Thus, limiting the usage of alkaline compounds in mycotoxin detoxification process.

Further, chitosan which is linear polysaccharides have been reported as potential inhibitor of fungi, bacteria and viruses (Agriopoulou *et al*., 2020). Its biocompatibility and antimicrobial properties makes it ideal for the preservation of foods (Zachetti *et al*., 2019). The application of 1 % chitosan encapsulated with 1 % lemon essential oil reduced the level of DON in wheat grains (Gunupuru *et al*., 2019). In addition, the synergistic effect of chitosan and water activity for controlling fungi growth and mycotoxin production of FBs and DON by *Fusarium* species on maize and wheat was reported. A decrease in DON and FBs production in irradiated maize and wheat grains following the application of low molecular weight chitosan with deacetylation above 70 % and a dose of 0.5 mg/g was observed (Zachetti *et al*., 2019)

Several studies reported the use of ozone (O_3) in degradation of various mycotoxin in food (Santos Alexandre *et al*., 2018). This technique does not leave any harmful residue after application. Majorly, it is used to disinfect cereals, fruits, vegetables or decontaminate the presence of mycotoxins. Its application was reported by Agriopoulou *et al*. (2016), this chemical (O_3) was found successful in the degradation of aflatoxins, particularly AFB_1 and AFG_1. This was attributed to the C8 and C9 double bond in their structures. However, AFG_1 proved to be most sensitive under optimum condition (55 $g.O_3.h^{-1}$ for 6h), ozone treatment showed a decrease in DON (29-32 %) and it modified form DON-3G (44 %). Significant microbial decline was noticed in durum wheat leaving chemical and rheological properties of semolina and pasta from ozonated wheat unaffected (Piemontese *et al*., 2018).

2.13.2 Biological Control

Researchers from different region of the world have made great achievement in the search for biological agents for mycotoxin detoxification (Hassan *et al*., 2018). The main advantages of this biological control measures are that, they prove to be more effective, irreversible, efficient and most importantly, they are environment friendly. This control measures offer an alternative approach to the control of mycotoxins since it can lead to the production of fewer or no toxic intermediates and end products. In this vein, the role of microbial agents and enzymes in the disinfection of mycotoxins is highlighted in this section.

The biodetoxification processes is a relatively new approach for mycotoxin reduction via non-pathogenic microbes or their enzymes via catabolic process. Several microorganism and specific kinds of isolated yeast have been effectively used for the management of mycotoxins in food and feed (Haque *et al*., 2020). Mechanism in the

removal of toxins by microorganism is still investigated and reasonable conclusions have been draw in relation to this approach.

Development of bacteria capable of biotransforming mycotoxins into non-toxic metabolites began in the 1980s. The first were *Flavobacterium aurantiacum* with the capacity to detoxify aflatoxin, *phenylobacterium* immobile was able to degrade OTA and *Gliocladium roseum* detoxified zearalenone via ring opening with subsequent decarboxylation (Nešic *et al*., 2021). Considering the toxic effect of trichothecenes, it is well known that 12, 13-epoxide ring is in charge of their toxic activity, so the removal of this epoxide group causes significant loss of toxicity (Foroud *et al*., 2019). Eubacterium BBSH 797 was the first isolated individual bacterial strain which is capable of biotransforming the epoxide group of trichothecenes.

As a way of biological control, probiotic yeast or products that contain yeast cell wall have also been implemented to defeat mycotoxins. Quite a number of yeast strains proved to be effective in transformation of this toxin to non-toxic or at least less toxic product, while some of them suppress the development of filamentous fungi (Pfliegler *et al*., 2015). Research report suggested that distillery yeast sludge possesses the ability to prevent the absorption of mycotoins in gastrointestinal (GI) tract and can be used as a poultry feed additives as it partially ameliorated the immuntoxic effect of mycotoxins (Khan *et al*., 2017). New strain of *T. mycotoxinivorans* capable of degrading ZEN and OTA into the non-toxic OTα has been used commercially to detoxify OTA in animal and poultry diet (Murugesan *et al*., 2015). More than 50 % degradation of patulin by *Rhodotorula mucilaginosa* (*R. mucilaginosa* JM19) shows the importance of this yeast in foods and raw materials (Li *et al*., 2019). Fungi species like *Rhizopus*, *Alternaria*, *Trichoderma*, *Dactylium*, *Penicillium*, *Peniophora* and *Mucor* have been shown to possess the ability to degrade different mycotoxins (Haque *et al*., 2020).

It was reported by Hackbart *et al*., (2014) that *Rhizopus oryzae* and *Trichoderma reesi* reduce aflatoxins. *Trichoderma* strains have also exerted significant antibiotics and parasitism ability, making them a potential candidates by forming coils around the *Fusarium* hyphae and penetrating it (Błaszczyk *et al*., 2017). Specific enzymes such as oxidase, peroxides, reductase, lactase, esterase, aminotransferase and carboxyl esterase have been purified from microbial systems (Loi *et al*., 2017). Furthermore, the mechanism of the enzyme action towards ZEN has been studied by different research groups (Vekiru *et al*., 2016) and detoxification strategies have been developed to disrupt its estrogenic activity. The prevalence ZEN detoxifying mechanism described so far is cleavage of lactone ring, which is catalysed by esterase. The reaction mechanism is irreversible because the resulting hydroxyketones spontaneously decarboxylate (Loi *et al*., 2017).

All enzymes included in this regulation are considered as processing aids, excepting invertase and lysozyme, which belong to the group of additives. Eligibility criteria for detoxifying treatments, including biotransformation, have been established for materials intended for animal nutrition in 2015. EU Regulations have been established regarding enzyme fumonisin esterase as feed additive for pigs and poultry (Commission Implementing Regulation (EU), 2018) and EFSA gave its scientific opinion in 2020 on safety and efficacy of fumonisin esterase from *Komagataella phaffii* DSM 32159 as a feed additive for all animal species, in accordance with Regulation (EC) No 1831/20031 which establishes the rules governing the community authorization of additives for use in animal nutrition (EFSA, 2020).

2.13.3 Physical control measures

Various physical means of treatment have been used naturally for the removal of mycotoxins after post-harvest practices. Some of these physical approaches include

sorting, grading and elimination of the affected part of many agricultural produce *(*Agriopoulou *et al*., 2020). Further, cleaning, washing, drying, segregation, milling, boiling, roasting, irradiation, extrusion microwave heating and peeling are also used as physical treatment methods for mycotoxin decontamination. Unquestionably, cleaning and sorting make up the first step of natural disinfection. Sorting techniques is usually regarded as the most superior means of disinfecting agricultural farm products since they pose no risk of producing degradable products (Chilaka *et al*., 2017).

In a study conducted by Chilaka *et al*. (2017), a decrease in percentage of total FBs content was observed to be 26 % and 69 % after purification process. After sorting, a decrease of 27 % to 93 % was observed for the reduced FB content. Processing techniques can reduce the level of mycotoxin but cannot completely inactivate them (Neme and Mohammed, 2017). The level of mycotoxin contamination can be reduced by softening, because the fungi accumulate on the surface of the granule. Storage conditions also play a vital role in detoxification of mycotoxin since they affect the overall growth of fungi. Primarily, storage under controlled condition such as packaging practices, ventilation, controlled temperature and appropriate air humidity reduce the growth of fungi and the accumulation of mycotoxins (Gonçalves *et al*., 2019). As a result of inadequate storage facilities approximately 20 to 50 % of crop losses were recorded in the developing countries.

Other physical means of controlling mycotoxin contamination have been reported in literature (Agriopoulou *et al*., 2020). Research indicated that zearalenone toxicity was reduced and its radiation was safe up to irradiation of 10 KGy when fruit juices of orange, tomato and pineapple infected with ZEN was treated with irradiated distilled water. A higher dose of radiation affected the quality of the fruit juices (Kalagatur *et al*., 2018). While irradiation was proposed as a promising approach to mycotoxin

detoxification, its detoxification remains questionable because it can cause physical, chemical and biological effects following potential molecular reactions (Shi *et al*., 2018). Mycotoxin binders were also reported to inhibit the absorption of mycotoxins as they bind to mycotoxins and do not allow their entry into the bloodstream from the gut. Adsorbent material such as activated carbon complex, non-digestible carbohydrates aluminosilicates and cholesterol has been employed as a mycotoxin binder. This standout as an alternative physical technique to degrade different microbes (Kamle *et al*., 2019).

2.14 Mycotoxin Regulations

Mycotoxins regulation is well established in many developed countries of the world to safe guard the consumers from the harmful effects posed by these compounds. A few number of Africa countries, Nigeria inclusive have fixed regulations of some toxins. For example, fifteen Africa countries including Algeria, Morocco, Nigeria, South Africa, Kenya and Ethiopia among others have been reported to have regulations for aflatoxins (FAO, 2004; Ferrant *et al*., 2012). Since the scientific report made by FAO 2004, there is little or no available improvement in mycotoxin regulation by Africa countries (FAO, 2004; Matumba *et al*., 2015b), and where they are in existence, they are either lacking or poorly enforced. This scenario thus leads to an increase in the level of exposure to mycotoxins above the limits set by health regulatory bodies (Alberts *et al*., 2017). The unavailability of mycotoxins legislation in some other developing countries may be due to lack of exposure and toxicological data of certain toxins, resources and capacity to obtain data and enforce regulations (Wild and Gong, 2010).

Nevertheless, the plan for keeping low level of mycotoxins in food, limiting their exposure rate, reducing healthcare cost and accessing high-value markets for food is the primary basis for establishment of mycotoxins regulations. In view of this, practical

enforcement of mycotoxins regulations in food is necessary and highly valued for export crops, but less relevant to a largely small scale and subsistence farmers (Matumba *et al*., 2015b), as the quality of food produce may be difficult to manage due to poor agricultural practices which could lead to high prevalence of mycotoxins.

The scientific community has proposed different strategies in controlling and mitigating *Fusarium* mycotoxin production through implementation of good agricultural practices (GAP), good manufacturing practices and hazard analysis and critical control points to address the problems posed by various toxins in foods (Chilaka *et al*., 2017). However, the degree of reduction in their concentration is dependent on the matrix type, the type of toxins as well as the processing method and different conditions employed in processing. Hitherto, European Union maximum limits for *Fusarium* toxins (deoxynivalenol, fumonisins, zearalenone and nivalenone) in cereals and cereal based products have been established but variation in tolerance level in different countries may sometimes be quite large due to different geographical and agronomical characteristics, state of industrialization, political strategy and economic achievement of individual country (Mallmann *et al*., 2020).

The existing mycotoxin regulations in Africa, concern only the aflatoxins, and Morocco had the most detailed mycotoxin regulation. Like aflatoxins, regulations for *Fusarium* toxins such as deoxynivalenol, fumonisins and others were included in the food regulations for only few countries. For instance, maximum limit for total fumonisins in corn and corn based food products intended for human consumption were first regulated in Switzerland with value at 1000 µg/kg. The standard limit of zearalenone has not been established in all countries but some nations in partnership with WHO have set the maximum acceptable levels to be between 50 and 1000 µg/kg in food, with a provisional daily intake of 0.5 µg/kg.bw/day (JECFA, 2000). Also, limit for DON in

foodstuffs has received attention from developed countries of the world and its tolerable daily intake and that of its metabolites is 1 µg/kg.bw/day for minimal exposure, and 8 µg/kg.bw/day for acute exposure (JECFA, 2001).

As previously stated, there are no national regulations or guidelines currently documented to mitigate the presence of *Fusarium* mycotoxins in foods in some Africa countries. In line with the above review, non-stop efforts are being made by legislative bodies in Africa, particular in Nigeria, to promote the harmonization of mycotoxins regulations and control measures of *Fusarium* mycotoxin contamination on foods, as well as facilitating international food trade based on sound scientific principles and risk analysis. Also, efforts are being made to prevent and/or minimize the food losses and safeguard the wholesomeness of food supply.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

The following analytical grade chemicals: deoxynivalenol solution - 200µg/ml (Sigma-Aldrich), dichloromethane (CH_2Cl_2), (Merck KGaA Germany), acetonitrile (CH_3CN) (LiChrosolv®, Merck KGaA Germany), zearalenone ELISA kit (AgraQuant), fumonisin ELISA kit (AgraQuant), sodium hydrogen carbonate ($NaHCO_3$), distilled water H_2O, nitrogen gas (N_2), N- hexane (C_6H_{14}), methanol (CH_2OH), (LiChrosolv®, Merck KGaA Germany), iso-octane and disodium sulphate salt (Na_2SO_4) with percentage purity in the range 99.5-99.9 % were obtained and used without further purification.Table 3.1 show the lists of analytical facilities used in this study

3.1.2 Equipments

Different analytical facilities were employed in the course of sample preparation and analysis of mycotoxins, these include; High Performance Liquid Chromatography (HPLC) (Model LC98II) equipped with UV/Vis detector, Centrifuge, Microscope (Model: Olumpus DHB), Water bath (Model: DK- 420), Laminar air flow hood (Model: LAFC-VI LKH00652G), Centrifuge (Adventure Pro Model: AV313), Chromatographic column (microsphere c-18) (150*4.6 mm), Thermostat incubator (Model: DNP-9052-1A), Weighing balance (JA103P/1704017), Mixer grinder (Model: VTCL-Smart 154250), Microplate reader (Model: Diatek DR-200Bs), Micropipette (Model: Dragon-YE5A530682 Microlux) Orbital shaker (Model: Celtech KJ-201BD) and Oven dryer (Model: Klarstein).

3.2 Study Area

Niger State is a state in central Nigeria and the largest state in the country with 76,363 square kilometres (29, 484 sq mi). It lies between 10°00′N 6°00′E of the coordinate. The state is categorized under hot and humid climate for most part of the year, especially between fifth and tenth month of every year (29.5 ℃ and 73.1 %) which is favourable for fungal growth and mycotoxin production. The state grows the largest quantity of maize and it is the second largest producer of sorghum throughout the country for commercial purpose (Merem *et al*., 2017).

Niger State local government areas were classified into four (4) microclimatic zones as illustrated in Figure 3.1.The state consist of wettest zone with annual rainfall greater than 1400mm, wet and dry zone having an annual rainfall between 1200-1400mm and 1000-1200mm respectively, while the driest zone have the annual rainfall less than 1000mm (Muhammad *et al*., 2019). The wettest zone (zone I) is made up of Suleja and Tafa, Wet zone (zone II) is made up of Borgu and Magama, dry zone (zone III) consist of Agaie, Agwara, Bida, Bosso, Edati, Gbako, Gurara, Katcha, Kontagora, Lapai, Lavun, Mashegu, Minna, Mokwa, Munya, Paiko, Rijau and Shiroro while the driest zone (zone IV) include Mariga, Rafi and Wushishi (Muhammad *et al*., 2019).

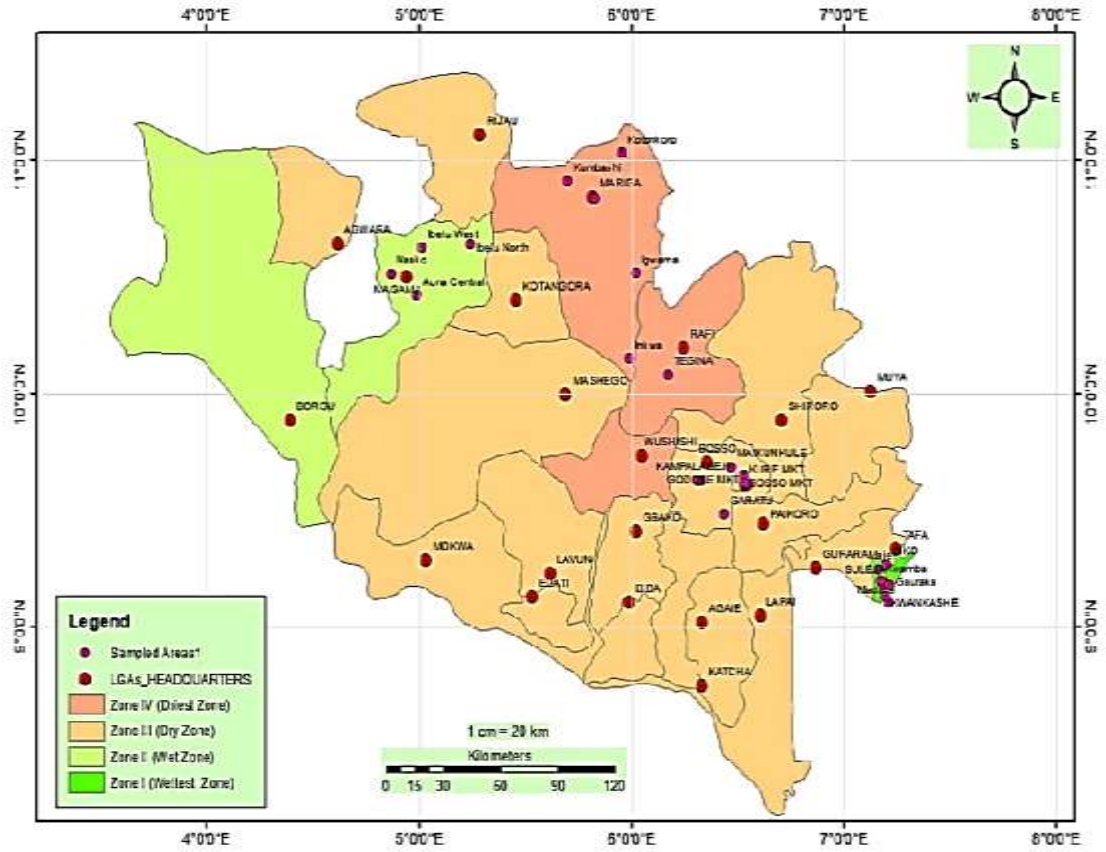


Figure 3.1: Micro-climatic Zones in Niger State from which Maize and Sorghum were Sampled.

3.2.1 Sample collection

A total of 480 samples consisting of 240 samples of maize and 240 samples of sorghum were collected from both silos and market in July 2019 from the four micro climatic zones of Niger state. This was pulled together to form sixty-four (64) composite samples, consisting of 16 white maize, 16 yellow maize, 16 red sorghum and 16 white sorghum samples. A total of eight (8) composite samples each from maize and sorghum were formed from the samples collected from the four micro-climatic zones. Samples were collected from Suleja, Magama, Bosso and Mariga. Based on the European Commission Regulation (2010), about 1 kg of each sample was collected, labelled, packaged with polythene bags and taken to the laboratory.

3.2.2 Sample Preparation

One kilogram of each sample was milled into fine powder using a mixer grinder and sub divided in order to produce a blended sub sample that served as a representative of the whole sample. Cleaning and decontamination of the equipment was performed using water and disinfectant after each sample milling step.

3.3 Fungal Isolation and Identification

Fungi isolation was carried out using plate dilution method as described by Kaufman *et al*. (1963) with slight modification (Njobeh *et al*., 2009). One gram of the milled sample was weighed into a sterile tube suspended in 9 mL ringer solution and shaken for 2 min on a vortex mixer. The suspension was serially diluted 10 folds.Aliquots (1 mL) of each tube from the 10^6 diluent were used for inoculation in triplicate on potato dextrose agar (PDA). The media was incubated for 7 days at 25 ℃ for enumeration of *Fusarium* species. Fungi growth and sporulation was observed using compound microscope and the plate count was noted. After incubation, the fungal colonies were counted using a colony counter and the number of colonies per gram of sample were counted and expressed in colony forming unit per gram as shown in Equation 3.1.

$$\mathrm{CFU/g} = \frac{\text{Number of colonies X Reciprocal of the dilution factor}}{\text{plating volumes (mL)}} \qquad 3.1$$

Before morphological identification of the isolates, colonies that resemble the representative isolate of *Fusarium species* were transferred (sub-cultured) onto sabouraud dextrose agar (SDA), potato dextrose agar (PDA) and malt extract agar (MEA). *Fusarium* species identification was carried out between the 3rd and 5th day of incubation. All isolates were identified on the basis of morphological characteristics of the colonies (conidial and conidiogenous cells) using the appropriate identification keys and atlas in literature (Pitt and Hocking, 2009; Oranusi and Olarewaju, 2013). The

summary of incidence rate, frequency and relative density of the isolated fungi species from maize and sorghum samples were calculated using Equation 3.2, 3.3 and 3.4 respectively.

$$\text{Incidence rate (\%)} = \frac{\text{Number of Isolates}}{\text{Total Number of Isolates}} \times 100 \quad 3.2$$

$$\text{Frequency (\%)} = \frac{\text{Number of Isolates}}{\text{Total Samples}} \times 100 \quad 3.3$$

$$\text{Relative density (\%)} = \frac{\text{Number of Isolates}}{\text{Total Incidence of Isolates}} \times 100 \quad 3.4$$

3.4 Mycotoxin Analysis

Subsequent to sample preparation, all analyses were carried out at the Central Research and Diagnostics Laboratory, Tanke, Ilorin, Kwara State. Deoxynivalenol and nivalenol extract were dried with nitrogen gas at the Central Research Laboratory, University of Ilorin, Kwara state. Enzyme Linked Immunosorbent Assay method was used for the extraction of fumonisins and zearalenol while the method of Patterson and Roberts, 1979 was used for extraction of deoxynivalenol.

3.4.1 Extraction of deoxynivalenol and nivalenol

About 12.5 g each of the samples was weighed and added into 250 mL conical flask, containing 50 mL of acetonitrile and water in ratio 9 to 1 (v/v). The conical flask was suspended on a shaker and stirred for 60 min. Thereafter, the mixture was filtered through a filter paper (Whatmann No 2) into a separating funnel. To an aliquot of the filtrate, 12 mL of iso-octane was added, this was swirled gently and the supernatant were discarded. In order to de-fat the samples, the procedure was repeated twice. The extract was poured into the separating funnel and 15 mL saturated solution of sodium hydrogen carbonate ($NaHCO_3$) was added. About 12 mL of dichloromethane (DCM) was added thrice for the extraction. In each of those times; the DCM layer was

coalesced together. Thereafter, both the DCM layer and saturated disodium hydrogencarbonate layer (aqueous layer) were separated.

The DCM layer was dried in a water bath at 50 ^{o}C, and the extract was reconstituted with 2 mL acetonitrile. The mixture was transferred into a dialysis tube and placed in boiling tube with 20 mL of 30 % acetone, and was agitated for 12 hrs. Thereafter, the dialysate was transferred into a separating funnel and the boiling tube was further treated with 5 ml of 30 % acetone and added to the dialysate. The dialysate was extracted three times with 10 mL of DCM and passed through a bed of disodium sulphate salt. The extract was reconstituted with 2 mL DCM and placed in an amber coloured vial and later dried under the influence of nitrogen (N_2) gas. The dried samples were further reconstituted in acetonitrile prior to HPLC analyses (Patterson and Roberts, 1979).

3.4.2 Quantification of deoxynivalenol and nivalenol using HPLC

Deoxynivalenol and nivalenol were quantified using HPLC modula system (model LC98II, Searchtech) with an ultraviolent detection. Chromatography separation was performed at column temperature of 30 ℃ for the two toxins. The system was accomplished with column types C-18, pressure max per flow rate of 420 pa x 0.8 ml/ml (deoxynivalenol/nivalenol) for separation and quantification of selected mycotoxins and internal standards. An injection volume of 20 µL was used for the prepared samples with the same mobile phase for the two toxins: that is, water: acetonitrile (90:10) for deoxynivalenol and nivalenol respectively. The same run time was used for HPLC analysis of each mycotoxin at wavelength 218 nm.

3.4.3 Method validation for high performance liquid chromatography (HPLC)

The methods of sampling and analysis for the official control of the levels of mycotoxins in food stuffs lay down by European Commission Regulation (EC) No

401/2006 of 23 February 2006 was adopted. According to the commission, the method was validated in terms of linearity (quantification), apparent recovery (% recovery) and sensitivity (limit of detection (LOD) and limit of quantification (LOQ)) using blank matrices of maize and sorghum (European Commission, 2006; Abia *et al*., 2013). External calibration curves were established based on the serial dilution of the mycotoxin standard solution. Linearity was determined by injecting mycotoxin standards at three different concentrations into the HPLC column. Calibrations curve between the different concentrations and correlation co-efficient (R^2) indicated good linearity with R^2 value of 0.9993 for the mycotoxin standards.

Percentage recoveries of the analytes were carried out by spiking three different samples (5 g of each) that were least contaminated with the analyte standard (100 µL of standard concentration). The spiked samples were left overnight in a fume cupboard at 30 ℃ for evaporation of the solvent to establish equilibrium between the sample matrix and the toxins. Mycotoxin from spiked samples was extracted by mycotoxin extraction method as described above. From each spiked sample, 20 µL of the extract was injected into the HPLC. The corresponding peak areas of the spiked samples were used for estimation of the apparent recovery by comparison with a standard of the same concentration prepared by dilution in pure solvent. The percentage recoveries were estimated using Equation 3.5 and the calibration parameters are presented in Table 3.1.

$$\% \text{ Recovery} = \frac{\text{Peak area spiked samples}}{\text{Peak area liquid standards}} \times 100 \qquad 3.5$$

Table 3.1: Calibration Parameters

Analytes	Calibration level (µg/kg)	Percentage recovery (%)	r^2	Equation of straight line
DON/NIV	12.5, 25.0, 50.0	87.7	0.9993	y = 2E+06x + 864270

Keys: DON = Deoxynivalenol, NIV = nivalenol, r^2 = correlation co-efficient

The sensitivity parameters (i.e. LOD and LOQ) for mycotoxins in the cereal grains were calculated from the signal to noise ratios (S/N) of the respective multiple reaction monitoring (MRM) chromatograms derived from the analysis of the spiked samples: LOD =3×S/N and LOD =10 ×S/N, respectively. Table 3.3 demonstrate the High Performance Liquid Chromatography operational conditions for deoxynivalenol and nivalenol.

3.5 Enzyme Linked Immunosorbent Assay for Fumonisin and Zearalenone

The enzyme linked immunosorbent assay (ELISA) method enabled the qualitative, semi-quantitative and quantitative determination of mycotoxins in food stuffs. The principles are binding assay that were based on monoclonal or polyclonal antibodies raised against toxins (antigens) and specific colour changes. The procedure adopted was based on the manufacturer's guideline (AgraQuant, 2019).

3.5.1 Extraction of fumonisins and zearalenone from maize and sorghum

A known mass (10 g) of each milled samples was weighed into labelled conical flasks. About 20 mL mixture of methanol and water (70:30) was added to the samples under continuous stirring in a mechanical shaker for 2 hrs. The reaction mixture was filtered through a Whatmann No. 2 filter paper. A portion of the filtrate (about 5 mL) was cleaned in a microfilter (5µm pore size), preconditioned with methanol and water in ratio 3:1 v/v. Afterwards, the filtrate was stored at 4 ^{o}C until further analyses (AgraQuant, 2019).

3.5.2 Assay procedure

All samples are prepared as stated in section (3.5.1) above. About 100 µL of the standard solution or prepared sample was added into appropriate wells of microtiter plate, 200 µL of HRP-conjugated antibody working solution were added into each well

separately. Thereafter, the microtiter plate was sealed with the cover membrane, and incubated for 15 minutes at room temperature. The content was dispensed (by aspiration or dumping) from each dilution well into a corresponding antibody coated micro well and washed repeatedly five times. Washing was done by filling each micro well with distilled de-ionized water using a multi-channel pipette. Complete removal of residual liquid at each step was ensured by striking the plate against an absorbent paper towel. Subsequently, 100 µL of substrate solutions was pipetted into each well. This was mixed gently by shaking the plate manually and incubated for 5 minutes at 25 °C. The reaction was allowed to develop in the dark and the plate was kept away from the drafts and other temperature fluctuations. Lastly, 100 µL of stop solution was added to each well and mixed gently by shaking the plate. After thorough mixing,the optical density of each well was measured using an ELISA microplate reader set to 450 nm. All samples, controls, and standards were assayed in duplicate. The range of quantification and percentage recoveries of the analytes were presented in Table 3.2.

Table 3.2: Validation Parameters for Enzyme-Linked Immunosorbent Assay Method

Mycotoxins	Limit of Detection (µg / kg)	Range of Quantification (µg / kg)	Percentage Recovery	Standard Conc. (µg / kg)	Wavelength
Fumonisin	200	250 – 5000	80%	0, 250, 500, 1000, 2500, 5000	450nm and 630 nm differential filter
Zearalenone	20(40) Maize (Sorghum)	40 – 1000	70%	0, 40, 100, 300, 1000	450nm and 630 nm differential filter

Source: AgraQuant (2009)

3.6 Estimation of Human Exposure to Mycotoxins

A simple questionnaire was administered to estimate human exposure to mycotoxins from maize and sorghum in Niger state in June 2019, a total of 120 respondents were required to fill the questionnaire in order to determine the exposure rate and risk

associated with mycotoxin contamination. Also, the survey was age- and gender-weighted and they represent adult population groups between the ages of 20-60 years (Appendix A). In this regards, the adult population groups were successfully interviewed through a quota sampling and completed the dietary intake questionnaires within 15-30 minutes. A portable scale was used to determine the weight of each respondent from the studied region. The weight of forms in which maize and sorghum are being consumed was also taken in grams. The questionnaire assessed whether the respondents consumed maize, sorghum or not, and explore the extent to which they consume their products (if they were consumed on a daily basis, weekly, monthly, quarterly or not at all).

3.6.1 Mycotoxins dietary intake

Since all the samples were contaminated with one or two mycotoxin(s), dietary exposure and risk characterization were calculated for all metabolites present in the samples. The estimated daily intake (EDI), percentage tolerable daily intake (% TDI) were evaluated by an integration of mycotoxins analysis data obtained from different samples analysed together with the consumption data of both gender with their mean body weight. In this study, the method employed by Rodriguez-Carrasco *et al*. (2013) and Adetunji *et al*. (2017); and confirmed by Joint FAO/WHO Expert Committee on Food Additives (JECFA) was adopted. The "estimated daily intake" (EDI) of individual metabolites was calculated using the formula presented in Equation 3.6.

$$\mathrm{EDI_m} = \frac{\mathrm{C_m \times k}}{\mathrm{b_w}} \qquad 3.6$$

Where; $\mathrm{EDI_m}$ is the estimated daily intake (µg/kg bw/day) for each mycotoxin m; C_m is the average level of a mycotoxin present in a sample type (µg/kg); k is the amount of the grains ingested on daily basis (g/day).

However, the formula presented in Equation 3.7 was implemented by multiplying the average level of each mycotoxin present in the maize and sorghum samples with the average maize and sorghum consumption in Niger state 331.96 g/person/day, 328.79 g/person/day "as estimated from the questionnaire" and then divided by mean body weight of 63.03, 64.29 and 61.58 kg for adult male, adult female and total population groups respectively.

3.6.2 Potential risk characterization

The calculated EDI_m was used to evaluate the potential risk of each mycotoxin. This was done by dividing the EDI_m with the tolerable daily intake (% of relevant TDI) (µg/kg bw/day) as designated in Equation 3.7 (Rodriguez-Carrasco *et al*., 2013).

$$\%\ TDI_m = \frac{EDI_m}{TDI} \times 100 \qquad 3.7$$

3.7 Data Analysis

Data were processed and calculated using SPSS statistics version 23 and Microsoft office Excel Professional Plus 2010 (Redmond, WA, USA). Mean, standard deviation (SD), range, tables and charts were used to present the results.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Frequency and average number of time people consume maize in Niger State, Nigeria

Based on the output from the respondents, Figure 4.1a revealed that 25 %, 25 %, 10 % and 45 % consume maize and maize products daily in wettest zone, wet zone, dry zone and driest zone respectively. The respondents consumed maize weekly from all the zones at higher rate when compared to daily consumption data (Wettest zone: 45 %; wet zone: 45 %; dry zone: 55 % and driest zone: 20 %) with the exception of driest zone that had higher percentile frequency of people consuming maize daily. Quite significant number of people; 5 and 15 % of the respondents from wettest zone and dry zone confirmed they do not consume maize at all, whereas no respondents established none consumption of maize and its products from wet zone and driest zone. Furthermore, it was clearly shown that for wettest zone, wet zone, dry zone and driest zone, the percentage frequency of people that consume maize and maize products monthly were 15, 20, 15 and 25 % respectively. Those that consume the products quarterly accounted for 10 % (wettest zone), 10 % (wet zone), 5 % (dry zone) and 10 % (driest zone).

On the average levels, those who consume maize and maize products daily take it once per day in each zone other than wet zone where they consume it more than one time a day (Figure 4.1b). Others consume it 5 times (wettest zone), 4 times (wet zone), 3 times (dry zone) and 5 times (driest zone) in a week. For an average consumption in a month, wet zone presented the highest value (8 times), while the least value was encountered in dry zone (5 times). However, the average number of time people consume maize and maize products within three months were estimated to be 4, 3, 6 and 5 times in wettest

zone, wet zone, dry zone and driest zone respectively. Inference from the questionnaire revealed that the average daily consumption of maize across the zones is 331.96 g.

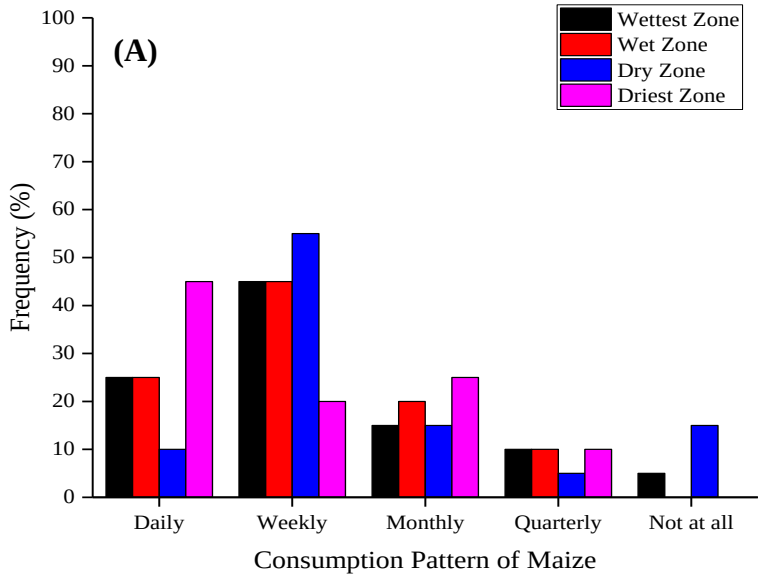


Figure 4.1 (a): Frequency of Maize Consumption from the Four Micro-Climatic Zones of Niger State.

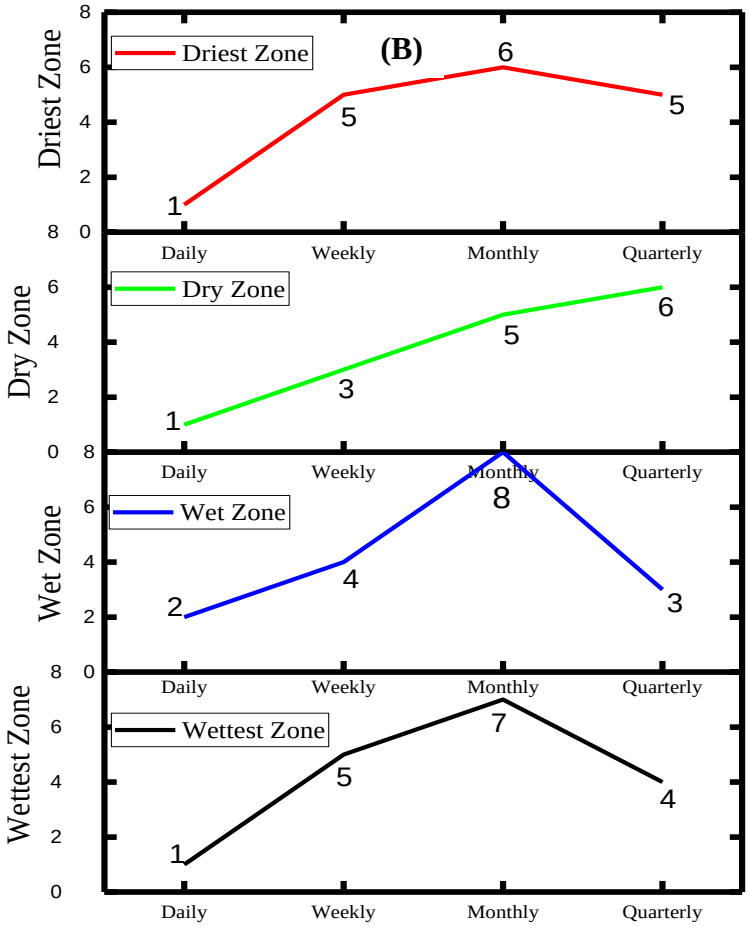


Figure 4.1 (b): Average Number of Time People Consume Maize in the Four Micro-Climatic Zones of Niger State

4.1.2 Frequency and average number of time people consume sorghum in Niger State, Nigeria

A slightly lower proportion of sorghum consumption data were obtained in relation to the maize consumption data. Figure 4.2 (a) revealed that 10 % (wettest zone), 20 % (wet zone), 5 % (dry zone) and 25 % (driest zone) of the population groups consumed sorghum daily. Large number of respondents consumed more sorghum in a week (than other categories) with percentage frequency of 40 % (wettest zone), 55 % (wet zone), 35 % (dry zone) and 45 % (driest zone). In a month, the percentage frequency of consumers that takes sorghum was estimated as follows: wettest zone (25 %) > wet zone (15 %) = dry zone (15 %) > driest zone (5 %). The outputs from the respondents also indicated that 10 % (wettest zone), 5 % (wet zone), 25 % (dry zone) and 15 % (driest zone) of sorghum consumers were found consuming the products fewer times in every three months (quarterly). It was observed that individuals who do not consume sorghum products at all were presents among the respondents. As such, dry zone (20 %) shows the highest percentage of people who do not consume sorghum and its products, while the least was estimated from the wet zone (5 %).

As presented in Figure 4.2 (b), it was clearly observed from all the zones that the average number of people who consume sorghum do that 3 times (wettest zone), 4 times (wet zone), 2 times (dry zone) and 4 times (driest zone) in a week. The average number of respondents who consumed sorghum monthly in wettest zone, wet zone, dry zone and driest zone, consume it 1 time, 7 times, 4 times and 5 times correspondingly. A few numbers of people consume it 3 times (wettest zone), 3 times (wetzone), 2 times (dry zone) and 4 times (driest zone) within 3 months. In addition, general ignorance of fungal infestation and mycotoxins contamination was also observed among the

respondents, although the population groups either acquired primary, secondary, tertiary or non-formal education, while some are not educated at all.

From the questionnaire, the average daily consumption of sorghum was recorded to be 328.79 g. The mean body weight of an adult maize and sorghum consumer was estimated to be 63.03 kg and 64.29 kg (male and female), while average body weight of the total population was recorded to be 61.58 kg. It can be observed that the average daily consumption rate of maize is higher than that of sorghum. In terms of frequency and quantity, maize is more consume than sorghum in Niger state. It is obvious that maize is more of a staple in wet zones than in dry zones. While in the case of sorghum, there is insignificant variation in the consumption pattern in the dry and wet zone.

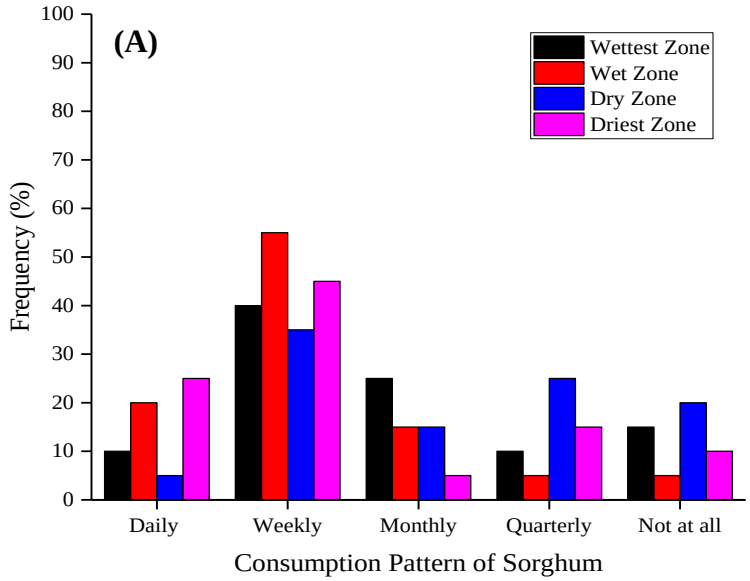


Figure 4.2 (a): Frequency of Sorghum Consumption from the Four Micro-Climatic Zones of Niger State.

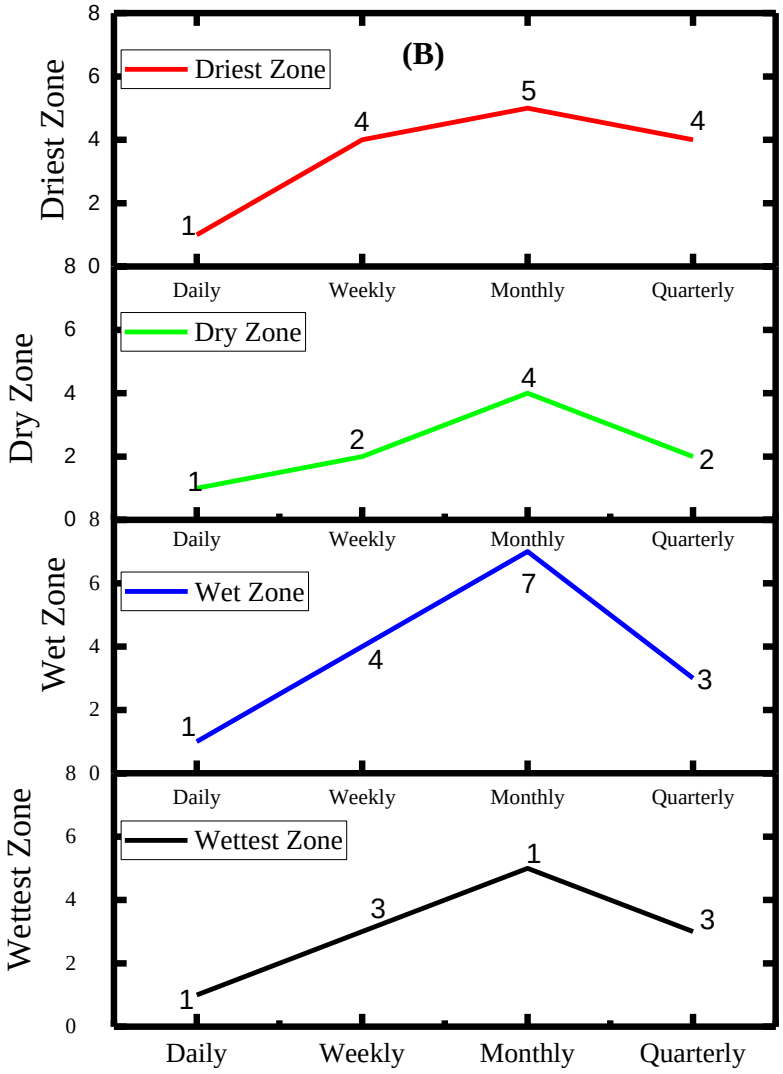


Figure 4.2 (b): Average Number of Time People Consume Sorghum in the Four Micro-Climatic Zones of Niger State

4.1.3 Colony forming units (CFU) of *Fusarium* spp found in maize and sorghum from the four micro-climatic zones

The rate of microbial contamination evaluated by the colony forming unit per gram (CFU/g) determined in sixty-four composites samples from the four micro-climatic zones of Niger state where maize and sorghum serve as the major staple are shown on Table 4.1. The result shows significant difference at $p < 0.05$. Yellow maize and red sorghum had the highest and the lowest level of CFU/g. Also, stored maize and market sorghum had the highest and least CFU/g in relation to the different sample types. The CFU contents in maize across the four micro-climatic zones is presented in decreasing order as follows: wet zone ($64.88 \times 10^6 \pm 17.91^c$) > driest zone ($20.13 \times 10^6 \pm 9.54^b$) > wettest zone ($19.50 \times 10^6 \pm 5.53^b$) > dry zone ($10.50 \times 10^6 \pm 2.96^a$), while that of sorghum is in the following order: wettest zone ($13.00 \times 10^6 \pm 5.98^b$) > driest zone ($5.00 \times 10^6 \pm 1.73^a$) > wet zone ($4.75 \times 10^6 \pm 1.25^a$) ≥ dry zone ($14.75 \times 10^6 \pm 1.23^a$). Maize was consistently more susceptible to fungi contamination than sorghum. The coloured variety of maize was more susceptibble to fungi contamination than the white variety, while the reverse is the case with sorghum. The fungal contamination of both grains were dependent on humidity with higher CFU/g recorded for wet zones than the drier zones for both crops. Also stored grains had higher fungal content than marketed samples

Table 4.1: Mean Colony Forming Unit per gram (CFU/g) of *Fusarium* spp in Different Sample Types, Varieties and across the Four Micro-climatic Zones

CFU/g of samples based on types, varieties and across the four micro-climatic zones			
CFU/g of samples based on varieties			
White Maize	Yellow maize	White sorghum	Red Sorghum
$24.19 \times 10^6 \pm 7.43^b$	$33.31 \times 10^6 \pm 9.86^c$	$8.06 \times 10^6 \pm 3.11^a$	$5.69 \times 10^6 \pm 1.28^a$
CFU/g of samples based on types			
Market Maze	Store Maize	Market Sorghum	Store Sorghum
$24.89 \times 10^6 \pm 6.92^b$	$32.63 \times 10^6 \pm 10.13^c$	$6.06 \times 10^6 \pm 2.15^a$	$7.69 \times 10^6 \pm 3.19^a$
CFU/g of maize across the zones			
Wettest zone	Wet zone	Dry zone	Driest zone
$19.50 \times 10^6 \pm 5.53^b$	$64.88 \times 10^6 \pm 17.91^c$	$10.50 \times 10^6 \pm 2.96^a$	$20.13 \times 10^6 \pm 9.54^b$
CFU/g of sorghum across the zones			
Wettest zone	Wet zone	Dry zone	Driest zone
$13.00 \times 10^6 \pm 5.98^b$	$4.75 \times 10^6 \pm 1.25^a$	$4.75 \times 10^6 \pm 1.23^a$	$5.00 \times 10^6 \pm 1.73^a$

Values with different superscripts across the row are significantly different ($p < 0.05$).

4.1.4 Frequency, incidence rate and relative density of *Fusarium* spp identified in different variety of maize samples

Five different *Fusarium* spp, *F. equiseti*, *F. graminearum*, *F. sporotrichioides*, *F. subglutinans* and *F. verticillioides* were identified from maize samples collected during the survey. With respect to the different varieties of maize samples analysed, *F. verticillioides* was observed to have the highest total number of isolate (21) of which white and yellow maize consist of 9 and 12 isolates with percentage incidence of 40.91 and 33.33 respectively. The next dominant species was observed to be *F. graminearum* and *F. sporotrichioides* with incidence rate of 7 (31.82 %) and 3 (13.64 %) in white maize, and 4 (11.11 %) and 8 (22.22 %) in yellow maize. Other species were observed to occur only in few numbers. As revealed in Table 4.2, the total percentile frequency of the isolated species was observed to be 68.77 % and 112.51 % for white maize and yellow maize, respectively. The total relative density of yellow maize was observed to be 62.07 % while that of white maize is 37.93 %. This implies that yellow maize is more vulnerable to fungi contamination than white maize.

Table 4.2: Distribution of Isolated *Fusarium* spp in Different Variety of Maize Samples

***Fusarium* species**	**Maize**						
				Frequency (%)		Relative Density (%)	
	White (n = 16)	Yellow (n = 16)	Total Incidence (n = 32)	White	Yellow	White	Yellow
F. verticillioides	9 (40.91)	12(33.33)	21	28.13	37.5	15.52	20.69
F. graminearium	7 (31.82)	4(11.11)	11	21.88	12.5	12.07	6.9
F.equiseti	1 (4.55)	7 (19.44)	8	3.13	21.88	1.72	12.07
F.sporotrichiodes	3 (13.64)	8 (22.22)	11	9.38	25	5.17	13.79
F. subglutinans	2 (9.09)	5 (13.89)	7	6.25	15.63	3.45	8.62
Total	22	36	58	68.77	112.51	37.93	62.07

Key: n = Number of samples

4.1.5 Frequency, incidence rate and relative density of *Fusarium* spp identified in maize samples from market and store

Fusarium spp contaminated maize samples collected from both market and store, with market samples being the most contaminated as revealed by the total frequency of species isolated in Table 4.3. Irrespective of the sample type, *F. verticillioides* contaminates both market and stored maize samples at high incidence rate of 38.24 % and 33.33 %, while the least predominant species isolated was found to be *F. subglutinans* (8.82 %) and *F. equiseti* (8.33 %). *Fusarium verticillioides* has the highest relative density of 22.41 % and it was observed in market sample while *F.equiseti* has the lowest density of 3.45 in stored maize. With respect to the total number of species isolated from both market and stored samples, it can be inferred that maize sample collected from market places were more colonized by fungi as compared to the stored samples.

Table 4.3: Distribution of Isolated *Fusarium* spp in Maize Samples from Market and Store Maize

Fusarium species	Market (n = 16)	Stored (n = 16)	Total occurrence (n = 32)	Frequency (%) Market	Stored	Relative Density (%) Market	Stored
F. verticillioides	13 (38.24)	8 (33.33)	21	40.63	25	22.41	13.8
F. graminearium	5 (14.71)	6 (25.00)	11	15.63	18.75	8.62	10.34
F.equiseti	6 (17.65)	2 (8.33)	8	18.75	6.25	10.34	3.45
F. sporotrichiodes	7 (20.58)	4 (16.67)	11	21.88	12.5	12.07	6.9
F. subglutinans	3 (8.82)	4 (16.67)	7	9.38	12.5	5.17	6.9
Total	34	24	58	106.27	75	58.61	41.39

Key: n = Number of samples

4.1.6 Frequency, incidence rate and relative density of *Fusarium* spp identified in maize from the four micro-climatic zones

The isolated species were observed to spread across the four micro-climatic zones. Among these species, *F. verticillioides* was the most commonly isolated species with the samples from wet and driest zone being the most contaminated with 6 isolates each, while dry zone has five (5) isolates. Of the 21 *F. verticillioides* isolated, wettest zone has the least number of isolate (4). Other *Fusarium* spp including *F. graminearum, F. equiseti, F. sporotrichioides and F. subglutinans* with total isolates of 11, 8, 11 and 7 were also recorded. As indicated in Table 4.4, the total frequency and relative density of all isolated species in descending order is as follows: wettest zone > driest zone 4 > dry zone > wet zone. In general, the most common *Fusarium spp* found in maize, regardless of the sample types, variety or zones were the *F. verticillioides* with frequency occurrence of 65.63 %.

Table 4.4: Distribution of Isolated *Fusarium* spp in Maize from the Four Micro-climatic Zones

	Maize												
***Fusarium* species**	Wettest (n = 8)	Wet (n = 8)	Dry (n = 8)	Driest4 (n = 8)	Total Incidence n = 32	Frequency (%)				Relative density (%)			
						Wettest	Wet	Dry	Driest	Wettest	Wet	Dry	Driest
F. verticillioides	4 (23.53)	6 (50.00)	5 (35.71)	6 (40.00)	21	12.5	18.75	15.63	18.75	6.9	10.34	8.62	10.34
F. graminearium	5 (29.41)	0 (0.00)	2 (14.29)	4 (26.67)	11	18.75	0	6.25	12.5	8.62	0	3.45	6.9
F.equiseti	3 (17.65)	3 (25.00)	2 (14.29)	0 (0.00)	8	9.38	9.38	6.25	0	5.17	5.17	3.45	0
F. sporotrichiodes	2 (11.76)	2 (16.67)	4 (28.57)	3 (20.00)	11	6.25	6.25	12.5	9.38	3.45	3.45	6.9	5.17
F. subglutinans	3 (17.65)	1 (8.33)	1 (7.14)	2 (13.33)	7	9.38	3.13	3.13	6.25	5.17	1.72	1.72	3.45
Total	17	12	14	15	58	56.26	37.51	43.76	46.88	29.31	20.68	24.14	25.86

Key: n = Number of samples

4.1.7 Frequency, incidence rate and relative density of *Fusarium* spp identified in sorghum samples from market and store

As shown in Table 4.5 below, the incidence rate of the five (5) *Fusarium species* isolated from market samples were evaluated to be 28.57, 21.43, 28.57, 14.29 and 7.14 % for *F. vericilllioides*, *F. nygamai*, *F. semitectum*, *F. solani* and *F. oxysporum*. On the other hand, the frequency of fungi contamination in stored sorghum samples were estimated in decreasing order: *F. verticillioides* (47.37) > *F. nygamai* (15.79), *F. oxysporum* (15.79) > *F.semitectum* (10.53) and *F. solani* (10.53). The observed relative density in Table 4.5 revealed that *F. verticillioides* (17.02 % of market sorghum grains and 19.15 % of stored sorghum grains) and *F. semitectum* (17.02 % of market sorghum and 4.26 % of stored sorghum grain) were the dominants spp. Total number of isolates for *F. nygamai*, *F. solan*i and *F. oxysporum* were 9, 6 and 5 respectively. An important observation made in this survey was that sorghum sample obtained from market places were contaminated than samples collected from the store.

Table 4.5: Distribution of Isolated *Fusarium* spp in Sorghum Samples from Market and Store

Fusarium species	Sorghum						
	Market (n = 16)	Stored (n = 16)	Total occurrence (n = 32)	Frequency (%) Market	Store	Relative Density (%) Market	Store
F. verticillioides	8 (28.57)	9 (47.37)	17	25	28.13	17.02	19.15
F. nygamai	6 (21.43)	3 (15.79)	9	18.75	9.38	12.77	6.38
F.semitectum	8 (28.57)	2 (10.53)	10	25	6.25	17.02	4.26
F. solani	4 (14.29)	2 (10.53)	6	12.5	6.25	8.51	4.26
F. oxysporum	2 (7.14)	3 (15.79)	5	6.25	9.38	4.26	6.38
Total	28	19	47	87.5	59.39	59.58	40.43

Key: n = Number of samples

4.1.8 Frequency, incidence rate and relative density of *Fusarium* spp identified in different varieties of sorghum samples

In the present study, mycological examination of 32 sorghum composite samples revealed the frequency of five (5) *Fusarium* species namely: *F. verticillioides*, *F. nygamai*, *F.semitectum*, *F. solani* and *F. oxysporum*. In both variety of sorghum samples, *F. verticillioides* was the most predominant species as indicated in Table 4.6 with incidence rate of 11 (42. 31) and 6 (28.57 %) in white and red sorghum. The dominants species in white sorghum was trailed by *F. semitectum*, 7 (26.92), while the incident rate of *F. verticillioides* observed in red sorghum was followed by *F. nygamai*, 5 (23.81 %). *Fusarium oxysporum* has the least occurrence of 1, with percentage incidence of 3.85 (white sorghum) and 19.05 (red sorghum). Similar trend was observed for both frequency of occurrence of the isolated species and the relative density. From this observation, it is worthy to say that sorghum white samples are more contaminated with *Fusarium spp* than sorghum red samples. Comparatively, maize are more vulnerable to *Fusarium* infestation than sorghum.

Table 4.6: Distribution of Isolated *Fusarium* spp in Different Varieties of Sorghum

***Fusarium* species**	**Sorghum**						
	White (n = 16)	Red (n = 16)	Total occurrence (n = 32)	Frequency (%) White	Frequency (%) Red	Relative Density (%) White	Relative Density (%) Red
F. verticillioides	11 (42.31)	6 (28.57)	17	34.38	18.75	23.4	12.77
F. nygamai	4 (15.38)	5 (23.81)	9	12.5	15.63	8.51	10.63
F.semitectum	7 (26.92)	3 (33.33)	10	21.88	9.38	14.89	6.38
F. solani	3 (11.54)	3(33.33)	6	9.38	9.38	6.38	6.38
F. oxysporum	1 (3.85)	4 (19.05)	5	3.13	12.5	2.13	8.51
Total	26	21	47	81.27	65.64	55.31	44.67

Key: n = Number of samples

4.1.9 Frequency, incidence rate and relative density of *Fusarium* spp identified in sorghum from the four micro-climatic zones

Fusarium vertcillioides constitutes 36.17 % of the 47 *Fusarium* isolates from the four zones. Being the most prevalent species isolated from the entire zone (with the exception of driest zone that had the highest number of *F. semitectum* isolates) it is known to be a primary producer of FUM and trichothecenes. Worthy of note is the fact that *F. oxysporum* has the least frequency of occurrence with percentile of 6.25, 3.13, 3.13 and 3.13 in wettest zone, wet zone, dry zone and driest zone respectively. The total relative densities of *Fusarium* spp isolated across the zones were in the following order: wettest zone (29.79) > driest zone (25.54) > dry zone (23.41) > wet zone (21.19). It could be deduced from the observation made from this study that wettest zone (zone 1) samples were the most contaminated.

Table 4.7: Distribution of isolated *Fusarium* spp in Sorghum from the Four Micro-climatic Zones

	Sorghum												
***Fusarium* species**	Wettest (n = 8)	Wet (n = 8)	Dry (n = 8)	Driest (n = 8)	Total Incidence (n = 32)	Frequency(%)				Relative density(%)			
						Wettest	WetDry	Driest		Wettest	Wet	Dry	Driest
F. verticillioides	4 (28.57)	6 (60.00)	4 (36.36)	3 (25.00)	17	12.5	18.75	12.5	9.38	8.51	12.77	8.51	6.38
F. nygamai	3 (21.43)	1 (10.00)	3 (27.27)	2 (16.67)	9	9.38	3.13	9.38	6.25	6.38	2.13	6.38	4.26
F.semitectum	2 (14.29)	2 (20.00)	2 (18.18)	4 (33.33)	10	6.25	6.25	6.25	12.5	4.26	4.26	4.26	8.51
F. solani	3 (21.43)	0 (0.00)	1 (9.09)	2 (16.67)	6	9.38	0	3.13	6.25	6.38	0	2.13	4.26
F. oxysporum	2 (14.29)	1 (10.00)	1 (9.09)	1 (8.33)	5	6.25	3.13	3.13	3.13	4.26	2.13	2.13	2.13
Total	14	10	11	12	47	43.76	31.26	34.39	37.51	29.79	21.29	23.41	25.54

Key: n = Number of samples

4.1.10 Incidence and concentration (µg/kg) of *Fusarium* mycotoxins in different varieties of maize and sorghum

The highest concentration of DON was detected in yellow maize (42.72±8.44 µg/kg), while the lowest mean concentration was recorded to be 1.08±0.33 µg/kg in red sorghum (Table 4.8). However, the highest number of samples positive for DON was detected in maize (12), while the lowest number of positive samples was found in sorghum (10). The observation made from this study implies that maize samples are more susceptible to DON contamination than sorghum samples. The mean concentration of FUM was higher in yellow maize samples (2009.91±952.83 µg/kg), followed by white maize (1375.85±368.24 µg/kg), red sorghum (872.72±185.22 µg/kg) and white sorghum samples (852.16±244.89 µg/kg). Meanwhile, its concentration was detected in 13 white maize samples while all yellow maize samples were positive for FUM. In sorghum samples, only 18 of the total (32) samples (10 white and 8 red samples) were found positive for FUM concentration.

As shown in Table 4.8, yellow maize had five (5) samples positive for nivalenol with mean concentration of 2.84±0.99 µg/kg. On the contrary, the least number of samples contaminated with NIV was found in red sorghum (2), with an average concentration of 0.10±0.02 µg/kg. White maize and white sorghum respectively had 4 and 6 samples positive for NIV contamination with mean concentration of 0.29±0.01 µg/kg and 1.11±0.03 µg/kg. The concentration of NIV in different varieties of maize and sorghum samples ranges between 0-1.90 µg/kg, 0-28.80 µg/kg, 0-12.85 µg/kg and 0-1.08 µg/kg for white maize, yellow maize, white sorghum and red sorghum. Zearalenone mean concentration was found higher in yellow maize sample (36.68±11.04 µg/kg) and it was consistent with other studied toxins whose mean concentration values were also found higher in yellow maize. This value was trailed by red sorghum (17.34±6.17 µg/kg),

white maize (15.95±3.21 µg/kg) and white sorghum (15.53±3.11 µg/kg) respectively. Moreover, the numbers of samples positive for ZEN were 2, 5, 2 and 1 for white maize; yellow maize, white sorghum and red sorghum correspondingly.

FUM is the most commonest *Fusarium* mycotoxin in both crops followed by DON, NIV and ZEN. Maize is more vulnerable to all the studied mycotoxins than sorghum. With regards to varieties, yellow maize had higher frequency and concentrations of all the studied toxins than the white variety. Conversely, white sorghum had higher occurrence of all the four toxins than the red variety; however, concentrations of FUM and ZEN were higher in the red than white variety.

Table 4.8: Incidence and Level of *Fusarium* Mycotoxins (µg/kg) in Different Varieties of Maize and Sorghum

Sample Types	Sample Parameters	DON	FUM	NIV	ZEN
White Maize	Total Samples	16	16	16	16
	No. of +ve Samples (% Contamination)	5(31.25)	13 (81.25)	4(25)	2 (12.50)
	Mean ±SD (µg/kg)	7.01±2.72[b]	1375.85±368.24[b]	0.29±0.01[a]	15.95±3.21[a]
	Range	0-85.29	0-4547.17	0-1.90	0-181.58
	EU Limit	1750	1000	Na	350
	No. of Cont. Samples above Max. Limit (%)	0	10 (62.50)	Na	0
Yellow Maize	Total Samples	16	16	16	16
	No. of +ve Samples (% Contamination)	7(43.75)	16 (100)	5(31.25)	5 (31.25)
	Mean ±SD (µg/kg)	42.72±8.44[c]	2009.91±952.83[c]	2.84±0.99[c]	36.68±11.04[b]
	Range	0-342.62	364.15-4889.31	0-28.80	0-235.79
	EU Limit	1750	1000	Na	350
	No. of Cont. Samples above Max. Limit (%)	0	13 (81.25)	Na	0
White Sorghum	Total Samples	16	16	16	16
	No. of +ve Samples (% Contamination)	6(37.5)	10 (62.50)	6 (37.50)	2 (12.50)
	Mean ±SD (µg/kg)	4.55±1.86[b]	852.16±244.89[a]	1.11±0.03[b]	15.53±3.11[a]
	Range	0-40.84	0-3269.81	0-12.85	0-190.00
	EU Limit	1250	1000	Na	100
	No. of Cont. Samples above Max. Limit (%)	0	8 (50.00)	Na	1 (6.25)
Red Sorghum	Total Samples	16	16	16	16
	No. of +ve Samples (% Contamination)	4(25)	8 (50.00)	2 (12.50)	1 (6.25)
	Mean ±SD (µg/kg)	1.08±0.33[a]	872.72±185.22[a]	0.10±0.02[a]	17.34±6.17[a]
	Range	0-8.46	0-3144.03	0-1.08	0-277.37
	EU Limit	1250	1000	Na	100
	No. of Cont. Samples above Max. Limit (%)	0	6 (37.50)	Na	1 (6.25)

Values with different superscripts down the column are significantly different ($p < 0.05$).

Keys: Mean = Mean concentration; SD = Standard deviation; DON = Deoxynivalenol; FUM = Fumonisins; NIV = Nivalenol; ZEN = Zearalenone; EU limit= European Union Maximum limits; Na = Not Applicable

4.1.11 Incidence and concentration (µg/kg) of *Fusarium* mycotoxins in different sample types

Similarly, higher concentration of DON was detected in market maize samples with a mean value of 28.53±6.65 and this was followed by maize samples collected from store

with mean concentration of 21.20±5.22 µg/kg. The least concentration of DON was detected in stored sorghum samples with average concentration of 1.18±0.27 µg/kg. When data was summarized; market maize has the highest number of samples that was positive for DON, while the least number of samples that were contaminated with DON was recorded in stored sorghum (Table 4.9). DON contaminations in maize sample were significantly higher ($p < 0.05$) than contamination in sorghum sample from both market and store. Considering fumonisins concentration detected based on sample type, market maize samples were observed to have the highest mean concentration (1786.38±462.26 µg/kg), followed by stored maize (1426.06±194.89 µg/kg), market sorghum (957.51±261.94 µg/kg) and stored sorghum (767.37±314.03 µg/kg).

Table 4.9, shows that market maize samples had the highest mean concentration of NIV (2.52±0.58 µg/kg), followed by market sorghum (0.99±0.13 µg/kg),stored maize (0.61±0.01 µg/kg) and stored sorghum (0.22±0.10 µg/kg). The same number of samples tested positive for NIV contamination in each sample type except for market maize that had five (5) positive samples. The concentration of NIV in market maize, stored maize, market sorghum and stored sorghum were in the range of 0-28.80 µg/kg, 0-6.56 µg/kg, 0-12.85 µg/kg and 0-1.22 µg/kg. The mean ZEN concentration with respect to sample type revealed that market maize samples had the highest mean concentration of 33.95±7.88 µg/kg, followed by market sorghum (29.21±4.61 µg/kg), stored maize (18.68±5.78 µg/kg) and stored sorghum (3.65±0.73 µg/kg). This result does not correspond to the higher number of *F. graminearium* isolates (prominent producer of zearalenone) that was found in stored maize. With regards to type of samples, the marketed samples of both crops had higher frequency of occurrence and mycotoxin contents than stored samples. Also, FUM appeared to be the dominant mycotoxins followed by ZEN, DON and NIV. In terms of concentration, the studied mycotoxins

followed the same pattern except for stored maize that had higher concentration in DON than ZEN.

Table 4.9: Incidence and Level of *Fusarium* Mycotoxins (µg/kg) in Different Sample Types

Sample Types	Sample Parameters	DON	FUM	NIV	ZEN
Market Maize	Total Samples	16	16	16	16
	No. of +ve Samples (% Contamination)	7(43.75)	16 (100)	5 (31.25)	4 (25.00)
	Mean ±SD (µg/kg)	28.53±6.65[b]	1786.38±462.26[c]	2.52±0.58[c]	33.95±7.88[c]
	Range	0-342.62	364.15-4889.31	0-28.80	0-235.79
	EU Limit	1750	1000	Na	350

	No. of Cont. Samples above Max. Limit (%)	0	13 (81.25)	Na	0
Stored Maize	Total Samples	16	16	16	16
	No. of +ve Samples (% Contamination)	5(31.25)	13 (81.25)	4 (25.00)	3 (18.75)
	Mean ±SD (µg/kg)	21.20±5.22^{b}	1426.06±194.89^{c}	0.61±0.01^{ab}	18.68±5.78^{b}
	Range	0-314.96	0-4452.20	0-6.56	0-181.58
	EU Limit	1750	1000	Na	350
	No. of Cont. Samples above Max. Limit (%)	0	10 (62.50)	Na	0
Market Sorghum	Total Samples	16	16	16	16
	No. of +ve Samples (% Contamination)	6(37.5)	10 (62.50)	4 (25.00)	2 (12.50)
	Mean ±SD (µg/kg)	4.45±1.39^{a}	957.51±261.94^{ab}	0.99±0.13^{b}	29.21±4.61^{bc}
	Range	0-40.84	0-3144.03	0-12.85	0-277.37
	EU Limit	1250	1000	Na	100
	No. of Cont. Samples above Max. Limit (%)	0	8 (50.00)	Na	2
Stored Sorghum	Total Samples	16	16	16	16
	No. of +ve Samples (% Contamination)	4(25)	8 (50.00)	4 (25.00)	1 (6.25)
	Mean ±SD (µg/kg)	1.18±0.27^{a}	767.37±314.03^{a}	0.22±0.10^{a}	3.65±0.73^{a}
	Range	0-8.46	0-3269.81	0-1.22	0-58.42
	EU Limit	1250	1000	Na	100
	No. of Cont. Samples above Max. Limit (%)	0	6 (37.50)	Na	0

Values with different superscripts down the column are significantly different ($p < 0.05$).

Keys: Mean = Mean concentration; SD = Standard deviation; DON = Deoxynivalenol; FUM = Fumonisins; NIV = Nivalenol; ZEN = Zearalenone; EU limit= European Union Maximum limits; Na = Not Applicable

4.1.12 Incidence and concentration (µg/kg) of multi-mycotoxins in maize and sorghum samples from the four zones

With regards to the mean concentration of DON in maize samples across the zones, Table 4.10 revealed that wettest zone had the highest mean concentration (47.38±9.57 µg/kg) followed by dry zone (39.79±13.03 µg/kg), driest zone (11.78±3.41 µg/kg) and wet zone (0.50±0.02 µg/kg) while sorghum samples across the zones had mean concentration of DON in the following order: wettest zone (5.63±3.77 µg/kg) > zone 3 (3.23±1.17 µg/kg) > zone 2 (1.35 ± 0.15 µg/kg) > zone 4 (1.06±0.36 µg/kg). The mean

concentration of fumonisins appeared to be highest in maize samples from the wettest zone (2113.29±754.73 µg/kg) while other mean concentration of maize samples across the zones were recorded to be 1611.92±709.13 µg/kg (driest zone) > 1539.95±865.48 µg/kg (dry zone) > 1449.99±505.66 µg/kg (wet zone). The same trend was observed in sorghum samples with the exception of the dry zone (816.67±276.92 µg/kg) that has mean concentration of fumonisins greater than the driest zone (770.44±140.17 µg/kg).

The highest mean concentration of NIV was observed in the driest zone (4.54±1.24 µg/kg), followed by wettest zone (1.43±0.35 µg/kg). Interestingly, the same concentration of NIV was observed in wet zone (0.15±0.01 µg/kg) and dry zone (0.15±0.01 µg/kg). On the contrary, wettest zone (1.89±0.65 µg/kg) had the highest mean concentration of NIV in sorghum samples and no sample was found positive for NIV in the dry zone. In the same vein, the highest mean concentration of ZEN in maize samples was observed in the wettest zone (35.46±9.88 µg/kg) while the least mean value was estimated from wet zone (17.89±6.73 µg/kg). Two samples each in three different zones were tested positive for zearalenone, while only one sample in the dry zone was contaminated with zearalenone. In the case of sorghum samples, none of the samples from wettest zone were contaminated with zearalenone. Whereas, one sample each was contaminated with zearalenone in the wet zone, dry zone and driest zone with mean concentrations of 7.30±2.14 µg/kg, 23.75±7.83 µg/kg and 34.67±12.33 µg/kg, respectively. In terms of climatic conditions, wetter zones had higher frequency of occurrence in maize samples than drier zones. On the other hand, sorghum samples present higher frequency of FUM and ZEN in drier zones than wetter zones.

Regarding the concentration of mycotoxins in maize across the zones, FUM, DON, and ZEN had higher mean concentration in the wettest zones with the exception of NIV that

present the highest mean concentration in the driest zone. In sorghum samples, the concentrations of FUM, DON and NIV were found higher in the wettest zone, while ZEN mean concentration was found higher in the driest zone. It can be observed from the results that none of the cereal grains regardless of the sample type, variety or locations (zones) were found contaminated with DON above the European Union (EU) maximum limits of 1750 µg/kg ad 1250 µg/kg for uprocessed maize and unprocessed cereals other than maize.

Further, the current study revealed that 57.81 % (maize) and 43.75 % (sorghum) of FUM contaminated samples were found to be above EU maximm regulatory limit of 1000 µg/kg for maize and other cereals intended for direct human consumption. The result also revealed that none of the studied maize samples were found contaminated with ZEN above EU maximum regulatory limit of 350 µg/kg for unprocessed maize, while only 6.25 % of the analysed sorghum samples were contaminated with ZEN above EU maximum limit of 100 µg/kg for unprocessed cereals other than maize. Presently, no maximum limit has been fixed for NIV in foods and feeds.

Table 4.10: Incidence and Level of *Fusarium* Mycotoxins (µg/kg) in Maize and Sorghum Samples from the Four Micro-climatic Zones

Zone	Maize								Sorghum							
	DON		FUM		NIV		ZEN		DON		FUM		NIV		ZEN	
	n (N) % Cont.	Mean±SD (Range) EU limit (No. of Cont. sample above EU limit)	n (N) % Cont.	Mean±SD (Range) EU limit (No. of Cont. sample above EU limit)	N (N) % Cont.	Mean±SD (Range) EU limit (No. of Cont. sample above EU limit)	n (N) % Cont.	Mean±SD (Range) EU limit (No. of Cont. sample above EU limit)	N (N) % Cont.	Mean±SD (Range) EU limit (No. of Cont. sample above EU limit)	n (N) % Cont.	Mean±SD (Range) EU limit (No. of Cont. sample above EU limit)	n (N) % Cont.	Mean±SD (Range) EU limit (No. of Cont. sample above EU limit)	n (N) % Cont.	Mean±SD (Range) EU limit (No. of Cont. sample above EU limit)
Wettest	5 (8) 62.5	47.39±9.57^{c} (0-342.62) 1750 (0)	7 (8) 87.50	2113.29±754.73 (0-4547.17) 1000 (5)	4 (8) 50	1.43±0.35^{b} (0-7.91) Na (Na)	2 (8) 25.0	35.46±9.88^{c} (0-235.79) 350 (0)	4 (8) 50.0	5.63±3.77^{b} (0-40.84) 1750 (0)	5 (8) 62.5	1270.44±907.96^{c} (0-3269.81) 1000 (5)	4 (8) 50.00	1.89±0.65^{b} (0-12.85) Na (Na)	0 (8) 0	0 (0) 100 (0)
Wet	1 (8) 12.5	0.50±0.02^{a} (0-4.03) 1750 (0)	8 (8) 100	1449.99±505.66 (879.87-2471.70) 1000 (7)	1 (8) 12.5	0.15±0.01^{a} (0-1.18) Na (Na)	2 (8) 25.0	17.89±6.73^{a} (0-73.68) 350 (0)	2 (8) 25.0	1.35±0.15^{a} (0-8.46) 1750 (0)	2 (8) 25.0	592.22±135.77^{a} (0-3144.03) 1000 (2)	1 (8) 12.50	0.11±0.01^{a} (0-0.87) Na (Na)	1 (8) 12.50	7.30±2.14^{b} (0-58.42) 100 (0)
Dry	2 (8) 25.0	39.79±13.03^{c} (0-314.96) 1750 (0)	7 (8) 87.50	1539.94±865.48 (0-4889.31) 1000 (5)	1 (8) 12.5	0.15±0.01^{a} (0-1.18) Na (Na)	2 (8) 25.0	29.21±8.12^{ab} (0-180.00) 350 (0)	2 (8) 25.0	3.23±1.17^{b} (0-19.14) 1750 (0)	6 (8) 75.0	816.67±276.92^{b} (0-1703.77) 1000 (4)	0 (8) 0.00	0 (0) Na (Na)	1 (8) 12.50	23.75±7.83^{c} (0-190.00) 100 (1)
Driest	4 (8) 50.0	11.78±3.41^{b} (0-85.29) I750 (0)	7 (8) 87.50	1611.92±709.13 (0-4301.89) 1000 (6)	3 (8) 37.5	4.54±1.24^{c} (0-28.80) Na (Na)	1 (8) 12.50	22.70±5.71^{ab} (0-181.58) 350 (0)	2 (8) 25.0	1.06±0.36^{a} (0-7.52) I750 (0)	5 (8) 62.5	770.44±140.17^{b} (0-2631.45) 1000 (3)	3 (8) 37.50	0.41±0.13^{a} (0-1.22) Na (Na)	1 (8) 12.50	34.67±12.33^{c} (0-277.37) 100 (1)
Total Sample	12 (32) 37.5	24.86±5.77 (0-342.62) 1750 (0)	29 (32) 90.63	1692.88±1034.24 (0-4889.31) 1000 (23)	9 (32) 28.13	1.57±2.13 (0-28.80) Na (Na)	7 (32) 21.88	26.32±18.72 (0-235.79) 350 (0)	10 (32) 31.25	2.82±1.45 (0-40.84) 1250 (0)	18 (32) 56.25	862.44±502.16 (0-3269.81) 1000 (14)	8 (32) 25.00	0.60±0.11 (0-12.85) Na (Na)	3 (32) 9.38	16.43±7.82 (0-277.37) 100 (2)

Values with different superscripts down the column are significantly different ($p < 0.05$).

Keys: Mean = Mean concentration; SD = Standard deviation; DON = Deoxynivalenol; FUM = Fumonisins; NIV = Nivalenol; ZEN = Zearalenone; EU limit= European Union Maximum limits; N = Number of Samples; n = Number of Positive Samples; % Cont. = Percentage Contamination; No. of Cont. sample above EU limit= Number of contaminated samples above EU limits; Na = Not Applicable

4.1.13 Results for potential risk characterization

Based on the calculated value, the estimated daily intake through consumption of maize presented higher EDI values for all the toxins as compared to sorghum samples, and the dietary intake of FUM was found higher followed by ZEN, DON and NIV (See Table 4.11). The same trend was observed for the dietary exposure assessment of sorghum. The highest dietary exposure of adult groups to all the toxins was observed for the total population groups, whereas male population groups recorded higher EDI values as against the female population groups. Regarding the exposure through maize consumption, it was clearly observed that the highest % TDI values were recorded for FUM compound among all the population groups followed by ZEN, DON and NIV toxins. The same scenario was noticed in the risk assessment of adult population groups consuming sorghum grains.

Table 4.11: Exposure Level and Risk Characterization of *Fusarium* Mycotoxins from Different Varieties of Maize and Sorghum in Adult Male and Female Population Groups based on Estimated Daily Intake

	Maize				Sorghum			
Mycotoxins	Mean Conc.	Estimated daily Intake ($x10^{-3}$) (EDI)	TDI (µg/kg)	Risk Characterization (% TDI)	Mean Conc.	Estimated daily Intake ($x10^{-3}$) (EDI)	TDI (µg/kg)	Risk Characterization (% TDI)
	(µg/kg)	(µg/kg. bw/day)			(µg/kg)	(µg/kg. bw/day)		
		Male (TP) Female		Male (TP) Female		Male (TP) Female		Male (TP) Female
DON	24.86	130.93 (134.01) 128.36	1	13.09 (13.40) 12.84	2.82	14.71 (15.06) 14.42	1	1.47 (1.51) 1.44
FUM	1692.88	8915.89 (9125.83) 8741.15	2	445.79 (456.29) 437.06	862.44	4498.84 (4604.77) 4410.66	2	224.94 (230.24) 220.53
NIV	1.57	8.27 (8.46) 8.11	0.7	1.18 (1.21) 1.16	0.60	3.13 (3.20) 3.07	0.7	0.45 (0.46) 0.44
ZEN	26.32	138.62 (141.88) 135.90	0.5	27.72 (28.38) 27.18	16.43	85.71 (87.72) 84.03	0.5	17.14 (17.54) 16.81

Keys: DON = Deoxynivalenol; FUM = Fumonisins; NIV = Nivalenol; ZEN = Zearalenone; EDI = Estimated daily intake; TDI = Tolerable daily intake; %TDI = Percentage tolerable daily intake; TP = Total population

4.1.14 Co-occurrence of *Fusarium* mycotoxins in maize and sorghum

Concerning the presence of several mycotoxins in the same sample, Table 4.12 and Table 4.13 summarizes the co-occurrence of *Fusarium* mycotoxins in maize and sorghum samples, respectively. In the present study, overall positive samples indicate that about 10 maize samples (31.25 %) were infested by one mycotoxin with occurrence of DON, FUM, NIV or ZEN while nineteen of the samples were contaminated with 2 toxins. The results suggest that 59.38 % of the maize samples showed co-occurrence incidence with combination of two different mycotoxins. Only three (3) maize samples have 3 different co-occurring mycotoxins with percentage incidence of 9.38. On the other hand, overall positive samples indicate that about 50 % of the sorghum samples were contaminated by one mycotoxin (DON, FUM, NIV or ZEN). Only eight (25 %) of the tested samples (sorghum) were estimated with at least two mycotoxins (DON/FUM, FUM/NIV and FUM/ZEN), 6.26 % of the analysed samples were contaminated by more than three toxins, (3.13 % for three toxins and 3.13 % of four toxins).

With regards to the maize and sorghum samples contaminated with two toxins, up to 9 and 5 samples respectively, were combination of DON and FUM toxin. Approximately 28 % and 16 % of maize and sorghum samples were combination of DON and FUM toxin. This indicates that the analysed samples are more vulnerable to higher value of joint occurrence of two mycotoxins. About sixteen percentile of all the analysed samples (7 maize sample and 3 sorghum sample) are contaminated with FUM/NIV. Also, FUM co-occur with ZEN in three maize samples. Whereas, no combination of FUM/ZEN was recorded in sorghum samples as indicated in Table 4.13. For the samples contaminated with more than two toxins, three maize samples were infested with 3 mycotoxin, with different combinations (DON/FUM/NIV, DON/FUM/ZEN and DON/NIV/ZEN), whereas only one combination (DON/FUM/ZEN) was detected in sorghum samples. Interestingly, combination of four (4) mycotoxins was recorded in only one sample, while no co-presence of four different mycotoxins or above was recorded in maize samples.

Table 4.12: Distribution Patterns of Co-occurring *Fusarium* Mycotoxins in Maize Samples from Niger State

No of co-occuring toxins	Co-occurring toxins	Number of samples	% co-occurrence
Two toxins	DON/FUM	9	28.13
	FUM/NIV	7	21.89
	FUM/ZEN	3	9.38
Three toxins	DON/FUM/NIV	1	3.13
	DON/FUM/ZEN	1	3.13
	DON/NIV/ZEN	1	3.13

Keys: DON = deoxynivalenol, FUM = fumonisins, NIV = nivalenol, ZEN = zearalenone

Table 4.13: Distribution Patterns of Co-occurring *Fusarium* Mycotoxins in Sorghum Samples from Niger State

No of co-occuring toxins	co-occurring toxins	Number of samples	% co-occurrence
Two toxins	DON/FUM	5	15.63
	FUM/NIV	3	9.38
Three toxins	DON/FUM/ZEN	1	3.13
Four toxins	DON/FUM/NIV/ZEN	1	3.13

Keys: DON = deoxynivalenol, FUM = fumonisins, NIV = nivalenol, ZEN = zearalenone

4.2 **Discussion**

The study provides one of the few recent most comprehensive documentation of *Fusarium* fungi and mycotoxin profiles of maize and sorghum from Niger State, the largest producer of maize and second largest producer of sorghum grain in Nigeria. It reports the existence of nine fungal species belonging to the genus *Fusarium* in stored and marketed grains of the white and yellow maize, and white and red sorghum varieties from the four microclimatic zones of Niger State, Nigeria. It also records the natural occurrence, concentrations and associated risks of dietary exposure to DON, FUM, NIV and ZEN toxins in the studied grains. Also, it is worth mentioning that the investigation revealed for the first time the incidence of NIV in maize and sorghum from the study area. The concentrations reported were not adjusted based on recovery rates.

The primary role of maize as a staple food in the studied region is comparable to that of other cereal grains including sorghum (Palacios-Rojas *et al*., 2020). Of the 22 countries where maize forms the highest percentage of calorie intake in the nation diets, 16 are in Africa, therefore explaining the high consumption rates of maize noticed from the studied region. Maize account for almost half of the calorie and protein consumed in eastern and southern Africa and one-fifth of the calories and protein consumed in West Africa (Galani *et al*., 2020). Report also confirmed that 208 million people in Sub Saharan Africa (SSA) depend on maize as a source of food security and economic wellbeing due to its increase calorific value (Palacios-Rojas *et al*., 2020). In terms of productivity, farmers preferred to plant maize than sorghum. Most of the maize is consumed within the household, while some sorghum is sold in light of their conventional processes and quality for income generating activities. Economic features

of maize (that is, easy to grow, harvest and store) have made it a competitive product, which has helped lower the price of other food staples (Ten Berge *et al*., 2019). Further, maize gives the highest conversion of dry substance to meat, milk and eggs compared to other cereal grains when used for the production of animal feeds. All these features account for high demand and consumption rate of maize than sorghum.

All the fungal species and mycotoxins recorded in this investigation have been shown as the commonest fungal and mycotoxin contaminants of maize and sorghum from Nigeria and elsewhere around the globe. Like (Wu *et al*., 2011) in United State, (Dorn *et al*., 2011) in Switzerland, in Saudi Arabia (Mahmoud *et al*., 2013), India (Sreenivasa *et al*., 2010), South-East Asian (Pitt and Hocking, 2009), Malaysia (Reddy and Salleh, 2011), Pakistan (Saleem *et al*., 2012), Brazil (Reddy *et al*., 2010), Qatar (Hassan *et al*., 2019), Makun *et al*., 2009, Chilaka *et al*., 2016, Garba *et al*., 2017, Muhammad *et al*., 2019 and Onyedum *et al*. (2020) in Nigeria. What is of major challenge is the high viability of the fungal spores in the studied region of Niger State. All the same, the presence of these *Fusarium* fungi and their respective mycotoxins in the studied samples is expected as their optimal conditions for growth and mycotoxin synthesis (24-30 °C, > 80 % relative humidity) are the prevailing weather conditions in most part of the year in Niger State. The vulnerability of maize to these fungi and toxins is hinged on the favourable climatic condition of the State. Nutrient composition of maize, as in, high carbohydrate content (66.6 gm/100 gm), 3.6 per 100 gm of fat and between 13-14.9/100 gm of moisture content (Enyisi *et al*., 2014) coupled with comparatively larger surface area (less kernel density) and encapsulated in a softer seed (less kernel hardness) coat than most cereals excellently supports fungal growth and mycotoxin production on the farm, during storage and after processing (Stössel, 1986). Similarly, the nutrient composition

of sorghum; 73.8 % of starch and a substantial amount of protein (12.3 %) with rich deposit of B-complex vitamins (niacin, riboflavin and pyridoxin), oil (3.6 %) and moisture (11-12 %) contents (FAO, 1994) makes it an excellent substrate for fungal growth and mycotoxin production. Therefore, the level of microbial contamination as evaluated by the colony forming unit (CFU/g) determined in 64 composite samples are shown in Table 4.1.

The variations in CFU levels observed in this study could be attributed to the fact that Niger state experiences extreme variation in monthly rainfall which could have high impact in generating conducive environment for the production of fungal spores (Yakubu *et al*., 2018). Also, the high rate of fungal count in stored samples is an indication of the fact that most of the storage facilities in use by farmers in the studied areas have limited impact in disrupting the environment, thereby favouring the proliferation of fungal spores. This implies that fungal growth could trigger the production of mycotoxins and subsequently manifest into the end products of poultry and other animals (Meat, milk and egg) that are fed with contaminated feed made from maize and sorghum from these storage facilities (Greco *et al*., 2014). Thus, this may consequently affect human health through their food chain. As reported by Gimeno *et al*. (2002), samples can be categorized as good (colony count range less than 3×10^4 CFU/g), regular (count range between 3×10^4 to 7×10^4 CFU/g) and bad (greater than 7×10^4 CFU/g), but the obtained results in the present study shows that none of the analysed samples fall within the "good" or "regular" range. This should be useful information and a source of concern for further studies due to the fact that maize and sorghum are the first and the third most consumed food crops throughout the four micro-climatic zones of Niger state (Hussaini, *et al*., 2009; Odusanya, 2018)

The contamination of maize and sorghum grains by members of *Fusarium* genus varied greatly from types, varieties and zones. Different reports have been documented in Nigeria and elsewhere (Muhammad *et al*., 2019; Hassan *et al*., 2019) on the level of *Fusarium* contamination in food crops including maize and sorghum. Hassan *et al*. (2019) found *F. verticillioides* as the major contaminants of marketed feed from Qatar. Among the nine (9) *Fusarium* spp isolated from both maize and sorghum samples in this study, *F. verticillioides*, which is a notorious producer of fumonisins, is not the only important toxin produced by *Fusarium* fungi associated with food crops worldwide (Kpodo *et al*., 2000; Marasas, 2001), but others such as *F. graminearium, F. solani* and *F. subglutinans* have been isolated from other commodities (Reddy *et al*., 2006) as reported in the current survey. So also in this study, *F. verticillioides* was the major contaminant of both maize and sorghum regardless of the sample types, varieties or zones. *F. graminearum and F. sporotrichioides* ascetically followed the dominants species. Other species like *F. equiseti* and *F. subglutinans* were isolated in few numbers. In general, yellow maize was more contaminated than white maize samples (See Table 4.2). It is a common knowledge that the differences between white and yellow maize is in their sugar, lutein, carotenoid and moisture contents. While the white maize usually referred as the sweet maize contains more sugar and moisture than the yellow variety, the former contains β-carotene which is responsible for its yellowness and serves as source of vitamin A. Quite a number of studies have shown higher levels of carbohydrates in yellow (62.38 %) than in the white variety (16.28 %), glucose, fructose, total detected reduced sugars and total detected sugar in yellow variety of maize than in white cultivars in Ibadan, Nigeria (Oladapo *et al*., 2017), Sudan (Elzeina, 2010) and Serbia (Zilic *et al*., 2011). Conversely, same workers and others have shown

that white maize contains higher concentrations of anti-nutritional and antimicrobial compounds namely oxalates, tannins saponins, flavonoids, alkaloids and phenols, phytates, phenolics and flavonoids (Okechukwu *et al*., 2013; Zilic *et al*., 2011; Oladapo *et al*., 2017), than the yellow maize in same countries. Meanwhile, it has been shown that bound phenolics are negatively related to *Fusarium* counts and fumonisins concentrations in maize cultivars (Ponce-Garcia *et al*., 2020). Therefore, the higher carbohydrates and sugars which are suitable substrates for fungi growth in yellow variety and the more antimicrobial compounds in the white maize account for the observed susceptibility of the yellow cultivar to fungal and mycotoxin contamination than the white cultivars. Nwogu and Nwankwo (1979) have also observed that yellow maize is more susceptible to microbial and mycotoxin contamination than white maize in Nigerian grown maize.

In the case of sorghum samples, white sorghum is susceptible to microbial and fungal infestation than the red variety because the former has significantly higher moisture and fat contents (Mohammed *et al*., 2019) than the later and these are principal favourable factors for fungal growth and mycotoxin production. The red colour of the grain's pericarp is essentially due to the presence of 3-deoxyanthocyanidins (Ali *et al.,* 2013; Wu *et al.*, 2012). It has also been observed that, the binding of proanthocyanidins with proteins, inhibition of hydrolytic enzymes, interactions to inactivate microbial adhesions and cell envelope transport proteins, and non-specific interaction with carbohydrates (Cowan *et al*., 1999) confers antimicrobial resistance to red sorghum. Furthermore, white grain food sorghum do not contain polyphenolic compounds such as tannins that could impact tolerance or resistance to insect and pest infestation (World Health Organisation (WHO), 2012), making it to be more vulnerable to fungal contamination.

Though, red grain types do not also contain any amount of tannins which occur in the pigmented inner layer (no testa), but exhibit higher amount of other phenolic compounds like anthocyanins, phystosteroids and polycosanols in its pericarp that could hinder the fungi growth (Waniska, 2000).

This study also revealed that market samples were more contaminated than stored samples. This observation denotes that farmers do not store their produce for long period of time before transporting to the market (Abass *et al*., 2014). Besides, there are common unwholesome trade practices observed during sampling that would exacerbate the fungi count and mycotoxins levels in marketed grains as compared to figures in stored samples as recorded in this work. Such unhygienic practices which are results of ignorance of food values chain actors to the existence of mycotoxins and non-enforcement of the legislated limits include transporting, drying and selling food commodities in open containers and mixing mouldy grains of low grades with high quality grain in order to maximize profits (Udomkun *et al*., 2017b).

Furthermore, the total frequency of all *Fusarium* isolated species was found to be highest in wettest zone (zone 1) when compared with other zones. This is because as moisture content increases, fungal infestation also increases (Embaby *et al*., 2013). Moreover, fungal growth and mycotoxin production are solely dependent on environmental temperature and humidity with hot and humid conditions exuberating them (Magan and Evans, 2000) which further explains the observed higher frequency and levels of fungal infestation and mycotoxin contamination in the wet than the drier zones. Be that as it may, high level of *F. verticillioides* reported from the present study could be related to the ability of these fungi to synthesize fumonisin toxins, which is

associated with high incidence of esophageal cancer in South Africa (Marasas *et al*., 1988, Sydenham *et al*., 1990) and China (Wang *et al*., 1995)

Hitherto, maize and maize products were found to have higher occurrence and mean concentration of FUM than any cereal or cereal based products as reported by Joint FAO/WHO Expert Committee on Food Additives in 2016 (JECFA, 2017). Numerous studies have reported FUM as the most dominant *Fusarium* mycotoxin in maize (Afolabi *et al.*, 2006; Adejumo *et al*., 2007; Chilaka *et al*., 2016; Chilaka *et al*., 2017; Jeff-Agboola and Omosanyin, 2017; Muhammad *et al*., 2019; Ezekiel *et al.*, 2020; Onyedum *et al*., 2020), at frequency of occurrence and concentrations similar to this work. Herein, FUM was detected in 90.63 % of the maize samples at mean concentration of 1692.88 µg/kg and range of 0 – 4889.31 µg/kg. Except for Chilaka *et al*. (2016) who reported high incidence rate of FUMs in maize samples at maximum concentration level of 8508 µg/kg, all the other workers recorded similar concentrations with the obtained results. About 71.88 % of the tested samples in this work had the toxin levels at above the EU acceptable limit. The incidence of FUM in sorghum was moderate as only slightly above half of the samples were contaminated but at high concentrations of up to 3269.81 µg/kg and 43.75 % had toxin content above the EU regulated limit of 1000 µg/kg. Such high concentration of FUM were found by Garba *et al.* (2017) who reported FUM with mean concentrations across the six agro-ecological zones of Nigeria of between 1170 and 1890 µg/kg. Onyedum *et al*. (2020) found the toxin in all the 20 samples analysed from the same study location at mean and range concentrations of 6198 µg/kg and 3700-8400 µg/kg respectively. FUM occurred in 8 out of 38 sorghum samples at low mean values of 17 µg/kg within the range of 188-248 µg/kg from Northern and Southern Nigeria (Gbashi *et al*., 2020). The toxin was also

found at very low incidence and safe level in 8 out of 110 samples at mean and maximum concentration of 83 µg/kg and 180 µg/kg respectively from Nigeria (Chilaka *et al*., 2016). In a comprehensive review of incidence of FUM in 824 sorghum samples from Brazil, Belgium, Ethiopia, Germany, Mexico and Tunisia, Astoreca *et al.* (2019) reported concentrations within the range of 0.1 and 2117.0 µg/kg but of all the samples only 3 from Ethiopia were above the EU legislated level.

The two trichothecenes analysed from the studied samples, DON and NIV have similar chemical structures as the only difference between them is the presence of oxygen at position 4 of the trichothecene structure and therefore, share the same mechanism of toxicity. The 9, 10 double bond and 12, 13 epoxide in the trichothecenes structure allow the toxins to bind to ribosomes and inhibit translation and consequently protein, RNA and DNA synthesis particularly in rapidly developing and dividing cells (Katika *et al*., 2015). It was also reported that the toxins activate protein kinases that mediate apoptosis and the consequence of these toxic actions are immunosuppression, weight loss and gastrointestinal disorder in mammals (Alassane-Kpembi *et al.,* 2015). DON occurred at low incidence of 37.50 % and 31.25 % (maize and sorghum) and safe concentrations (mean concentration of 24.86 and 2.82 µg/kg). It can be observed from the obtained results that none of the cereal grains, regardless of the sample type, variety or location were found contaminated with DON above the EU maximum limit of 1750 µg/kg and 1250 µg/kg, for unprocessed maize and unprocessed cereals other than maize. Although there is paucity of information on the occurrence of DON in Nigeria sorghum. However, the percentile occurrence of DON in the maize grains analysed is in agreement with the study carried out by Adejumo *et al*. (2007) who reported low incidence rate of DON in Nigeria maize. Chilaka *et al*. (2017) reported high occurrence

of DON of up to 3842 µg/kg in maize in Cameroon and explained that such alarming levels of contamination is as a result of the impact of climate change, flood and drought coupled with deplorable agricultural infrastructures in Sub Saharan Africa.

While NIV were found at relatively low frequency of 28.13 % (maize) and 25 % (sorghum) with mean concentration of 1.57 and 0.60 µg/kg respectively, it could be observed that yellow maize had the highest mean concentration of NIV with respect to the data presented in Table 4.8. Also, closer observation of NIV concentration in the cereal grains reveal that market samples (maize and sorghum) had higher mean values when compared to their corresponding items. This result can be linked to the fact that samples which has not been properly stored are presented for sales in the market and were accidentally purchased during sampling. In relation to the sample zones, the highest mean concentration of NIV in maize samples was estimated from the driest zone, while the least value was observed from wet zone. The observed trend is attributed to the fact that NIV concentration has always been reported in cereal grains from regions that are characterized by relatively drier and warmer climate (Leggieri *et al*., 2020). Hitherto, high incidence rate of NIV have been reported to be present in Nigeria maize and the report of Adetunji *et al*. (2014) stated that 54.3 % of the total sample quantified were contaminated with NIV. On the contrary, the present study recorded lower percentage occurrence of NIV in maize which is comparable to the findings reported by Akinmusire *et al*. (2019). There are quite a few studies on the occurrence of NIV concentration in sorghum grains in Nigeria. Chilaka *et al*. (2016) did not detect NIV but found DON in only 3 of 110 sorghum samples from Nigeria at mean concentration of 100 µg/kg. Ediage *et al.* (2015) did not find DON and NIV in sorghum of Africa origin marketed in Belgium and Germany. These workers advanced that

trichothecenes are associated to temperate climate and hence their low incidence in unfavourable tropical climates such as in Nigeria. It is noteworthy that no DON and NIV producers were isolated in this work which might also account for their low prevalence in the studied grain samples.

Zearalenone is a non-steroidal estrogen that causes endocrine disruptions in estrogen responsive tissues of mammals. It causes testicular germ cell depletion, altered testis morphology, reduced serum testosterone concentration in male animals (Kim *et al*., 2003), while inducing estrogenic symptoms such as uterine enlargement, vulvovaginitis (Zinedine *et al*., 2007) in female animals which all culminates to infertility. Its disruption of endocrine secretions explains it roles in cervical cancer in Middle East (Zinedine *et al.,* 2017) and precocious pubertal changes in children in Puerto Rico, mammary hypertrophy in females and feminization of male (Peraica *et al*., 1999, Massart and Saggese, 2010). All the 21.88 % of maize samples contaminated with zearalenone had concentrations between 0 and 235.79 µg/kg and the levels were all below the EU legislated levels of 350 µg/kg for unprocessed maize. Similarly, ZEN was detected in only 3 of the 32 sorghum composite samples with two of the samples having ZEN above EU legislated limit of 100 µg/kg for unprocessed grain other than maize. The observed low frequency of ZEN in maize samples also agrees with the result obtained by Chilaka *et al*. (2016); Maziero and Bersot (2010) in Nigeria and Brazil respectively. The review article of Chilaka *et al*. (2017) which reported concentration levels of up to 2044 µg/kg in 321 maize samples from Nigeria and ZEN content of up to 14990 µg/kg in 807 maize samples from ten African countries also substantiates the fact that the aforementioned estrogenic effects of ZEN can cause health challenges in Nigeria and other parts of Africa. Gbashi *et al*. (2020) also found ZEN in 11 of 38

sorghum samples at concentration range of 4.8-22 µg/kg. Chilaka *et al*. (2016) also obtained similar results as ZEN was detected in 1 % of 110 sorghum samples with concentrations in all the samples being less than the EU maximum limit of 100 µg/kg. Likewise, Ediage *et al* (2015) found the mycotoxin in 1 of the 10 sorghum tested samples at concentration of 90 µg/kg. However, Garba *et al.* (2017) found zearalenone at high frequency and concentration of up to 3092 µg/kg from Nigerian grown sorghum that were kept for over a year before analysis. Prolonged storage period exuberate mycotoxin level (Magan and Evans, 2000).

Worthy of note is the fact that, risk assessment is the scientific evaluation of the probable daily intake of toxins or potential adverse health effects resulting from human exposure to the toxins. However, the view point is the primary basis for the establishment of guideline and regulations. The availability of consumption data and mycotoxins occurrence in different sample types/variety across the zones provides the required information for risk and exposure assessment.

Careful scrutiny of the obtained results reveals that the highest risk was estimated for fumonisins toxin. With a % TDI of 456.29 and 230.24 for FUM in maize and sorghum samples respectively, it implies that Nigerians consume FUM at concentration level that is more than four and two times the tolerable daily limit via consumption of maize and sorghum, respectively (Table 4.11). Adetunji *et al*. (2017) reported a lower national % TDI of 172.8 in maize, which is almost twice the safe daily dietary intake level. Onyedum *et al.* (2020) also reported EDI and % TDI (% 167.9) of sorghum samples which was found above the acceptable levels. However, this toxin (FUM) disrupts sphingolipid metabolism by inhibiting the enzyme ceramide synthase and the cellular mechanisms behind FB_1-induced toxicity include the induction of oxidative stress,

apoptosis and cytotoxicity, as well as alterations in cytokine expression (Stockmann-Juvala and Savolainen (2008). These mechanisms of action elicit neurotoxicity, hepatotoxicity and nephrotoxicity in animals causing equine leukoencephalomalacia porcine pulmonary oedema in pigs, liver and kidney damage in laboratory rats, human esophageal and liver cancers and neural tube defects in human babies (Sun *et al.,* 2011) and therefore, the presence of this toxin at unsafe limits in the studied samples intended for human consumption and consequent unacceptable high dietary exposure raises serious economic and public health concern.

Regardless of the sample types, varieties or zones, maize (13.40 %) samples were found to have higher percentage TDI values of DON than sorghum (1.51 %) and generally, all the percentage TDI value obtained in this study were found below the recommended TDI limit (1 µg/kg.bw/day). Also, the dietary intake levels of NIV were found below the tolerable limits across all the population groups. Even though the risk associated with NIV in this study was low, it was still higher than that of other countries such as France (Sirot *et al*., 2013) and Iran (Yazdanpanah *et al.,* 2012). Its presence in the grains should not be negligible because of the health related risk associated with the consumption of NIV at low doses. Chronic exposure in small amount and additive and synergistic interactions with other mycotoxins could also pose public concern (Smith *et al*., 2016).

Irrespective of the population groups, the calculated dietary exposure through maize (28.38 % TDI) and sorghum (17.54 % TDI consumption to ZEN toxin was lower than the tolerable daily intake (0.5 µg/kg.bw/day). Though the entire maize samples had higher EDI values compared to the corresponding sorghum samples. This is due to the

fact that sorghum samples are less susceptible to mycotoxin contamination when compared to maize counterpart. Using the European Food Safety Authority Tolerable daily intake standard of 0.25 µg/kg TDI, Adetunji *et al.* (2017) showed a % TDI of between 0.0 and 164.8 in maize across Nigeria. Taking into account the studies carried out by several other authors on the exposure level of ZEN in food grains, it is important to highlight the results obtained by Sirot *et al*. (2013). The authors assessed the exposure to zearalenone in the French adult and child population. Nevertheless, the toxin (ZEN) has been associated with gynecomastia with testicular atrophy in rural males in Southern Africa (Shephard, 2008) and general infertility in both human beings and animals (Sherif *et al.*, 2009).

Further, the simultaneous occurrence of multimycotoxins in maize and sorghum, and indeed other cereals are in line with previous studies (Makun *et al*., 2011, Chilaka *et al*., 2016, Ogara *et al*., 2017, Onyedum *et al*., 2020). Co-occurrence of mycotoxin is due to the colonization of sample by fungus producing more than one toxins and or infestation of sample by different fungi species producing different mycotoxins as seen in this work. Indeed most of the fungi isolated in the current study are multimycotoxin producers; *F. verticillioides* (FUM and moniliformin), *F. graminearium* (FUM and ZEN), *F. semitectum* (ZEN), *F. nygamai* (FUM), *F. solani* (fusarins and fusaric acid), *F. oxysporum* (T-2 and moniliformin) (Jennessen *et al*., 2005; Ismaiel and Papenbrock, 2015; Kostic *et al*., 2019). The combined toxic effects of mycotoxins in these combinations could be additive, synergistic or antagonistic (Miller *et al*., 1995). As observed in the present study, 91 % of all the analysed samples were contaminated with at least one *Fusarium* toxin and 9 % of the samples are free of contamination. The co-occurrence data of the current study thus shows that the observed phenomenon are in

congruent with the trends reported for frequent contamination of *Fusarium* toxins in cereal products across sub-saharan Africa (Adekoya *et al*., 2018; Chilaka *et al*., 2016). Though there is general paucity of data on the combined effects of higher multiple mycotoxins. Even the work of Smith *et al.* (2016) comprehensively recorded the natural simultaneous occurrence of mycotoxins in food and feeds, the combined toxic effects of the mycotoxins were focused mostly on the *Fusarium* toxins with information on the interaction between four mycotoxins as observed in the present survey missing. Nevertheless, it has generally been reported that the simultaneous exposure to many mycotoxins elicits mostly additive or synergistic effects (Speijers and Speijers, 2004, Smith *et al*., 2016). Onyedum *et al*. (2020) reported the co-occurrence of AFT/OTA/FUMs, AFT/OTA, AFT/FUM and OTA/FUMs in various samples from north-central Nigeria staples. Adetunji *et al*. (2017) reported co-presence of AFB_1, $FUMB_1$ and ZEN contamination in 67.93 % and 17 % of stored maize grains in Nigeria. Chilaka *et al*. (2016) revealed the co-presence of *Fusarium* mycotoxin in cereals and processed products (ogi) from Nigeria. From their study, they observed that 60 %, 19 %, 30 % and 93 % of maize, sorghum, millet and ogi respectively were contaminated with at least two mycotoxins.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Fusarium verticillioides which is a prominent producer of fumonisins was the most predominant species isolated regardless of the sample type, variety or micro-climatic zones. This report from 64 composites samples derived from 480 maize and sorghum samples from Niger state indicated that 57.81 % of the analysed samples had fumonisins at concentrations and human daily intake levels above the regulatory limits set by Europeann Union and Codex Alimentarius Commission, and are therefore usafe for human consumption and would be rejected by interational trade. The high prevalence of FUM emphasizes the necessity of continuous evaluation of the toxin in maize and sorghum respectively. The detectable levels for deoxynivalenol and zearalenone in all samples were lower than the maximum limit set by European Union except for fewer sorghum samples that were found contaminated with zearalenone above EU limit.

The uavailability of international regulatory limits for nivalenol in food commodities indicates that prompt attention is required to reduce its toxicocological effects. In

general, the high cosumption rate of maize and sorghum in the studied region may have significant impact on the level of dietary mycotoxin exposure. The present study also revealed that different *Fusarium* mycotoxins contaminated the analysed samples. Thus, the anticipated synergistic and additive effect of the simultaneous occurrence of the observe mycotoxins will further complicate the deplorable health conditions.

5.2 Recommendations

Since synergistic and/or combined effects of co-occurrence of mycotoxins is still unknown, it is necessary to carry out further investigation to understand the underlying forces involving mycotoxin production and contamination. Therefore, there is urgent need to provide special intervention plans to reduce mycotoxin occurrence and contents in food samples. In light of the obtained data, the following recommendations were made:

1. The obvious adverse public health and economic implications necessitates increased surveillance and urgent regulation of mycotoxins in foods and feeds.
2. Further research is required to provide a source of ready-to-use data for implementation of good agricultural practices when considering the consumption rate and contamination level of maize and sorghum in Niger state. It is therefore, a call for scientists to derive and deploy effective mycotoxin mitigation agricultural and manufacturing strategies along food value chains in order to reduce exposure to these toxins.

3. It is also recommended that longitudinal follow up studies to determine the association between dietary mycotoxin exposure and health/disease outcomes in African communities be conducted intensively.

REFERENCES

Abarca, M. L., Bragulat, M. R., Castellá, G. & Cabañes, F. J. (2019). Impact of some environmental factors on growth and ochratoxin A production by *Aspergillus niger* and *Aspergillus welwitschiae*. *International Journal of Food Microbiology*, 291, 10-16.

Abass, A. B., Ndunguru, G., Mamiro, P., Alenkhe, B., Mlingi, N. & Bekunda, M. (2014). Post-harvest food losses in a maize-based farming system of semi-arid savannah area of Tanzania. *Journal of Stored Products Research*, 57, 49-57.

Abbas, H. K., Shier, W. T. & Cartwright, R. D. (2007). Effect of temperature, rainfall and planting date on aflatoxin and fumonisin contamination in commercial Bt and non-Bt corn hybrids in Arkansas. *Phytoprotection*, 88(2), 41-50.

Abbas, H. K., Williams, W. P., Windham, G. L., Pringle, H. C., Xie, W. & Shier, W. T. (2002). Aflatoxin and fumonisin contamination of commercial corn (*Zea mays*) hybrids in Mississippi. *Journal of Agricultural and Food Chemistry*, 50(18), 5246-5254.

Abbas, H. K., Yoshizawa, T. & Shier, W. T. (2013). Cytotoxicity and phytotoxicity of trichothecene mycotoxins produced by *Fusarium* spp. *Toxiconology*, 74, 68-75.

Abdel-Azeem, A. M., Abdel-Azeem, M. A., Darwish, A. G., Nafady, N. A. & Ibrahim, N. A. (2019). *Fusarium*: biodiversity, ecological significances, and industrial

applications. In Recent Advancement in White Biotechnology Through Fungi. *Springer*, 1, 201-261.

Abia, W. A., Warth, B., Sulyok, M., Krska, R., Tchana, A. N., Njobeh, P. B., Dutton, M.F. & Moundipa, P. F. (2013). Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Food Control*, 31(2), 438-453.

Adejumo, T. O., Hettwer, U. & Karlovsky, P. (2007). Occurrence of *Fusarium* species and trichothecenes in Nigerian maize. *International Journal of Food Microbiology*, 116(3), 350-357.

Adejuwon, J. O. (2018). Assessment of the changing pattern in maize cultivation in Sokoto-Rima River Basin, Nigeria. *Jouirnal of Applied Sciences and Environmental Management*, 22(9), 1433-1437.

Adekoya, I., Obadina, A., Adaku, C. C., De Boevre, M., Okoth, S., De Saeger, S. & Njobeh, P. (2018). Mycobiota and co-occurrence of mycotoxins in South African maize-based opaque beer. *International Journal of Food Microbiology*, 270, 22-30.

Adetunji, M. C., Atanda, O. O. & Ezekiel, C. N. (2017). Risk assessment of mycotoxins in stored maize grains consumed by infants and young children in Nigeria. *Children*, 4(7), 58, doi: 10.3390/children4070058.

Adetunji, M., Atanda, O., Ezekiel, C.N., Sulyok, M., Warth, B., Beltrán, E., Krska, R., Obadina, O., Bakare, A. & Chilaka, C.A. (2014). Fungal and bacterial metabolites of stored maize (*Zea mays, L.*) from five agro-ecological zones of Nigeria. *Mycotoxin Research*, 30(2), 89-102.

Afolabi, C. G., Bandyopadhyay, R., Leslie, J. F. & Ekpo, E. J. (2006). Effect of sorting on incidence and occurrence of fumonisins and *Fusarium verticillioides* on maize from Nigeria. *Journal of Food Protection*, 69(8), 2019-2023.

Afshar, P., Shokrzadeh, M., Raeisi, S. N., Ghorbani-HasanSaraei, A. & Nasiraii, L. R. (2020). Aflatoxins biodetoxification strategies based on probiotic bacteria. *Toxicon*, 178, 50-58.

Afzal, H., Shazad, S., Qamar, S. & Nisa, S. Q. U. (2013). Morphological identification of *Aspergillus* species from the soil of Larkana District (Sindh, Pakistan). *Asian Journal of Agriculture and Biology*, 1(3), 105-117.

AgraQuant (2019). Mycotoxin ELISA Test Kit. Romer labs Singapore plc. Ltd-Asia.

Agriopoulou, S., Koliadima, A., Karaiskakis, G. & Kapolos, J. (2016). Kinetic study of aflatoxins' degradation in the presence of ozone. *Food Control*, 61, 221-226.

Agriopoulou, S., Stamatelopoulou, E. & Varzakas, T. (2020). Advances in occurrence, importance, and mycotoxin control strategies: Prevention and detoxification in foods. *Foods*, 9 (2), 137.

Ahamed, S., Foster, J. S., Bukovsky, A. & Wimalasena, J. (2001). Signal transduction through the Ras/Erk pathway is essential for the mycoestrogen zearalenone-induced cell- cycle progression in MCF-7 cells. *Molecular Carcinogenesis*, 30, 88-98.

Ahmed, N. E., Agla, S. A., Idris, M. O. & Elhussein, S. A. (2008). Fungi associated with stored Sorghum grains and their effects on grain quality. *Life science*, 2(3), 723-729.

Ajeigbe, H. A., Herbert, B. & Oni, C. A. (2018). Hand book on recipes of sorghum and sorghum based foods in Nigeria. *International Crops Research Institute for the Semi- Arid Tropics*, 1- 44.

Akinmusire, O. O., El-Yuguda, A. D., Musa, J. A., Oyedele, O. A., Sulyok, M., Somorin, Y. M. & Krska, R. (2019). Mycotoxins in poultry feed and feed ingredients in Nigeria. *Mycotoxin Research*, 35(2), 149-155.

Akinseye, F. M., Ajegbe, H. A., Kamara, A. Y., Adefisan, E. A. & Whitbread, A. M. (2020). Understanding the response of sorghum cultivars to nitrogen applications in the semi-arid Nigeria using the agricultural production systems simulator. *Journal of Plant Nutrition*, 43(6), 834-850.

Alassane-Kpembi, I., Kolf-Clauw, M., Gauthier, T., Abrami, R., Abiola, F. A., Oswal, I. P. & Puel, 0. (2013). New insights into mycotoxin mixtures: The toxicity of low doses of type B trichothecenes on intestinal epithelial cells is synergistic. *Toxicology and Applied Pharmacology*, 272(1), 191-198.

Alassane-Kpembi, I., Puel, O. & Oswald, I. P. (2015). Toxicological interactions between the mycotoxins deoxynivalenol, nivalenol and their acetylated derivatives in intestinal epithelial cells. *Archives of Toxicology*, 89, 1337-1346.

Alberts, J. F., Lilly, M., Rheeder, J. P., Burger, H. M., Shephard, G. S. & Gelderblom, W. C. A. (2017). Technological and community-based methods to reduce mycotoxin exposure. *Food Control*, 73, 101-109.

Alexander, N. J., McCormick, S. P., Waalwijk, C., van der Lee, T. & Proctor, R. H. (2011). The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium*. *Fungal Genetics and Biology*, 48(5), 485-495.

Alexander, N. J., Proctor, R. H. & McCormick, S. P. (2009). Genes, gene clusters, and biosynthesis of trichothecenes and fumonisins in *Fusarium*. *Toxin Reviews*, 28(2-3), 198-215.

Ali, R. B., Atangwho, I. J., Kuar, N., Ahmad, M., Mahmud, R. & Asmawi, M. Z. (2013). In vitro and in vivo effects of standardized extract and fractions of *Phaleria macrocarpa* fruits pericarp on lead carbohydrate digesting enzymes. *BMC Complementary and Alternative Medicine*, 13(1), 1-11.

Al-Taher, F., Cappozzo, J., Zweigenbaum, J., Lee, H. J., Jackson, L. & Ryu, D. (2017). Detection and quantitation of mycotoxins in infant cereals in the US market by LC-MS/MS using a stable isotope dilution assay. *Food Control*, 72, 27-35.

Aly, A. H., Debbab, A. & Proksch, P. (2011). Fungal endophytes: Unique plant inhabitants with great promises. *Applied Microbiology and Biotechnology*, 50, 3-19.

Anjorin, S. T., Fapohunda, S., Sulyok, M. & Krska, R. (2016). Natural co-occurrence of emerging and minor mycotoxins on maize grains from Abuja, Nigeria. *Annals of Agricultural and Environmental Sciences*, 1(1), 23-26. doi:http://dx.doi.org/10.20936/AAE/160104.

Antonissen, G., Devreese, M., De Baere, S., Martel, A., Van Immerseel, F. & Croubels, S. (2017). Impact of Fusarium mycotoxins on hepatic and intestinal mRNA expression of cytochrome P450 enzymes and drug transporters, and on the pharmacokinetics of oral enrofloxacin in broiler chickens. *Food and Chemical Toxicology*, 101, 75-83.

Askun, T. (2018). Introductory chapter: *Fusarium*: Pathogenicity, infections, diseases, mycotoxins and management. *Intech Open*, 1, 1-12. doi.org/15772/intech.76507

Astoreca, A. L., Emateguy, L. G. & Alconada, T. M. (2019). Fungal contamination and mycotoxins associated with sorghum crop: Its relevance today. *European Journal of Plant Pathology*, 1-12.

Atanda, O., Makun, H. A., Ogara, I. M., Edema, M., Idahor, K. O., Eshiett, M. E. & Oluwabamiwo, B. F. (2013). Fungal and mycotoxin contamination of Nigerian foods and feeds. *Mycotoxin and Food Safety in Developing Countries*, 3-38.

Atanda, O., Ndenn, J. & Diedhiou, P. (2015). The economic impact of aflatoxins in West Africa: The case of Nigeria, Gambia and Senegal. *Partnership for Aflatoxin Control in Africa* (PACA). 32-48

Austen, B., Mc Carthy, H., Wilkins, B., Smith, A. & Duncombe, A. (2001). Fatal disseminated *Fusarium* infection in acute lymphoblastic leukaemia in complete remission. *Journal of Clinical Pathology*, 54, 488-490. doi: 10.1136/jcp.54.6.488.

Avantaggiato, G., Quaranta, F., Desiderio, E. & Visconti, A. (2003). Fumonisin contamination of maize hybrids visibly damaged by sesamia. *Journal of the Science of Food and Agriculture*, 83(1), 13-18.

Ayalew, A. (2010). Mycotoxins and surface and internal fungi of maize from Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, 10(9).

Ayalew, A., Fehrmann, H. Lepschy, J., Beck, R. & Abate, D. (2006). Natural occurrence of mycotoxins in staple cereals from Ethiopia. *Mycopathologia*, 162(1), 57-63.

Ayelign, A. & De Saeger, S. (2020). Mycotoxins in Ethiopia: Current status, implications to food safety and mitigation strategies. *Food Control*, 113, 107163.

Bacon, C. W., Yates, I., Hinton, D. M. & Meredith, F. (2001). Biological control of *Fusarium moniliforme* in maize. *Environmental Health Perspective*, 109 (2), 325-327.

Bahadur, B., Rajam, M. V., Sahijram, L. & Krishnamurthy, K. V. (2015). Plant diversity, organization, function and improvement. *Plant Biology and Biotechnology*, 1(7), 197-215.

Bandyopadhyay, R., Frederickson, D. E., McLaren, N. W., Odvody, G. N. & Ryley, M. J. (1998). Ergot: A new disease threat to sorghum in the Americas and Australia. *Plant Disease*, 82(4), 356-367.

Bankole, S. A. & Adebanjo, A. (2003). Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. *African Journal of Biotechnology*, 2(9), 254-263. doi: http://doi.org/10.4314/ajb.v2i9.14833.

Bankole, S. A. & Mabekoje, O. O. (2004). Occurrence of aflatoxins and fumonisins in preharvest maize from south-western Nigeria. *Food additives and contaminants*, 21(3), 251-255.

Bankole, S. A., Mabekoje, O. O. & Enikuomehin, O. A. (2003). *Fusarium* spp. and fumonisin B1 in stored maize from Ogun State, Nigeria. *Tropical Science*, 43(2), 76-79.

Barger, G. (1937). The alkaloids of ergot. *Analyst*, 62(734), 340-354.

Bartók, T., Tölgyesi, L., Szekeres, A., Varga, M., Bartha, R., Szécsi, Á., Bartók, M. & Mesterházy, Á. (2010). Detection and characterization of twenty eight isomers of fumonisin B_1 (FB_1) mycotoxin in a solid rice culture infected with *Fusarium verticillioides* by reversed-phase high-performance liquid chromatography/ electrospray ionization time-of-flight and ion trap mass spectrometry. *Rapid Communication and Mass Spectrometry*, 24(1), 35-42.

Bennett, J. W. & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*, 16, 497-516.

Bennett, J. W. (2010). An overview of the genus *Aspergillus. Aspergillus*: *Molecular Biology and Genomics,* 1-17.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S. D., Haesaert, G., Karlovsky, P., Oswald, I. P., Seefelder, W., Speijers, G. & Stroka, J. (2013). Masked mycotoxins: A review. *Molecular Nutrition & Food Research,* 57 (1), 165-186. doi: 1.1002/mnfr.201100764.

Berthiller, F., Schuhmacher, R., Adam, G. & Krska, R. (2009). Formation, determination and significance of masked and other conjugated mycotoxins. *Analytical and Bioanalytical Chemistry,* 395(5), 1243-1252.

Beyerle, J., Frei, E., Stiborova, M., Habermann, N. & Ulrich, C. M. (2015). Biotransformation of xenobiotics in the human colon and rectum and its association with colorectal cancer. *Drug Metabolism Reviews,* 47(2), 199-221.

Bezuidenhout, S. C., Gelderblom, W. C. A., Gorstallman, C. P., Horak, R. M., Marasas, W. F. O., Spiteller, G. & Vleggaar, R. (1988). Structure elucidation of the fumonisins, mycotoxins from *Fusarium*-moniliforme. *Journal of Chemical Society, Chemical Communication* 1730, 743-745.

Bhat, R. V., Shetty, P. H., Amruth, R. P. & Sudershan, R. V. (1997). A food borne disease outbreak due to the consumption of moldy sorghum and maize containing fumonisin mycotoxins. *Journal of Toxicology: Clinical Toxicology,* 35(3), 249-255.

Bhat, R., Rai, R. V. & Karim, A. A. (2010). Mycotoxins in food and feed: Present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety,* 9(1), 57-81.

Binder, E. M. (2007). Managing the risk of mycotoxins in modern feed production. *Animal Feed Science and Technology,* 133, 149-166.

Binder, E. M., Tan, L. M., Chin, L. J., Handl, J. & Richard, J. (2007). Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal Feed Science and Technology,* 137(3-4), 265-282.

Birr, T., Jensen, T., Preußke, N., Sönnichsen, F. D., De Boevre, M., De Saeger, S., Hasler, M., Verreet, J. A. & Klink, H. (2021). Occurrence of *Fusarium* mycotoxins and their modified forms in forage maize cultivars. *Toxins,* 13(2), 110.

Blackwell, B. A., Edwards, O. E., Fruchier, A., ApSimon, J. W. & Miller, J. D. (1996). NMR structural studies of fumonisin B_1 and related compounds from *Fusarium moniliforme*. *Fumonisins in Food,* 75-91.

Blandino, M., Haidukowski, M., Pascale, M., Plizzari, L., Scudellari, D. & Reyneri, A. (2012). Integrated strategies for the control of *Fusarium* headblight and deoxynivalenol contamination in winter wheat. *Field Crops Resources*, 133, 139-149.

Błaszczyk, L., Basińska-Barczak, A., Ćwiek-Kupczyńska, H., Gromadzka, K., Popiel, D. & Stępień, Ł. (2017). Suppressive Effect of Trichoderma spp. on toxigenic Fusarium species. *Polish Journal of Microbiology*, 66, 85-100.

Bojja, R. S., Cerny, R. L., Proctor, R. H. & Du, L. (2004). Determining the biosynthetic sequence in the early steps of the fumonisin pathway by use of three gene-disruption mutants of *Fusarium verticillioides*. *Journal of Agricultural and Food Chemistry*, 52(10), 2855-2860.

Broekaert, N., Devreese, M., De Baere, S., De Backer, P. & Croubels, S. (2015). Modified *Fusarium* mycotoxins unmasked: From occurrence in cereals to animal and human excretion. *Food and Chemical Toxicology*, 80, 17-31. doi: 10.1016/j.fct.2015.02.015.

Brown, D. W., Butchko, R. A., Baker, S. E. & Proctor, R. H. (2012b). Phylogenomic and functional domain analysis of polyketide synthases in *Fusarium*. *Fungal Biology*, 116(2), 318-331.

Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G. & White, T. C. (2012a). Hidden killers: Human fungal infections. *Science Translational Medicine*, 4(165), 165. doi:10.1126/scitranslined.3004404.

Bryla, M., Waskiewicz, A., Ksieniewicz-wozniak, E., Szymczyk, K. & Jedrzejczak, R, (2018). Modified *Fusarium* mycotoxins in cereals and their product-metabolism, occurrence and toxicity. An updated review. *Molecules*, 23(4), 963. doi: 10.3390/molecules23040963.

Bulder, A. S., Arcella, D., Bolger, M., Carrington, C., Kpodo, K., Resnik, S., Riley, R.T., Wolterink, G. & Wu, F. (2012). Fumonisins (addendum). *Safety Evaluation of Certain Food Additives and Contaminants*, 65, 325-794.

Bullerman, L. B., Schroeder, L. L. & Park, K. Y. (1984). Formation and control of mycotoxins in food. *Journal of Food Protection*, 47(8), 637-646.

Butchko, R. A., Plattner, R. D. & Proctor, R. H. (2003). FUM13 encodes a short chain dehydrogenase/reductase required for C-3 carbonyl reduction during fumonisin biosynthesis in *Gibberella moniliformis*. *Journal of Agricultural and Food Chemistry*, 51(10), 3000-3006.

Canady, R. A., Coker, R. D., Egan, S. K., Krska, R., Olsen, M., Resnik, S. & Schlatter, J. (2001). T-2 and HT-2 toxins. Safety evaluation of certain mycotoxins in food. *WHO Food additives Series*, 47, 557-597.

Cano-Sancho, G., Valle-Algarra, F. M., Jiménez, M., Burdaspal, P., Legarda, T. M., Ramos, A. J., Sanchis, V. & Marín, S. (2011). Presence of trichothecenes and co-occurrence in cereal-based food from Catalonia (Spain). *Food Control*, 22(3-4), 490-495.

Center for Disease Control and Prevention (CDC), (1999). Update: Multistate outbreak of listeriosis—United States, Morbidity and Mortality *Weekly Report* 47, 1085 – 1086.

Chala, A., Taye, W., Ayalew, A., Krska, R., Sulyok, M. & Logrieco, A. (2014). Multimycotoxin analysis of sorghum (*Sorghum bicolor L.* Moench) and finger millet (*Eleusine coracana L. Garten*) from Ethiopia. *Food Control*, 45, 29-35.

Chen, G., Hinds, J., Zobel, E., Rosario-Lebron, A. & Hooks, C. R. R. (2015). Evaluation of prophylactic sprays on pest abundance, foliar damage and yield in winter wheat. *International Journal of Pest Management*, 61, 161-170.

Chilaka, C. A., De Boevre, M., Atanda, O. O. & De Saeger, S. (2016). Occurrence of *Fusarium* mycotoxins in cereal crops and processed products (Ogi) from Nigeria. *Toxins*, 342(8), 1-18.

Chilaka, C. A., De Boevre, M., Atanda, O.O. & De Saeger, S. (2017). The status of *Fusarium* mycotoxins in sub-Saharan Africa: A review of emerging trends and post-harvest mitigation strategies towards food control. *Toxins*, 9(1), 19.

Chilaka, C. A., De Kock, S., Phoku, J. Z., Mwanza, M., Egbuta, M. A. & Dutton, M. F. (2012). Fungal and mycotoxin contamination of South African commercial maize. *Journal of Food, Agriculture and Environment*, 10(2), 296-303.

Choudhary, A. K. & Kumari, P. (2010). Management of mycotoxin contamination in pre-harvest and post-harvest crops: Present status and future prospects. *Journal of Phytology*, 2(7), 37-52.

Claeys, L., Romano, C., De Ruyck, K., Wilson, H., Fervers, B., Korenjak, M., Zavadil, J., Gunter, M. J., De Saeger, S., De Boevre, M. & Huybrechts, I. (2020). Mycotoxin exposure and human cancer risk: A systematic review of epidemiological studies. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 1449-1464.

Codex Alimentarius Commission (2002). ‘‘Proposed draft code of practice for the prevention (reduction) of mycotoxin contamination in cereals, including annexes on ochratoxin A, zearalenone, fumonisins and tricothecenes.” *Codex Committee on Food Additives and Contaminants, Thirty-fourth Session*.

Cole, R. J., Doner, J. W. & Holbrook, C. C. (1995). Advances in mycotoxin elimination and resistance. Advances in Peanut Science. *American Peanut Research and Education Society, Stillwater, Ok,* 456-474.

Coleman, J., Blake-Kalff, M. & Davies, E. (1997). Detoxification of xenobiotics by plants: Chemical modification and vacuolar compartmentation. *Trends in Plant Science*, 2, 144-151.

Commission Implementing Regulation (EU) 2018/1568 of 18 October 2018 Concerning the authorisation of a Preparation of Fumonisin Esterase Produced by Komagataella Phaffii (DSM 32159) as a Feed Additive for All Pigs and All Poultry Species. Available online: https://eur-lex.europa.eu/legalcontent /EN/TXT/PDF/?uri=CELEX:32018R1568&from=EN.

Cortinovis, C., Pizzo, F., Spicer, L. J. & Caloni, F. (2013). *Fusarium* mycotoxins: Effects on reproductive function in domestic animals- A review. *Theriogenology*, 80(6), 557-564.

Cotton, T. K. & Munkvold, G. P. (1998). Survival of *Fusarium moniliforme*, *F. proliferatum* and *F. subglutinans* in maize stalk residue. *Phytopathology*, 88, 550-555.

Council for Agriculture Science and Technology (CAST) (2003). Mycotoxins: Risks in plant, animal, and human systems. *Task Force Report*, 139. Ames, Iowa.

Covarelli, L., Beccari, G., Prodi, A., Generotti, S., Etruschi, F., Juan, C., Ferrer, E. & Mañes, J. (2015). *Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. *Journal of the Science of Food and Agriculture*, 95, 540-551.

Cowan, D., Pettersson, D. R. & Ross, G. M. (1999). Investigations into the effect of xylanases and pectinases on broilers performance in sorghum based diets with low levels of wheat. *In Proceedings of the Australia Poultry Science Symposium* 11, 112-115

Crippin, T., Renaud, J. B., Sumarah, M. W. & Miller, J. D. (2019). Comparing genotype and chemotype of *Fusarium graminearum* from cereals in Ontario, Canada. *PloS one*, 14(5), e0216735.

Cullen, W. (1789). A Treatise of the Materia Medica: By William Cullen, In Two Volumes 2, 1-5.

D'Mello, J. P. F. & Mac Donald, A. M. C. (1997). Mycotoxins. *Animal Feed Science and Technolology*, 69, 155-166.

da Silva, S. A., Pereira, R. G. F. A., de Azevedo Lira, N., da Glória, E. M., Chalfoun, S. M. & Batista, L. R. (2020). Fungi associated to beans infested with coffee berry borer and the risk of ochratoxin A. *Food Control*, 113, 107204.

Darbre, P. D., Byford, J. R., Shaw, L. E., Horton, R. A., Pope, G. S. & Sauer, M. J. (2002). Oestrogenic activity of isobutylparaben in vitro and in vivo. *Journal of Applied Toxicology: An International Journal*, 22(4), 219-226.

De Angelis, E., Monaci, L. & Visconti, A. (2014). Investigation on the stability of deoxynivalenol and DON-3-glucoside during gastro-duodenal in vitro digestion of a naturally contaminated bread model food. *Food Control*, 43, 270-275.

De Morais C. L., Pinheiro, S. S., de Carvalho, C. W. P., Queiroz, V. A. V., de Menezes, C. B., Moreira, A. V. B., de Barros, F. A. R., Awika, J. M., Martino, H. S. D. & Pinheiro-Sant'Ana, H. M. (2015). Phenolic compounds profile in sorghum processed by extrusion cooking and dry heat in a conventional oven. *Journal of Cereal Science*, 65, 220-226.

Dill-Macky, R. & Jones, R. K. (2000). The effect of previous crop residues and tillage on *Fusarium* head blight of wheat. *Plant Disease*, 84(1), 71-76.

Dong, C., Davis, R. J. & Flavell, R. A. (2002). MAP kinases in the immune response. *Annual Review of Immunology*, 20(1), 55-72.

Dorn, B., Forrer, H. R., Jenny, E., Wettstein, F. E., Bucheli, T. D. & Vogelgsang, S. (2011). *Fusarium* species complex and mycotoxins in grain maize from maize hybrid trials and from grower's fields. *Journal of Applied Microbiology*, 111(3), 693-706.

Dowd, P. F. (2003). Insect management to facilitate preharvest mycotoxin management. *Journal of Toxicology*: *Toxin Reviews*, 22(2-3), 327-350.

Dowswell, C. (2019). Maize in the third world. CRC Press.

Du, K., Wang, C., Liu, P., Li, Y. & Ma, X. (2017). Effects of dietary mycotoxins on gut microbiome. *Protein and peptide letters*, 24(5), 397-405.

Edia, H. (2018). Sorghum production, cultivation and economic importance in Nigeria. Retrieved from www.blog.farmcrowdy.com.

Ediage, E. N., Van Poucke, C. & De Saeger, S. (2015). A multi-analyte LC-MS/MS method for the analysis of 23 mycotoxins in different sorghum varieties: The forgotten sample matrix. *Food Chemistry*, 177, 397-404.

European Food Safety Authority (EFSA) Panel on Contaminants in Food Chain (CONTAM). (2014). Scientific opinion on the risks to human and animal health related to the presence of beauvericin and enniatins in food and feed. *European Food Safety Authority Journal*, 12(8), 3802.

European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain. (2011). Scientific Opinion on the risks for public health related to the presence of zearalenone in food. *European Food Safety Authority Journal*, 9(6), 2197.

European Food Safety Authority (EFSA). Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). (2020). Safety and efficacy of

fumonisin esterase from *Komagataella Phaffii* DSM 32159 as a feed additive for all animal species. *EFSA Journal*, 18, e06207.

El-sharkaway, S. H., Selim, M. I., Afifi, M. S. & Halaweish, F. T. (1991). Microbial transformation of zearalenone to α-zearalenone sulfate. *Applied Environmental Microbiology,* 57, 549-552.

Elzeina, H. E. (2010). The nutritive value of newly developed maize (*Zea mays .L*.) inbred lines. Unpublished.

Embaby, E. M., Reda, M., Abdel-Wahhab, M. A., Omara, H. & Mokabel, A. M. (2013). Occurrence of toxigenic fungi and mycotoxins in some legume seeds. *Journal of Agricultural Technology,* 9(1), 151-164.

Enyisi, S. I., Umoh, V. J., Whong, C. M. Z., Abdullahi, I. O. & Alabi, O. (2014). Chemical and nutritional value of maize products obtained from selected markets. *African Journal of Food Science and Technology*, 5, 100-104.

Enyiukwu D. N., Awurum, A. N. & Nwaneri, J. A. (2014) Mycotoxins in stored agricultural products: Implications to food safety and health and prospects of plant-derived pesticides as novel approach to their management. *Greener Journal of Microbiology and Antimicrobials*, 2(3), 32-48

Eskola, M., Kos, G., Elliott, C. T., Hajšlová, J., Mayar, S. & Krska, R. (2020). Worldwide contamination of food-crops with mycotoxins: Validity of the widely cited 'FAO estimate'of 25 %. *Critical Reviews in Food Science and Nutrition*, 60(16), 2773-2789.

European Commission (2006). Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of European Union*, 70, 12-34.

European Commission (2007). Commission Regulation (EC) No. 1126/2007 on maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Official Journal of European Union,* 255, 14-17.

European Commission Regulation (2010). EC. NO./65/2010 of 26 February 2010 amending regulation (EC). NO.1881/2016. Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of European Union*, 150, 8-12.

European Food Safety Authority (EFSA) (2017). Risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. *EFSA Journal*, 15, 4718.

Ezekiel, C. N., Kraak, B., Sandoval-Denis, M., Sulyok, M., Oyedele, O. A., Ayeni, K. I., Makinde, O. M., Akinyemi, O. M., Krska, R., Crous, P. W. & Houbraken, J.

(2020). Diversity and toxigenicity of fungi and description of *Fusarium madaense* sp. nov. from cereals, legumes and soils in north-central Nigeria. *MycoKeys*, 67, 95.

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W. F. O. & Wingfield, M. J. (2006). Impact of indigenous storage systems and insect infestation on the contamination of maize with fumonisins. *African Journal of Biotechnology*, 5(7), 546-552.

Fandohan, P., Gnonlonfin, B., Hell, K., Marasas, W. F. O. & Wingfield, M. J. (2005). Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa. *International Journal of Food Microbiology*, 99(2), 173-183.

FAO. (2016). Maize, rice, wheat: A guide to sustainable cereal production. *Food and Agriculture Organization of the United Nations*, *Rome*, ISBN: 978-92-5-108519-6.

Ferrante, M., Sciacca, S. & Conti, G. O. (2012). Carcinogen role of food by mycotoxins and knowledge gap. *Carcinogen,* InTech, Rijeka, Croatia, 133-162.

Ferrigo, D., Raiola, A. & Causin, R. (2016). *Fusarium* toxins in cereals: occurrence, legislation, factors promoting the appearance and their management. *Molecules*, 21(5), 627.

Filtenborg, O., Frisvad, J. C. & Thrane, U. (1996). Moulds in food spoilage. *International Journal of Food Microbiolgy*, 33, 85-102.

Food and Agriculture Organization (FAO) (1994). Components of sweet sorghum stem juice. In: Integrated energy systems in China - the cold northeastern region experience. FAO corporate document repository. Retrived.from.http:// www.fao. org/docrep/T4470E/t4470e07.htm#4.1.%20components%20of%20sweet%20sorghum%20stem%20juice on 06/04/2012.

Food and Agriculture Organization (FAO) (2004). Worldwide regulations for mycotoxins in food and feed in 2003. *FAO Food and Nutrition Paper* 81. Food and Agriculture Organization of the United Nations, Rome, Italy.

Food and Agriculture Organization of the United Nations statistics (FAOSTAT) (2018). *Food and Agriculture Organization of the United Nations, Roma, Italy*.

Foroud, N. A., Baines, D., Gagkaeva, T. Y., Thakor, N., Badea, A., Steiner, B., Bürstmayr, M. & Bürstmayr, H. (2019). Trichothecenes in cereal grains–An update. *Toxins*, 11(11), 634.

Fouche, T., Claassens, S. & Maboeta, M. (2020). Aflatoxins in the soil ecosystem: An overview of its occurrence, fate, effects and future perspectives. *Mycotoxin Research*, 1-7.

Freire, L., Furtado, M. M., Guerreiro, T. M., da Graça, J. S., da Silva, B. S., Oliveira, D. N., Catharino, R. R. & Sant'Ana, A. S. (2019). The presence of ochratoxin A does not influence *Saccharomyces cerevisiae* growth kinetics but leads to the formation of modified ochratoxins. *Food and Chemical Toxicology*, 133, 110756.

Fremy, J. M., Alassane-Kpembi, I., Oswald, I. P., Cottrill, B. & Van Egmond, H. P. (2019). A review on combined effects of moniliformin and co-occurring *Fusarium* toxins in farm animals. *World Mycotoxin Journal*, 12(3), 281-291.

Frisvad, J. C., Houbraken, J., Popma, S. & Samson, R. A. (2013). Two new *Penicillium* species *Penicillium buchwaldii* and *Penicillium spathulatum*, producing the anticancer compound asperphenamate. *Federation of European Microbiological Societies*: *Microbiology Letters*, 339(2), 77-92.

Fromme, H., Gareis, M., Volkel, W. & Gottschalk, C. (2016). Overall internal exposure to mycotoxins and their occurrence in occupational and residential settings - An overview. *International Journal of Hygiene and Environmental Health*, 219(2), 143-165. doi: 10.1016/ j.ijheh.2015.11.004.

Fumero, M. V., Reynoso, M. M. & Chulze, S. (2015). *Fusarium temperatum* and *Fusarium subglutinans* isolated from maize in Argentina. *International Journal of Food Microbiology*, 199, 86-92.

Fung, F. & Clark, R. F. (2004). Health effects of mycotoxins: A toxicological overview. *Journal of Toxicology: Clinical Toxicology*, 42(2), 217-234.

Gaffoor, I. & Trail, F. (2006). Characterization of two polyketide synthase genes involved in zearalenone biosynthesis in Gibberella zeae. *Applied and Environmental Microbiology*, 72(3), 1793-1799.

Gajęcka, M., Jakimiuk, E., Zielonka, Ł., Obremski, K. & Gajęcki, M. (2009). The biotransformation of chosen mycotoxins. *Polish Journal of Veterinary Sciences*, 12, 293-303.

Galani, Y. J. H., Orfila, C. & Gong, Y. Y. (2020). A review of micronutrient deficiencies and analysis of maize contribution to nutrient requirements of women and children in Eastern and Southern Africa. *Critical Reviews in Food Science and Nutrition*, 1-24.

Gamanya, R. & Sibanda, L. (2001). Survey of *Fusarium moniliforme* (*F. verticillioides*) and production of fumonisin B_1 in cereal grains and oilseeds in Zimbabwe. *International Journal of Food Microbiology*, 71(2-3), 145-149.

Garba, M. H., Makun, H. A., Jigam, A. A., Muhammad, H. L. & Patrick, B. N. (2017). Incidence and toxigenicity of fungi contaminating sorghum from Nigeria. *World Journal of Microbiology and Biotechnology*, 4, 105-114.

Gareis, M., Bauer, J. Thiem, J., Plank, G., Grabley, S. & Gedek, B. (1990). Cleavage of zearalenone-glycoside, a "Masked" mycotoxin during digestion in swine. *Journal of Veterinary Medicine Series B,* 37(3), 236-240. doi: 10.1111/j.1439-450.1990.tb01052.x.

Garvey, G. S., McCormick, S. P., Alexander, N. J. & Rayment, I. (2009). Structural and functional characterization of TRI3 trichothecene 15-O-acetyltransferase from *Fusarium sporotrichioides*. *Protein Science*, 18(4), 747-761.

Gbashi, S., Madala, N. E., De Saeger, S., De Boevre, M., Adekoya, I., Adebo, O. A. & Njobeh, P. B. (2018). The socio-economic impact of mycotoxin contamination in Africa. Fungi and mycotoxins-their occurrence, impact on health and the economy as well as pre-and postharvest management strategies. *World Journal of Microbiology and Biotechnology* , 1-20.

Gbashi, S., Njobeh, P. B., Madala, N. E., De Boevre, M., Kagot, V. & De Saeger, S. (2020). Parallel validation of a green-solvent extraction method and quantitative estimation of multi-mycotoxins in staple cereals using LC-MS/MS. *Scientific Reports*, 10(1), 1-16.

Gill, H. & Vasundhara, M. (2019). Isolation of taxol producing endophytic fungus *Alternaria brassicicola* from non-Taxus medicinal plant *Terminalia arjuna*. *World Journal of Microbiology and Biotechnology*, 35(5), 1-8.

Gimeno, A. (2002). Los Hongos y las Micotoxinas en la Alimentación Animal; Conceptos, Problemas, Control Y Recomendaciones, Online available at http:// www.engormix.com/MA-micotoxinas/articulos/criterios-calidad-micologica-t353/p0.htm.

Glenn, A. E. (2007). Mycotoxigenic Fusarium species in animal feed. *Animal Feed Science and Technology*, 137(3-4), 213-240.

Gonçalves, A., Gkrillas, A., Dorne, J. L., Dall'Asta, C., Palumbo, R., Lima, N. & Giorni, P. (2019). Pre-and postharvest strategies to minimize mycotoxin contamination in the rice food chain. *Comprehensive Reviews in Food Science and Food Safety*, 18(2), 441-454.

Gorst-Allman, C. P., Steyn, P. S., Vleggar, R. & Rabie, C. J. (1985). Structure elucidation of a novel trichothecene glycoside using H-1 and C-13 Nuclear Magnetic-Resonance Spectroscopy. *Journal of the Chemical Society,* Perkin Transactions, 1, 1553-1555.

Gratz, S. W., Dinesh, R., Yoshinari, T., Holtrop, G., Richardson, A. J., Duncan, G., MacDonald, S., Lloyd, A. & Tarbin, J. (2017). Masked trichothecene and zearalenone mycotoxins withstand digestion and absorption in the upper GI tract but are efficiently hydrolyzed by human gut microbiota in vitro. *Molecular Nutrition & Food Research*, 61(4), 1600680.

Greco, M. V., Franchi, M. L., Rico Golba, S. L., Pardo, A. G. & Pose, G. N. (2014). Mycotoxins and mycotoxigenic fungi in poultry feed for food-producing animals. *The Scientific World Journal*, 14-20.

Grenier, B. & Oswald, I. (2011). Mycotoxin co-contamination of food and feed: Meta-analysis of publications describing toxicological interactions. *World Mycotoxin Journal*, 4, 285-313.

Gunupuru, L. R., Patel, J. S., Sumarah, M. W., Renaud, J. B., Mantin, E. G. & Prithiviraj, B. (2019). A plant biostimulant made from the marine brown algae *Ascophyllum nodosum* and chitosan reduce *Fusarium* head blight and mycotoxin contamination in wheat. *PLoS One*, 14(9), e0220562.

Hackbart, H. C. S., Machado, A. R., Christ-Ribeiro, A., Prietto, L. & Badiale-Furlong, E. (2014). Reduction of aflatoxins by Rhizopus oryzae and Trichoderma reesei. *Mycotoxin Research*, 30(3), 141-149.

Haighton, L. A., Lynch, B. S., Magnuson, B. A. Pan, Y., Huang, L., Peng, D., Wang, X. & Nestmann, E. R. (2012). A reassessment of risk associated with dietary intake of ochratoxin A based on a lifetime exposure model. *Critical Reviews in Toxicology*, 42(2), 147-168.

Hansen, F. T., Sørensen, J. L., Giese, H., Sondergaard, T. E. & Frandsen, R. J. (2012). Quick guide to polyketide synthase and nonribosomal synthetase genes in *Fusarium*. *International Journal of Food Microbiology*, 155(3), 128-136.

Haque, M. A., Wang, Y., Shen, Z., Li, X., Saleemi, M. K. & He, C. (2020). Mycotoxin contamination and control strategy in human, domestic animal and poultry: A Review. *Microbial Pathogenesis*, 142, 104095.

Haratian, M., Sharifnabi, B., Alizadeh, A. & Safaie, N. (2008). PCR analysis of the Tri13 gene to determine the genetic potential of *Fusarium graminearum* isolates from Iran to produce nivalenol and deoxynivalenol. *Mycopathologia*, 166, 109-116.

Hassan, Y. I. & Zhou, T. (2018). Addressing the mycotoxin deoxynivalenol contamination with soil-derived bacterial and enzymatic transformations targeting the C3 carbon. *World Mycotoxin Journal*, 11(1), 101-112.

Hassan, Z. U., Al Thani, R., Balmas, V., Migheli, Q. & Jaoua, S. (2019). Prevalence of *Fusarium* fungi and their toxins in marketed feed. *Food Control*, 104, 224-230.

Heilos, D., Rodríguez, C. Y., Englinger, B., Timelthaler, G., Van Schoonhoven, S., Sulyok, M., Boecker, S., Süssmuth, R. D., Heffeter, P., Lemmens-Gruber, R. & Dornetshuber-Fleiss, R. (2017). The natural fungal metabolite beauvericin exerts anticancer activity in vivo: A pre-clinical pilot study. *Toxins*, 9(9), 258.

Hendricks, K. (1999). Fumonisins and neural tube defects in south Texas. *Epidemiology*, 198-200.

Hertveldt, K. (2016). Mycotoxins occurrence in Nigerian cereal crops (sorghum and millet): Faculty of pharmaceutical sciences, Ghent University. Unpublished.

Hillman, G. (1978). On the origins of domestic rye: Secale cereale: the finds from Aceramic Can Hasan III in Turkey. *Anatolian Studies*, 28, 157-174.

Hofrichter, M. (2010). The Mycota: A comprehensive treatise on fungi as experimental systems for basic and applied research: *Industrial Applications, 2nd edition; Springer-Verlag: New York.*

Hossen, S. M., Yoshida, M., Nakagawa, H., Nagashima, H., Okadome, H., Nakajima, T. & Kushiro, M. (2012). Distribution of nivalenol in milling fractions of severely *Fusarium*-infected Japanese soft winter wheat grains. *Journal of Japanese Society of Mycoloxicology*: *Mycotoxins*, 62(2), 77-82.

Hove, M., De Boevre, M., Lachat, C., Jacxsens, L., Nyanga, L. K. & De Saeger, S. (2016). Occurrence and risk assessment of mycotoxins in subsistence farmed maize from Zimbabwe. *Food Control*, 69, 36-44.

Howard, P. C., Eppley, R. M., Stack, M. E., Warbritton, A., Voss, K.A., Lorentzen, R. J., Kovach, R. M. & Bucci, T. J. (2001). Fumonisin B_1carcinogenicity in a two-year feeding study using F344 Rats and B6C3F1 Mice. *Environmental Health Perspectives*, 109, 277-282.

Huang, D., Cui, L., Sajid, A., Zainab, F., Wu, Q., Wang, X. & Yuan, Z. (2019). The epigenetic mechanisms in *Fusarium* mycotoxins induced toxicities. *Food and Chemical Toxicology*, 123, 595-601.

Hussaini, A. M., Timothy, A. G., Olufunmilayo, H. A., Ezeikel, A. S. & Godwin, H. O. (2009). Fungi and some mycotoxins found in mouldy sorghum in Niger State, Nigeria. *World Journal of Agricultural Sciences*, 5(1), 5-17.

Hyde, K. D., Maharachchikumbura, S. S., Hongsanan, S., Samarakoon, M. C., Lücking, R., Pem, D., Harishchandra, D., Jeewon, R., Zhao, R.L., Xu, J. C. & Elgorban,

A. M. (2017). The ranking of fungi: A tribute to David L. Hawksworth on his 70th birthday. *Fungal Diversity*, 84(1), 1-23.

Inderbitzin, P., Shoemaker, R. A., O'Neill, N. R., Turgeon, B. G. & Berbee, M. L. (2006). Systematics and mating systems of two fungal pathogens of opium poppy: The heterothallic *Crivellia papaveracea* with a *Brachycladium penicillatum* asexual state and a homothallic species with a *Brachycladium papaveris* asexual state. *Botany*, 84(8), 1304-1326.

International Agency for Research on Cancer (IARC) (1993). Monographs on the evaluation of the carcinogenic risk of chemicals to human: Some naturally occurring substances. Food items and constituents, heterocyclic aromatic amines and mycotoxins. *International Agency for Research on Cancer*, 397-488.

International Agency for Research on Cancer (IARC) (2002). Monographs on the evaluation of carcinogenic risks to humans. Some traditional herbal medicines, some mycotoxins, naphthalene and styrene. *International Agency for Research on Cancer*, 82, 171-300.

International Agency for Research on Cancer, IARC (2012). *IARC monographs on the evaluation of carcinogenic risks to humans*, 1-104.

Ismaiel, A. A. & Papenbrock, J. (2015). Review mycotoxins: producing fungi and mechanisms of phytotoxicity. *Agriculture*, 5, 492-537.

Jaćević, V., Dumanović, J., Lazarević, M., Nepovimova, E., Resanović, R., Milovanović, Z., Wu, Q. & Kuča, K. (2020). Antidotal Potency of the Novel, Structurally Different Adsorbents in Rats Acutely Intoxicated with the T-2 Toxin. *Toxins*, 12(10), 643.

Jajić, I., Dudaš, T., Krstović, S., Krska, R., Sulyok, M., Bagi, F. & Stankov, A. (2019). Emerging *Fusarium* mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin in Serbian maize. *Toxins*, 11(6), 357.

Jakovac-Strajn, B., Pavšič-Vrtač, K., Ujčič-Vrhovnik, I., Vengušt, A. & Tavčar-Kalcher, G. (2010). Microbiological and mycotoxicological contamination in Slovenian primary grain production. *Toxicological and Environmental Chemistry*, 92(8), 1551-1563.

Jeff-Agboola, Y. A. & Omosanyin, T. R. (2017). Occurrence of toxigenic moulds isolated in maize (Zea mays) from Okitipupa metropolis, Ondo State, Nigeria. *International Journal of Food Safety, Nutrition, Public Health and Technology*, 9(4), 28.

Jennessen, J., Nielsen, K. F., Houbraken, J., Lyhne, E. K., Schnurer, J., Frisvad, J. C. & Samson, R. A. (2005). Secondary metabolite and mycotoxin production by the

Rhizopus microsporus group. *Journal of Agriculture and Food Chemistry*, 53(5), 1833-1840.

Jestoi, M. (2008). A review: Emerging *Fusarium* mycotoxins - fusaproliferin, beauvericin, enniatins and moliniformin. *Food Science and Nutrition*, 48(1), 21-49.

Jin, J., Beekmann, K., Ringø, E., Rietjens, I. M. & Xing, F. (2021). Interaction between food-borne mycotoxins and gut microbiota: A review. *Food Control*, 107998.

Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2001). Safety evaluation of certain mycotoxins in food. Deoxynivalenol, HT-2 and T-2 toxin. *FAO Food and Nutrition Paper* 74. http://www.inchem.org/documents /jecfa/ jecmono/v47je01.htm

Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2017). Evaluation of certain contaminants in Food 83rd Report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series*, No. 1002.

Joint FAO/WHO Expert Committee on Food Additives (JECFA). (2000). Zearalenone. In: Safety evaluation of certain food additives and contaminants. *WHO Food Additives Series*, 44, 393482

Jonsson, M., Jestoi, M., Anthoni, M., Welling, A., Loivamaa, I. & Hallikainen, V. (2016). *Fusarium* mycotoxin enniatin B: Cytotoxic effects and changes in gene expression profile. *Toxicology in Vitro*, 34, 309-320. Doi:10.1016/j.tiv.2016.04.017.

Kalagatur, N. K., Mudili, V., Kamasani, J. R. & Siddaiah, C. (2018). Discrete and combined effects of Ylang-Ylang (Cananga odorata) essential oil and gamma irradiation on growth and mycotoxins production by *Fusarium graminearum* in maize. *Food Control*, 94, 276-283.

Kamala, A., Ortiz, J., Kimanya, M., Haesaert, G., Donoso, S., Tiisekwa, B. & De Meulenaer, B. (2015). Multiple mycotoxin co-occurrence in maize grown in three agro-ecological zones of Tanzania. *Food Control*, 54, 208-215.

Kamle, M., Mahato, D. K., Devi, S., Lee, K. E., Kang, S. G. & Kumar, P. (2019). Fumonisins: Impact on agriculture, food, and human health and their management strategies. *Toxins*, 11(6), 328.

Katika, M. R., Hendriksen, P. J., van Loveren, H. & ACM Peijnenburg, A. (2015). Characterization of the modes of action of deoxynivalenol (DON) in the human jurkat T-cell line. *Journal of Immunotoxicology*, 12(3), 206-216.

Kaufman, D. D., Williams, L. E. & Sumner, C. B. (1963). Effect of plating medium and incubation temperature on growth of fungi in soil-dilution plates. *Canadian Journal of Microbiology*, 9(6), 741-751.

Keller, M. D., Bergstrom, G. C. & Shields, E. J. (2014). The aerobiology of *Fusarium graminearum. Aerobiologia*, 30, 123-136.

Keller, N. P. (2015). Translating biosynthetic gene clusters into fungal armor and weaponry. *Nature Chemical Biology*, 11(9), 671-677.

Kemboi, D. C., Antonissen, G., Ochieng, P. E., Croubels, S., Okoth, S., Kangethe, E. K., Faas, J., Lindahl, J. F. & Gathumbi, J. K. (2020). A review of the impact of mycotoxins on dairy cattle health: Challenges for food safety and dairy production in sub-Saharan Africa. *Toxins*, 12(4), 222.

Khalil, A. M. A. & Hashem, A. H. (2018). Morphological changes of conidiogenesis in two *Aspergillus* species. *Journal of Pure and Applied Microbiology*, 12(4), 2041-2048.

Khan, A., Aalim, M. M., Khan, M. Z., Saleemi, M. K., He, C., Khatoon, A., & Gul, S. T. (2017). Amelioration of immunosuppressive effects of aflatoxin and ochratoxin A in White Leghorn layers with distillery yeast sludge. *Toxin Reviews*, 36(4), 275-281.

Khonga, E. B. & Sutton, J. C. (1988). Inoculum production and survival of Gibberella zeae in maize and wheat residues. *Canadian Journal of Plant Pathology*, 10(3), 232-239.

Kiaya, V. (2014). Post-harvest losses and strategies to reduce them. *Technical Paper on Postharvest Losses, Action Contre la Faim* (ACF), 25.

Kim, J. E., Son, H. & Lee, Y. W. (2018). Biosynthetic mechanism and regulation of zearalenone in *Fusarium graminearum*. *JSM Mycotoxins*, 68(1), 1-6.

Kim, S. L., Snook, M. E. & Lee, J. O. (2003). Radical scavenging activity and cytotoxicity of maysin (C-glycosylflavone) isolated from silks of *Zea mays L*. *한국작물학회지*, 48(5), 392-396.

Kim, Y. T., Lee, Y. R., Jin, J., Han, K. H., Kim, H., Kim, J. C., Lee, T., Yun, S. H. & Lee, Y. W. (2005). Two different polyketide synthase genes are required for synthesis of zearalenone in Gibberella zeae. *Molecular Microbiology*, 58(4), 1102-1113.

Kimanya, M. E., De Meulenaer, B., Tiisekwa, B., Ndomondo-Sigonda, M., Devlieghere, F., Van Camp, J. & Kolsteren, P. (2008). Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in

rural villages of Tanzania. *Food Additives and Contaminants*, 25(11), 1353-1364.

Kimanya, M. E., Shirima, C. P., Magoha, H., Shewiyo, D. H., De Meulenaer, B., Kolsteren, P. & Gong, Y. Y. (2014). Co-exposures of aflatoxins with deoxynivalenol and fumonisins from maize-based complementary foods in Rombo, Northern Tanzania. *Food Control*, 41, 76-81.

Kirinčič, S., Škrjanc, B., Kos, N., Kozolc, B., Pirnat, N. & Tavčar-Kalcher, G. (2015). Mycotoxins in cereals and cereal products in Slovenia–Official control of foods in the years 2008–2012. *Food Control*, 50, 157-165.

Kos, J., Hajnal, E. J., Škrinjar, M., Mišan, A., Mandić, A., Jovanov, P. & Milovanović, I. (2014). Presence of Fusarium toxins in maize from Autonomous Province of Vojvodina, Serbia. *Food Control*, 46, 98-101.

Kostelanska, M., Dzuman, Z., Malachova, A., Capouchova, I., Prokiniva, E., Skerikova, A. & Hajslova, J. (2011). Effects of milling and baking technologies on levels of deoxynivalenol and its masked form deoxynivalenol-3-glucoside. *Journal of Agricultural and Food Chemistry*, 59(17), 9303-9312. doi:10.1021/jf202428f.

Kostelanska, M., Hajslova, J., Zachariasova, M., Malachova, A., kalachova, K., Poustka, J., Fiala, J., Scott, P. M., Berthiler, F. M. & Krska, R. (2009). Occurrence of deoxynivalenol and its major conjugates, deoxynivalenol-3-glucosides, in beer and some brewing Intermediates. *Journal of Agricultural and Food Chemistry*, 57, 3187-3194.

Kostic, A. Ž., Milinčić, D. D., Petrović, T. S., Krnjaja, V. S., Stanojević, S. P., Barać, M. B., Tešić, Ž. L. & Pešić, M. B. (2019). Mycotoxins and mycotoxin producing fungi in pollen. *Toxins*, 11(2), 64.

Kpodo, K. A. (2001). Fusaria and Fumonisins in Maize and Fermented Maize Products from Ghana (Doctoral dissertation, Royal Veterinary and Agricultural University, Department of Dairy and Food Science, Food Microbiology).

Kpodo, K., Thrane, U. & Hald, B. (2000). Fusaria and fumonisins in maize from Ghana and their co-occurrence with aflatoxins. *International journal of food microbiology*, 61(2-3), 147-157.

Kumar, V., Basu, M. S. & Rajendran, T. P. (2008). Mycotoxin research and mycoflora in some commercially important agricultural commodities. *Crop Protection*, 27(6): 891-905.

Kunishige, K., Kawate, N., Inaba, T. & Tamada, H. (2017). Exposure to zearalenone during early pregnancy causes estrogenic multitoxic effects in mice. *Reproductive Sciences*, 24(3), 421-427.

Lacey, T. (1986). Factors affecting mycotoxin production. In: Mycotoxins and Phycotoxins *.6th international IUPAC symposium on Mycotoxins and Phycotoxins*, Pretoria, South Africa. 67-76

Lancova, K., Hajslova, J., Poustka, J., Krplova, A., Zachariasova, M., Dostalek, P. & Sachambaula, L. (2008). Transfer of *Fusarium* mycotoxins and "Masked" deoxynivalenol (deoxynivalenol-3-glucoside) from field barley through malt to beer. *Food Additives and Contaminants*, 25, 732-744.

Lanyasunya, T. P., Wamae, L. W., Musa, H. H., Olowofeso, O. & Lokwaleput, I. K. (2005). The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. *Pakistan Journal of Nutrition*, 4, 162-169.

Laskin, J. D., Heck, D. E. & Laskin, D. L. (2002). The ribotoxic stress response as a potential mechanism for MAP kinase activation in xenobiotic toxicity. *Toxicological Sciences*, 69(2), 289-291.

Lattanzio, V. M. T., Ciasca, B., Haidukowski, M., Infantino, A., Visconti, A. & Pascale, M. (2013). Mycotoxin profile of *Fusarium langsethiae* isolated from wheat in Italy: Production of type-A trichothecenes and relevant glucosyl derivatives. *Journal of Mass Spectrometry*. 48, 1291-1298.

Lavkor, I. & Var, I. (2017). The control of aflatoxin contamination at harvest, drying, pre-storage and storage periods in peanut: The new approach. *Aflatoxin-control, analysis, detection and health risks*, 45-64.

Lawrence, D. P., Gannibal, P. B., Dugan, F. M. & Pryor, B. M. (2014). Characterization of *Alternaria* isolates from the infectoria species-group and a new taxon from *Arrhenatherum*, *Pseudoalternaria arrhenatheria* sp. nov. *Mycological Progress*, 13(2), 257-276.

Lee, T., Han, Y. K., Kim, K. H., Yun, S. H. & Lee, Y. W. (2002). Tri13 and Tri7 determine deoxynivalenol-and nivalenol-producing chemotypes of Gibberella zeae. *Applied and Environmental Microbiology*, 68(5), 2148-2154.

Leggieri, M. C., Lanubile, A., Dall'Asta, C., Pietri, A. & Battilani, P. (2020). The impact of seasonal weather variation on mycotoxins: Maize crop in 2014 in northern Italy as a case study. *World Mycotoxin Journal*, 13(1), 25-36.

Lene, L. (2010). The importance of fungi for a more sustainable future on our planet. *Fungal Biology Reviews British Mycological Society*, 24, 90-92.

Li, P., Zhou, Q., Wang, T., Zhou, H., Zhang, W., Ding, X. & Zhang, Q. (2016). Development of an enzyme-linked immunosorbent assay method specific for the detection of g-group aflatoxins. *Toxins*, 8(1), 5.

Li, X., Tang, H., Yang, C., Meng, X. & Liu, B. (2019). Detoxification of mycotoxin patulin by the yeast Rhodotorula mucilaginosa. *Food Control*, 96, 47-52.

Li, Y. C., Ledoux, D. R., Bermudez, A. J., Fritsche, K. L. & Rottinghaus, G. E. (2000). The individual and combined effects of fumonisin B_1 and moniliformin on performance and selected immune parameters in turkey poults. *Poultry Science*, 79, 871-878.

Lincy, S. V., Chandrashekar, A., Narayan, M. S., Sharma, R. & Thakur, R. P. (2011). Natural occurrence of trichothecene-producing Fusaria isolated from India with particular reference to sorghum. *World Journal of Microbiology and Biotechnology*, 27(4), 981-989.

Lindblad, M., Gidlund, A., Sulyok, M., Börjesson, T., Krska, R., Olsen, M. & Fredlund, E. (2013). Deoxynivalenol and other selected *Fusarium* toxins in Swedish heat-Occurrence and correlation to specific *Fusarium* species. *International Journal of Food Microbiology*, 167(2), 284-291.

Liu, Y. & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. *Environmental Health Perspectives*, 118(6), 818-824.

Liu, Z., Zhang, G., Zhang, Yi., Jin, Q., Zhao, J. & Li, J. (2016). Factors controlling mycotoxin contamination in maize and food in the Hebei province, China. *Agronomy for Sustainable Development*, 36(2), 39

Logrieco, A., Rizzo, A., Ferracane, R. & Ritieni, A. (2002). Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. *Applied and Environmental Microbiology*, 68 (1), 82-85. doi: 10.1128/AEM.68.1.82-85.2002.

Loi, M., Fanelli, F., Liuzzi, V. C., Logrieco, A. F. & Mulè, G. (2017). Mycotoxin biotransformation by native and commercial enzymes: Present and future perspectives. *Toxins*, 9(4), 111.

Ma, L. J., Geiser, D. M., Proctor, R. H., Rooney, A. P., O'Donnell, K., Trail, F., Gardiner, D. M., Manners, J. M. & Kazan, K. (2013). *Fusarium* pathogenomics. *Annual Review of Microbiology*, 67, 399-416.

Magan, N. & Evans, P. (2000). Volatiles as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage. *Journal of Stored Products Research*, 36(4), 319-340.

Mahmoud, M. A., Al-Othman, M. R. & Abd El-Aziz, A. R. M. A. (2013). Mycotoxigenic fungi contaminating corn and sorghum grains in Saudi Arabia. *Pakistan Journal of Botany*, 45(5), 1831-1839.

Makun, H. A., Dutton, M. F., Njobeh, P. B., Mwanza, M. & Kabiru, A. Y. (2011). Natural multi-occurrence of mycotoxins in rice from Niger state, Nigeria. *Mycotoxins Resources*, 27(2), 97-104. doi: http://doi.org/10.1007/s12550-010-0080-5.

Makun. H. A., Timothy, A. G., Olufunmilayo, H. A., Ezekiel, A. S. & Godwin, H. O. (2009). Fungi and some mycotoxins found in mouldy sorghum in Niger State, Nigeria. *World Journal of Agricultural Sciences*, 5(1), 5-17.

Malachova, A., Cerkal, R., Ehrenbergerova, J., Dzuman, Z., Vaculova, K. & Hajslova, J. (2010). *Fusarium* mycotoxins in various barley cultivars and their transfer into malt. *Journal of the Science of Food and Agriculture*, 90, 2495-2505.

Mallebrera, B., Juan-Garcia, A., Font, G. & Ruiz, M. J. (2016). Mechanisms of beauvericin toxicity and antioxidant cellular defense. *Toxicology Letters*, 246, 28-34.

Mallmann, C. A., Tyska, D., Almeida, C. A., Oliveira, M. S. & Gressler, L. T. (2020). Mycotoxicological monitoring of breakfast and infant cereals marketed in Brazil. *International Journal of Food Microbiology*, 331, 108628.

Mannaa, M. & Kim, K. D. (2017). Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. *Mycobiology*, 45(4), 240-254.

Manova, R. & Mladenova, R. (2009). Incidence of zearalenone and fumonisins in Bulgarian cereal production. *Food Control*, 20(4), 362-365.

Manshor, N., Rosli, H., Ismail, N., Saleeh, B. & Zakaria, L. (2012). Diversity of *Fusarium* species from highland areas in Malaysia. *Tropical Life Species Research*, 23(2), 1-15.

Marasas, W. F. (2001). Discovery and occurrence of the fumonisins: A historical perspective. *Environmental Health Perspectives*, 109(2), 239-243.

Marasas, W. F. O. (1996). Fumonisin: History, worldwide occurrence and impact. *Advances in Experimental Medicine*, 392, 1-17.

Marasas, W. F. O., Jaskiewics, K., Venter, F. S. & Van Schalkwyk, D. J. (1988). *Fusarium moniliforme* contamination of maize in oesophageal cancer areas in Transkei. *South African Medicine Journal*, 74, 110-114.

Marasas, W. F., Riley, R. T., Hendricks, K. A., Stevens, V. L., Sadler, T. W., Gelineau-van Waes, J., Missmer, S. A., Cabrera, J., Torres, O., Gelderblom, W. C. & Allegood, J. (2004). Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: A potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *Journal of Nutritions*, 134(4), 711-716.

Massart, F. & Saggese, G. (2010). Oestrogenic mycotoxin exposures and precocious pubertal development. *International Journal of Andrology*, 33(2), 369-376.

Mateo, E. M., Gómez, J. V., Romera, D., Tarazona, A., Gimeno-Adelantado, J. V., Mateo-Castro, R. & Jiménez, M. (2018). Comparative study of different cereals as substrates for T-2 and HT-2 production by *Fusarium langsethiae*. *International Journal of Food Engineering*, 4(1), 56-59

Matossian, M. K. (1981). Mold poisoning: An unrecognized English health problem, 1550–1800. *Medical history*, 25(1), 73-84.

Matumba, L., Sulyok, M., Monjerezi, M., Biswick, T. & Krska, R. (2015a). Fungal metabolites diversity in maize and associated human dietary exposures relate to micro-climatic patterns in Malawi. *World Mycotoxin Journal*, 8(3), 269-282

Matumba, L., Van Poucke, C., Njumbe Ediage, E., Jacobs, B. & De Saeger, S. (2015b). Effectiveness of hand sorting, flotation/washing, dehulling and combinations thereof on the decontamination of mycotoxin-contaminated white maize. *Food Additives & Contaminants: Part A*, 32(6), 960-969.

Maziero, M. T. & Bersot, L. D. S. (2010). Micotoxinas em alimentos produzidos no Brasil. *Revista Brasileira de Produtos Agroindustriais*, 12(1), 89-99.

McCormick, S. P., Alexander, N. J. & Proctor, R. H. (2006). Fusarium Tri4 encodes a multifunctional oxygenase required for trichothecene biosynthesis. *Canadian Journal of Microbiology*, 52(7), 636-642.

McCormick, S. P., Alexander, N. J., Trapp, S. E. & Hohn, T. M. (1999). Disruption of TRI101, the gene encoding trichothecene 3-O-acetyltransferase, from *Fusarium sporotrichioides*. *Applied and Environmental Microbiology*, 65(12), 5252-5256.

McCormick, S. P., Stanley, A. M., Stover, N. A. & Alexander, N. J. (2011). Trichothecenes: from simple to complex mycotoxins. *Toxins*, 3(7), 802-814.

McKerracher, M. (2016). Bread and surpluses: The Anglo-Saxon 'bread wheat thesis' reconsidered. *Environmental Archaeology*, 21(1), 88-102.

McLean, M. & Berjak, P. (1987). Maize grains and their associated mycoflora-a micro-ecological consideration. *Seed Science and Technology*, 15(3), 831-850.

Merem, E. C., Twumasi, Y., Wesley, J., Isokpehi, P., Shenge, M., Fageir, S., Crisler, M., Romorno, C., Hines, A., Hirse, G. & Ochai, S. (2017). Analysing rice production issues in the Niger state area of Nigeria's middle belt. *Food and Public Health*, 7(1), 7-22. doi: 10.5923/j.fph.20170701.02.

Mesnil, M., Crespin, S., Avanzo, J. L. & Zaidan-Dagli, M. L. (2005). Defective gap junctional intercellular communication in the carcinogenic process. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1719(1-2), 125-145.

Miller, J. D. (2016). Mycotoxins in food and feed: A Challenge for the twenty-first century. *Biology of Microfungi*, 469-493.

Miller, J. D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Lloyd Seaman, W., Seifert, K. A., Turkington, K. & Voldeng, H. (1998). Effect of tillage practice on *Fusarium* head blight of wheat. *Canadian Journal of Plant Pathology*, 20, 95-103.

Miller, J. D., Savard, M. E., Schaafsma, A. W., Seifert, K. A. & Reid, L. M. (1995). Mycotoxin production by *Fusarium moniliforme* and *Fusarium proliferatum* from Ontario and occurrence of fumonisin in the 1993 corn crop. *Canadian Journal of Plant Pathology*, 17, 233-239.

Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Merrill, A. H., Rothman, K. J. & Hendricks, K. A. (2006). Exposure to fumonisins and the occurrence of neutral tube defects along the Texas-Mexico border. *Environmental Health Perspectives*, 114, 237-241.

Mogensen, J. M., Larson, T. O. & Nielson, K. F. (2010). Widespread occurrence of the mycotoxin fumonisin B-2 in wine. *Journal of Agricultural and Food Chemistry*, 58(8), 4853-4857.

Mohammed, Z. S., Mabudi, A. H., Murtala, Y., Jibrin, S., Sulaiman, S. & Salihu, J. (2019). Nutritional analysis of three commonly consumed varieties of sorghum (*Sorghum bicolor L.*) in Bauchi State, Nigeria. *Journal of Applied Sciences and Environmental Management*, 23(7), 1329-1334.

Moore, G. G. (2014). Sex and recombination in aflatoxigenic *Aspergilli*: global implications. *Frontiers in microbiology*, 5, 32.

Moretti, A. N. (2009). Taxonomy of *Fusarium* genus: A continuous fight between lumpers and splitters. *Zbornik Matice srpske za prirodne nauke*, (117), 7-13.

Morgan, M. K., Fitzgerald S. D., Rottinghaus, G. E., Bursian, S. J. & Aulerich, R. J. (1999). Toxic effects to mink of moniliformin extracted from *Fusarium fujikuroi* culture material. *Veterinary and Human Toxicology* 1(1), 1-5.

Muhammad, H. K., Apeh, D. O., Muhammad, H. L., Olorunmowaju, Y. B., Ifeji, E. & Makun, H. A. (2019). Mycoflora of maize in Niger state, Nigeria. *Advanced Research in Life Sciences*, 3(1), 40-45.

Mukanga, M., Derera, J., Tongoona, P. & Laing, M. D. (2010). A survey of pre-harvest ear rot diseases of maize and associated mycotoxins in south and central Zambia. *International Journal of Food Microbiology*, 141(3), 213-221.

Mulunda, M., Dzoma, B., Nyirenda, M. & Bakunzi, F. (2013). Mycotoxins occurrence in selected staple food in main markets from Lubumbashi, Democratic Republic of Congo. *Journal of Food, Agriculture and Environment,* 11, 51-54.

Munkvold, G. P. (2017). *Fusarium* species and their associated mycotoxins. *Mycotoxigenic fungi*, 51-106.

Munkvold, G., Stahr, H. M., Logrieco, A., Moretti, A. & Ritieni, A. (1998). Occurrence of fusaproliferin and beauvericin in *Fusarium*-contaminated livestock feed in Iowa. *Applied and Environmental Microbiology*, 64(10), 3923-3926.

Murugesan, G. R., Ledoux, D. R., Naehrer, K., Berthiller, F., Applegate, T. J., Grenier, B., Phillips, T.D. & Schatzmayr, G. (2015). Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. *Poultry Science*, 94(6), 1298-1315.

Mwajita, M. R., Murage, H., Tani, A., Esther, M., Kahangi, E. M. & Makonde, H. M. (2014). Molecular characterization of bacteria and fungi from rice growing regions in Kenya. *International Journal of Biosciences*, 5, 7-14.

Nakagawa, H., Sakamoto, S., Sago, Y. & Nagashima, H. (2013). Detection of type A trichothecene di-glucosides produced in corn by high-resolution liquid chromatograhy-orbitrap mass spectrometry. *Toxins (Basel),* 5(3), 590-604.

Nathanail, A. V., Syvahuoko, J., Malachova, A., Jestoi, M., Varga, E., Michlmayr, H., Adam, G., Sievilainen, E., Berthiller, F. & Peltonen, K. (2015). Simultaneous determination of major type A and B trichothecenes, zearalenone and certain modified metabolites in Finnish cereal grains with a novel liquid chromatography-tandem mass spectrometric method. *Analytical and Bioanalytical Chemistry*, 407 (16), 4745-4755. doi: 10.1007/s00216-015-8676-4.

Neme, K. & Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies. A review. *Food Control*, 78, 412-425.

Nesic, K., Habschied, K. & Mastanjević, K. (2021). Possibilities for the Biological Control of Mycotoxins in Food and Feed. *Toxins,* 13(3), 198.

Nicolardot, B., Bouziri, L., Bastian, F. & Ranjard, L. (2007). A microcosm experiment to evaluate the influence of location and quality of plant residues on residue decomposition and genetic structure of soil microbial communities. *Soil Biology and Biochemistry*, 39, 1631-1644.

Nikiema, P. N., Worrillow, L., Traore, A. S., Wild, C. P. & Turner*, P. C. (2004). Fumonisin contamination of maize in Burkina Faso, West Africa. *Food Additives and Contaminants*, 21(9), 865-870.

Njobeh, P. B., Dutton, M. F., Koch, S. H., Chuturgoon, A. A., Stoev, S. D. & Mosonik, J. S. (2010). Simultaneous occurrence of mycotoxins in human food commodities from Cameroon. *Mycotoxin Research*, 26(1), 47-57.

Njobeh, P. B., Dutton, M. F., Koch, S. H., Chuturgoon, A., Stoev, S. & Seifert, K. (2009). Contamination with storage fungi of human food from Cameroon. *International Journal of Food Microbiology*, 135(3), 193-198.

Nucci, M. & Anaissie, E. (2007). *Fusarium* infections in immuno-compromised patients. *Clinical Microbiology Reviews*, 20 (4), 695-704.

Nwogu, E. O. & Nwankwo, F. I. (1979). A survey of the quality of yellow maize and white maize sold in Port Harcout markets. *NSPRI Technical Report,* 9, 83-85.

O'Riordan, M. J. & Wilkinson, M. G. (2008). A survey of the incidence and level of aflatoxin contamination in a range of imported spice preparations on the Irish retail market. *Food Chemistry*, 107(4), 1429-1435.

Odusanya, R. (2018). Maize production in Nigeria: Is it profitable in 2018? Retrieved from https: // www.legit.ng/1152358-Maize production-nigeria-profitable-2018. Html.

Ogara, I. M., Zarafi, A. B., Alabi, O., Banwo, O., Ezekiel, C. N., Warth, B., Sulyok, M. & Krska, R. (2017). Mycotoxin patterns in ear rot infected maize: A comprehensive case study in Nigeria. *Food Control*, 73, 1159-1168.

Ohara, T. & Tsuge, T. (2004). FoSTUA, encoding a basic helix-loop-helix protein, differentially regulates development of three kinds of asexual spores, macroconidia, microconidia, and chlamydospores, in the fungal plant pathogen *Fusarium oxysporum*. *Eukaryotic cell*, 3(6), 1412-1422.

Okechukwu, U. P. C., Okwesili, N. F. C., Parker, J. E., Abubakar, B., Emmanuel, O. C., Christian, O. E. & Celestine, A. A. (2013). Anti-nutrients survey of three maize varieties consumed in Abakaliki metropolis Nigeria. *International Journal of Pharma Medicine and Biological Sciences*, 2(2), 12-17.

Oladapo, A., Adepeju, A., Akinyele, A. & Adepeju, D. (2017). The proximate, functional and anti-nutritional properties of three selected varieties of maize (yellow, white and popcorn) flour. *International Journal of Science,* 1, 23-26.

Ominski, K. H., Marquardt, R. R., Sinha, R. N. & Abramson, D. (1994). Ecological aspects of growth and mycotoxin production by storage fungi. Mycotoxin in grains: Compounds other than aflatoxin. Eagan press, USA, 287-305.

Ono, E. Y. S., Moreno, E. C., Ono, M. A., Rossi, C. N., Saito, G. H., Vizoni, É., Sugiura, Y. & Hirooka, E. Y. (2011). Effect of cropping systems and crop successions on fumonisin levels in corn from Northern Paraná State, Brazil. *European Journal of Plant Pathology*, 131, 653-660.

Onyedum, S. C., Adefolalu, F. S., Muhammad, H. L., Apeh, D. O., Agada, M. S., Imienwanrin, M. R. & Makun, H. A. (2020). Occurrence of major mycotoxins and their dietary exposure in North-Central Nigeria staples. *Scientific African*, 7, 00188.

Oranusi, S. U. & Olarewaju, S. A. (2013). Mycoflora and aflatoxin contamination of some foodstuffs. *International Journal of Biotechnology and Allied Fields (IJBAF)*, 1(1), 9-18.

Ostry, V., Malir, F., Toman, J. & Grosse, Y. (2017). Mycotoxin as human carcinogens-the IARC monographs classification. *Mycotoxin Research*, 33(1), 65-73.

Ouanes, Z., Ayed-Boussema, I., Baati, T., Creppy, E. E. & Bacha, H. (2005). Zearalenone induces chromosome aberrations in mouse bone marrow: preventive effect of 17-β-estradiol, progesterone and vitamin E. *Genetic Toxicology and Environmental Mutagenesis*, 565, 139-149.

Ouanes-Ben Othmen, Z., Essefi, S. & Bacha, H. (2008). Mutagenic and epigenetic mechanisms of zearalenone: Prevention by Vitamin E. *World Mycotoxin Journal*, 1(3), 369-374.

Palacios, S. A., Del Canto, A., Erazo, J. & Torres, A. M. (2021). *Fusarium cerealis* causing *Fusarium* head blight of durum wheat and its associated mycotoxins. *International Journal of Food Microbiology*, 109161.

Palacios-Rojas, N., McCulley, L., Kaeppler, M., Titcomb, T. J., Gunaratna, N. S., Lopez-Ridaura, S. & Tanumihardjo, S. A. (2020). Mining maize diversity and improving its nutritional aspects within agro-food systems. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 1809-1834.

Parkin, D. M. (2006). The global health burden of infection-associated cancers in the year 2002. *International journal of cancer*, 118(12), 3030-3044.

Patriarca, A., da Cruz Cabral, L., Pavicich, M. A., Nielsen, K. F. & Andersen, B. (2019). Secondary metabolite profiles of small-spored *Alternaria* support the new phylogenetic organization of the genus. *International Journal of Food Microbiology*, 291, 135-143.

Patterson, D. S. & Roberts, B. A. (1979). Mycotoxins in animal feedstuffs: Sensitive thin layer chromatographic detection of aflatoxin, ochratoxin A, sterigmatocystin, zearalenone, and T-2 toxin. *Journal of the Association of Official Analytical Chemists*, 62(6), 1265-1267.

Peigné, J., Messmer, M., Aveline, A., Berner, A., Mäder, P., Carcea, M., Narducci, V. Samson, M. F., Thomsen, I. K. & Celette, F. (2013). Wheat yield and quality as influenced by reduced tillage in organic farming. *Organic Agriculture*, 4, 1–13.

Peraica, M., Radić, B., Lucić, A., & Pavlović, M. (1999). Toxic effects of mycotoxins in humans. *Bulletin of the World Health Organization*, 77(9), 754.

Perrone, G. & Susca, A. (2017). *Penicillium* species and their associated mycotoxins. Mycotoxigenic fungi, 107-119.

Pestka, J. J. & Smolinski, A. T. (2005). Deoxynivalenol: Toxicology and potential effects on humans. *Journal of Toxicology and Environmental Health*, Part B, 8(1), 39-69.

Pestka, J. J. (2007). Deoxynivalenol: Toxicity, mechanisms and animal health risks. *Animal Feed Science Technology*, 137, 283-298.

Pestka, J. J., Zhou, H. R., Moon, Y. & Chung, Y. J. (2004). Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. *Toxicology letters*, 153(1), 61-73.

Pfliegler, W. P., Pusztahelyi, T. & Pócsi, I. (2015). Mycotoxins–prevention and decontamination by yeasts. *Journal of Basic Microbiology*, 55(7), 805-818.

Pfohl-Leszkowicz, A., Petkova-Bocharova, T., Chernozemsky, I. N. & Castegnaro, M. (2002). Balkan endemic nephropathy and associated urinary tract tumours: a review on aetiological causes and the potential role of mycotoxins. *Food Additives & Contaminants*, 19(3), 282-302.

Pfordt, A., Ramos Romero, L., Schiwek, S., Karlovsky, P. & von Tiedemann, A. (2020). Impact of environmental conditions and agronomic practices on the prevalence of *Fusarium* species associated with ear-and stalk rot in maize. *Pathogens*, 9(3), 236.

Phoku, J. Z., Dutton, M. F., Njobeh, P. B., Mwanza, M., Egbuta, M. A. & Chilaka, C. A. (2012). *Fusarium* infection of maize and maize-based products and exposure of a rural population to fumonisin B_1 in Limpopo Province, South Africa. *Food Additives & Contaminants: Part A*, 29(11), 1743-1751.

Piacenti, K. C., Rocha, L. O., Savi, G. D., Carnielli-Queiroz, L., Fontes, L. D. & Correa, B. (2019). Assessment of toxigenic *Fusarium* species and their mycotoxins in brewing barley grains. *Toxins*, 11(31), 1 - 14.

Piemontese, L., Messia, M. C., Marconi, E., Falasca, L., Zivoli, R., Gambacorta, L. & Solfrizzo, M. (2018). Effect of gaseous ozone treatments on DON, microbial contaminants and technological parameters of wheat and semolina. *Food Additives & Contaminants*: Part A, 35(4), 761-772.

Pinotti, L., Ottoboni, M., Giromini, C., Dell' ortho, V. & Cheli, F. (2016). Mycotoxin contamination in the EU feed supply chain: A focus on cereal by products. *Toxins*, 8(2), 45. doi: 10.3390/toxins8020045.

Pitt, J. I. & Hocking, A. D. (2009). Primary keys and miscellaneous fungi. In *Fungi and food spoilage*, 53-143. Springer, Boston, MA.

Pitt, J. I., Wild, C., Baan, R., Gelderblom, W., Miller, J. D., Riley, R. T. & Wu, F. (2012). Improving Public Health through Mycotoxin Control: Chapter 3 - sampling and sample preparation methods for determining concentrations of mycotoxins in foods and feeds. *IARC Scientific Publications*, 158, 39–51

Placinta, C. M., D'Mello, J. F. & Macdonald, A. M. C. (1999). A review of worldwide contamination of cereal grains and animal feed with *Fusarium mycotoxins*. Animal feed science and technology, 78(1-2), 21-37.

Pleadin, J., Vahčić, N., Perši, N., Ševelj, D., Markov, K. & Frece, J. (2013). Fusarium mycotoxins' occurrence in cereals harvested from Croatian fields. *Food control*, 32(1), 49-54.

Ponce-García, N., Ortíz-Islas, S., García-Lara, S. & Serna-Saldivar, S. O. (2020). Physical and chemical parameters, *Fusarium verticillioides* growth and fumonisin production in kernels of nine maize genotypes. *Journal of Cereal Science*, 96, 103128.

Prosperini, A., B errada, H., Ruiz, M. J., Caloni, F., Coccini, T., Spicer, L. J., Perego, M. C. & Lafranconi, A. (2017). A review of the mycotoxin enniatin B. *Frontiers in Public Health*, 5, 304. doi: 10.3389/fpubh.2017.00304.

Prosperini, A., Meca, G., Font, G. & Ruiz, M. J. (2012). Study of the cytotoxic activity of beauvericin and fusaproliferin and bioavailability in vitro on Caco-2 cells. *Food Chemistry and Toxicology*, 50, 2356-2361.

Raja, H. A. Baker, T. R. Little, J. G. & Oberlies, N. H. (2017). DNA barcoding for identification of consumer-relevant mushrooms: A partial solution for product identification. *Food Chemistry*, 214, 383-392.

Rao, V. K., Ramana, M. V., Girisham, S. & Reddy, S. M. (2013). Culture media and factors influencing ochratoxin A production by two species of *Penicillium* isolated from poultry feeds. *National Academy Science Letters*, 36(1), 101-110.

Ratnadass, A., Marley, P. S., Hamada, M. A., Ajayi, O., Cissé, B., Assamoi, F. & Tenkouano, A. (2003). Sorghum head-bugs and grain molds in West and Central Africa: I. Host plant resistance and bug–mold interactions on sorghum grains. *Crop Protection*, 22(6), 837-851.

Reddy, K. R. N. & Salleh, B. (2011). Co-occurrence of moulds and mycotoxins in corn grains used for animal feeds in Malaysia. *Journal of Animal and Veterinary Advamces*, 10(5), 668-673.

Reddy, K. R. N., Reddy, C. S., Mangala, U. N. & Muralidharan, K. (2006). Site of infection of *Aspergillus* species in seeds of rice cultivars. *Journal of Mycology and Plant Pathology*, 36, 271-277.

Reddy, K. R. N., Salleh, B., Saad, B., Abbas, H. K., Abel C. A. & Shier, W. T. (2010). An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Reviews*, 29(1), 3-26.

Ritieni, A., Monti, S. M., Randazzo, G., Logrieco, A., Moretti, A., Peluso, G., Ferracane, R. & Fogliano, V. (1997). Teratogenic effects of fusaproliferin on chicken embryos. *Journal of Agriculture and Food Chemistry*, 45, 3039-3043.

Rodrigues, T. T. M. S., Berbee, M. L., Simmons, E. G., Cardoso, C. R., Reis, A., Maffia, L. A. & Mizubuti, E. S. G. (2010). First report of *Alternaria tomatophila* and *A. grandis* causing early blight on tomato and potato in Brazil. *New Disease Reports*, 22, 28-28.

Rodriguez-Carrasco, Y., José Ruiz, M., Font, G. & Berrada, H. (2013). Exposure estimates to *Fusarium* mycotoxins through cereals intake. *Chemosphere,* 93, 2297-2303.

Rossi, V., Scandolara, A. & Battilani, P. (2009). Effect of environmental conditions on spore production by *Fusarium verticillioides*, the causal agent of maize ear rot. *European Journal of Plant Pathology*, 123, 159-169.

Russell, L., Cox, D. F., Larsen, G., Bodwell, K. & Nelson, C. E. (1991). Incidence of molds and mycotoxins in commercial animal feed mills in seven Midwestern states 1988-1989. *Journal of Animal Science*, 69(1), 5-12.

Sadiq, M. S., Yakasai, M. T., Ahmad, M. M., Lapkene, T. Y. & Abubakar, M. (2013). Profitability and production efficiency of small-scale maize production in Niger State, Nigeria. *IOSR Journal of Applied Physics*, 3(4), 19-23.

Salamini, F., Özkan, H., Brandolini, A., Schäfer-Pregl, R. & Martin, W. (2002). Genetics and geography of wild cereal domestication in the near east. *Nature Reviews Genetics*, 3(6), 429-441.

Saleem, M. J., Bajwa, R., Hannan, A. & Qasier, T. A. (2012). Maize seed storage mycoflora in Pakistan and its chemical control. *Pakistan Journal of Botany*, 44(2), 807-812.

Sandermann, Jr. H. (1992). Plant metabolism of xenobiotics. *Trends in Biochemical Sciences*, 17(2), 82-84.

Sandermann, Jr. H. (1994). Higher plant metabolism of xenobiotics: "The green liver" concept. *Pharmacogenetics,* 4(5), 225-241.

Sandermann, Jr. H. (2004). Bound and unextractable pesticidal plant residues: Chemical characterization and consumer exposure. *Pest Management Science: Formerly Pesticide Science*, 60(7), 613-623.

Sangare-Tigori, B., Moukha, S., Kouadio, H. J., Betbeder, A. M., Dano, D. S. & Creppy, E. E. (2006). Co-occurrence of aflatoxin B_1, fumonisin B_1, ochratoxin A and zearalenone in cereals and peanuts from Côte d'Ivoire. *Food additives and contaminants*, 23(10), 1000-1007.

Sanhueza, C. E. P. & Degrossi, M. C. (2004). Morniliformin, a *Fusarium* mycotoxin. *Review Mexican Mycology*, 19, 103-112.

Santini, A., Meca, G., Uhlig, S. & Ritieni, A. (2012). Fusaproliferin, beauvericin and enniatins: Occurrence in food-A review. *World Mycotoxin Journal*. 5(1), 71-81. doi:10.3920/wmj2011.1331.

Santos Alexandre, A. P., Vela-Paredes, R. S., Santos, A. S., Costa, N. S., Canniatti-Brazaca, S. G., Calori-Domingues, M. A. & Augusto, P. E. D. (2018). Ozone treatment to reduce deoxynivalenol (DON) and zearalenone (ZEN) contamination in wheat bran and its impact on nutritional quality. *Food Additives & Contaminants:* Part A, 35(6), 1189-1199.

Santos, A. C. D. S., Trindade, J. V. C., Lima, C. S., Barbosa, R. D. N., da Costa, A. F., Tiago, P. V. & de Oliveira, N. T. (2019). Morphology, phylogeny, and sexual stage of *Fusarium caatingaense* and *Fusarium pernambucanum*, new species of the *Fusarium incarnatum-equiseti* species complex associated with insects in Brazil. *Mycologia*, 111(2), 244-259.

Schelstraete, W., Devreese, M. & Croubels, S. (2020). Comparative toxicokinetics of *Fusarium* mycotoxins in pigs and humans. *Food and Chemical Toxicology*, 137, 111140.

Schertz, H., Dänicke, S., Frahm, J., Schatzmayr, D., Dohnal, I., Bichl, G., Schwartz-Zimmermann, H. E., Colicchia, S., Breves, G., Teifke, J. P. & Kluess, J. (2018). Biomarker evaluation and toxic effects of an acute oral and systemic fumonisin exposure of pigs with a special focus on dietary fumonisin esterase supplementation. *Toxins,* 10(7), 296.

Schneweis, I., Meyer, K., Engelhardt, G. & Bauer, J. (2002). Occurrence of zearalenone-4-β-D-glucopyranoside in wheat. *Journal of Agricultural and Food Chemistry,* 50 (6), 1736-1738. doi: 10.1021/jf010802t.

Schollenberger, M., Muller, H. M., Rufle, M., Suchy, S. & Drochner, W. (2008). Redistribution of 16 *Fusarium* toxins during commercial dry milling of maize. *Cereal Chemistry*, 85, 557-560.

Schoustra, S. E., Debets, A. J., Rijs, A. J., Zhang, J., Snelders, E., Leendertse, P. C., Melchers, W.J., Rietveld, A.G., Zwaan, B. J. & Verweij, P. E. (2019). Environmental hotspots for azole resistance selection of *Aspergillus fumigatus*, the Netherlands. *Emerging Infectious Diseases*, 25(7), 1347.

Scientific Committee on Food. (1999). Opinion on *Fusarium* toxins Part1: Deoxynivalenol (DON). *Retrieved from http://ec.europa.eu/food/fs/scf/out44-en.pdf.*

Scudamore, K. A. & Patel, S. (2000). Survey for aflatoxins, ochratoxin A, zearalenone and fumonisins in maize imported into the United Kingdom. *Food Additives & Contaminants*, 17(5), 407-416.

Sebastià, N., Meca, G., Soriano, J. M. & Mañes, J. (2012). Presence of *Fusarium* emerging mycotoxins in tiger-nuts commercialized in Spain. *Food Control*, 25, 631-635.

Sengupta, A. & Dick, W. A. (2015). Bacterial community diversity in soil under two tillage practices as determined by pyrosequencing. *Microbiological Ecology*, 70, 853-859.

Seo, J. A., Proctor, R. H. & Plattner, R. D. (2001). Characterization of four clustered and coregulated genes associated with fumonisin biosynthesis in *Fusarium verticillioides*. *Fungal Genetics and Biology*, 34(3), 155-165.

Sergent, T., Ribonnet, L., Kolosova, A., Garsou, S., Schaut, A., De Saeger, S., Van Peteghem, C., Larondelle, Y., Pussemier, L. & Schneider, Y. J. (2008). Molecular and cellular effects of food contaminants and secondary plant components and their plausible interactions at the intestinal level. *Food and Chemical Toxicology*, 46(3), 813-841.

Sewald, N., von Gleissenthall, J. L., Schuster, M., Muller, G. & Aplin, R. T. (1992). Structure elucidation of a plant metabolite of 4-desoxynivalenol. *Tetrahedron-Asymmetry*, 3 (7), 953-960. doi: 10.1016/ S0957-4166(00)82193-X.

Shank, R. A., Foroud, N. A., Hazendonk, P., Eudes, F. & Blackwell, B. A. (2011). Current and future experimental strategies for structural analysis of trichothecene mycotoxins-A prospectus. *Toxins* 3, 1518-1553.

Sharma, R., Thakur, R. P., Senthilvel, S., Nayak, S., Reddy, S. V., Rao, V. P. & Varshney, R. K. (2011). Identification and characterization of toxigenic *Fusaria* associated with sorghum grain mold complex in India. *Mycopathologia*, 171(3), 223-230.

Shephard, G. S. (2008). Determination of mycotoxins in human foods. *Chemical Society Reviews*, 37(11), 2468-2477.

Shephard, G. S., Burger, H. M., Gambacorta, L., Krska, R., Powers, S. P., Rheeder, J. P., Solfrizzo, M., Sulyok, M., Visconti, A. Warth, B. & van der Westhuizen, L. (2013). Mycological analysis and multimycotoxins in maize from rural subsistence farmers in the former Transkei, South Africa. *Journal of Agriculture and Food Chemistry*, 61(34), 8232-8240.

Sherif, S. O., Salama, E. E. & Abdel-Wahhab, M. A. (2009). Mycotoxins and child health: The need for health risk assessment. *International journal of hygiene and environmental health*, 212(4), 347-368.

Shi, H., Li, S., Bai, Y., Prates, L. L., Lei, Y. & Yu, P. (2018). Mycotoxin contamination of food and feed in China: Occurrence, detection techniques, toxicological effects and advances in mitigation technologies. *Food Control*, 91, 202-215.

Simsek, S., Ovando-Martinez, M., Ozsisli, B., Whitney, K. & Ohm, J. B. (2013). Occurrence of deoxynivalenol and deoxynivalenol-3-glucoside in hard red spring wheat grown in the USA. *Toxins*, 5(12), 2656-2670.

Sirot, V., Fremy, J. M. & Leblanc, J. C. (2013). Dietary exposure to mycotoxins and health risk assessment in the second French total diet study. *Food and Chemical Toxicology*, 52, 1-11.

Smith, D. & Ryan, M. J. (2009). Fungal sources for new drug discovery. *Mc Graw-Hill Year book of Science and Technology*, 131-133.

Smith, M. C., Gheux, A., Coton, M., Madec, S., Hymery, N. & Coton, E. (2018). In vitro co-culture models to evaluate acute cytotoxicity of individual and combined mycotoxin exposures on Caco-2, THP-1 and HepaRG human cell lines. *Chemico-biological Interactions*, 281, 51-59.

Smith, M. C., Madec, S., Coton, E. & Hymery, N. (2016). Natural co-occurrence of mycotoxins in foods and feeds and their in vitro combined toxicological effects. *Toxins*, 8(4), 94.

Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L. & Kizek, R. (2010). Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology*, 3(3), 94-99.

Soliman, H. M. (2003). Mycoflora and mycotoxins of cereal grains in Delta, Egypt. *Mycobiology*, 31(4), 183-190.

Somda, I., Leth, V. & Sereme, P. (2007). Evaluation of lemongrass, eucalyptus and neem aqueous extracts for controlling seed-borne fungi of sorghum grown in Burkina Faso Publikation: Bidrag til tidsskrift› Tidsskriftartikel› Forskning.

Somma, S., Petruzzella, A. L., Logrieco, A. F., Meca, G., Cacciola, O. S. & Moretti, A. (2014). Phylogenetic analyses of *Fusarium graminearum* strains from cereals in Italy, and characterisation of their molecular and chemical chemotypes. *Crop and Pasture Science*, 65, 52-60.

Speijers, G. J. A. & Speijers, M. H. M. (2004). Combined toxic effects of mycotoxins. *Toxicology letters*, 153(1), 91-98.

Sreenivasa, M., Dass, R. & Janardhana, G. (2010). Survey of postharvest fungi associated with sorghum grains produced in Karnataka (India). *Journal of Plant Protection Research*, 50(3), 335-339.

Ssepuuya, G., Van Poucke, C., Ediage, E. N., Mulholland, C., Tritscher, A., Verger, P. & De Saeger, S. (2018). Mycotoxin contamination of sorghum and its contribution to human dietary exposure in four sub-Saharan countries. *Food Additives & Contaminants: Part A*, 35(7), 1384-1393.

Stanciu, O., Juan, C., Miere, D., Dumitrescu, A., Bodoki, E., Loghin, F. & Mañes, J. (2017). Climatic conditions influence emerging mycotoxin presence in wheat grown in Romania– A 2-year Survey. *Crop protection*, 100, 124-133.

Stockmann-Juvala, H. & Savolainen, K. (2008). A review of the toxic effects and mechanisms of action of fumonisin B_1. *Human & Experimental Toxicology*, 27(11), 799-809.

Stoev, S. D., Diakov, L., Koynarski, V. & Angelov, A. (2010). Special pathology and diagnostics of mycoses, mycotoxicoses, parasitoses, intoxications and avitaminoses; Publishing House CD Contrast, *Stara Zagora, Bulgaria*, 1-239.

Stössel, P. (1986). Aflatoxin contamination in soybeans: Role of proteinase inhibitors, zinc availability, and seed coat integrity. *Applied and Environmental Microbiology*, 52(1), 68-72.

Sudakin, D. L. (2003). Trichothecenes in the environment: Relevance to human health. *Toxicology Letters*, 143, 97–107.

Sulyok, M., Berthiller, F., Krska, R. & Schuhmacher, R. (2006). Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Communication Mass Spectrometry*. 20, 2649-2659.

Summerell, B. A., Laurence, M. H., Liew, E. C. & Leslie, J. F. (2010). Biogeography and phylogeography of Fusarium: A review. *Fungal Diversity*, 44(1), 3-13.

Sun, G., Wang, S., Hu, X., Su, J., Huang, T., Yu, J., Tang, L., Gao, W. & Wang, J. S. (2007). Fumonisin B_1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. *Food Additives and Contaminants*, 24(2), 181-185.

Sun, G., Wang, S., Hu, X., Su, J., Zhang, Y., Xie, Y., Zhang, H., Tang, L. & Wang, J.S., (2011). Co-contamination of aflatoxin B_1 and fumonisin B_1 in food and human dietary exposure in three areas of China. *Food Additives and Contaminants*. 28, 461-470.

Sy-Cordero, A. A., Pearce, C. J. & Oberlies, N. H. (2012). Revisiting the enniatins: A review of their isolation, biosynthesis, structure determination and biological activities. *Journal of Antibiotics*, 65, 541-549.

Sydenham, E. W., Thiel, P. G., Marasas, W. F.O., Shephard, G. S., Van Schalkwyk, D. J. & Koch, K. R. (1990). Natural occurrence of some *Fusarium* mycotoxins in corn from low and high esophageal cancer prevalence frequency areas of the Transkei, *Southern African Journal of Agriculture and Food Chemistry*, 38, 1900-1903.

Szécsi, Á., Szekeres, A., Bartók, T., Oros, G., Bartók, M. & Mesterházy, Á. (2010). Fumonisin B1-4-producing capacity of Hungarian *Fusarium verticillioides* isolates. *World Mycotoxin Journal*, 3(1), 67-76.

Taniwaki, M. H., Pitt, J. I. & Magan, N. (2018). *Aspergillus* species and mycotoxins: Occurrence and importance in major food commodities. *Current Opinion in Food Science*, 23, 38-43.

Tao, Y., Xie, S., Xu, F., Liu, A., Wang, Y., Chen, D. & Yuan, Z. (2018). Ochratoxin A: Toxicity, oxidative stress and metabolism. *Food and Chemical Toxicology*, 112, 320-331.

Tatay, E., Espín, S., García-Fernández, A. J. & Ruiz, M. J. (2018). Estrogenic activity of zearalenone, α-zearalenol and β-zearalenol assessed using the E-screen assay in MCF-7 cells. *Toxicology Mechanisms and Methods*, 28(4), 239-242.

Ten Berge, H. F., Hijbeek, R., van Loon, M. P., Rurinda, J., Tesfaye, K., Zingore, S. Craufurd, P., van Heerwaarden, J., Brentrup, F., Schröder, J. J. & van Ittersum, M. K. (2019). Maize crop nutrient input requirements for food security in sub-Saharan Africa. *Global Food Security*, 23, 9-21.

Thiel, P. G., Meyer, C. J. & Marasas, W. F. O. (1982). Natural occurrence of moniliformin together with deoxynivalenol and zearalenone in Transkeian corn. *Journal of Agricultural and Food Chemistry*, 30, 308-317.

Tian, Y., Yu, D., Liu, N., Tang, Y., Yan, Z. & Wu, A. (2020). Confrontation assays and mycotoxin treatment reveal antagonistic activities of Trichoderma and the fate of Fusarium mycotoxins in microbial interaction. *Environmental Pollution*, 267, 115559.

Tima, H., Brückner, A., Mohácsi-Farkas, C. & Kiskó, G. (2016). *Fusarium* mycotoxins in cereals harvested from Hungarian fields. *Food Additives & Contaminants*: Part B, 9(2), 127-131.

Tollenson, W. H., Dooley, K. L., Sheldon, W. C., Thurman, J. D., Bucci, T. J. & Howard, P. C. (1996). The mycotoxin fumonisin induces apoptosis in cultured human cells and in livers and kidneys of rats. Fumonisin in food: Advances in experimental medicine and biology. *Plenum Press*, New York, 237-250.

Tolosa, J, Font, G., Mañes, J. & Ferrer, E. (2017). Mitigation of enniatins in edible fish tissues by thermal processes and identification of degradation products. *Food Chemistry and Toxicology*, 101:67-74. doi:10.1016/j.fct.2016.12.039.

Tonshin, A. A., Teplova, V. V., Andersson, M. A. & Salkinoja-Salonen, M. S. (2010). The *Fusarium* mycotoxins enniatins and beauvericin cause mitochondrial dysfunction by affecting the mitochondrial volume regulation, oxidative phosphorylation and ion homeostasis. *Toxicology*, 276(1), 49-57. doi:10.1016/j.tox.2010.07.001.

Tran, V. N., Viktorová, J. & Ruml, T. (2020). Mycotoxins: Biotransformation and bioavailability assessment using caco-2 cell monolayer. *Toxins*, 12(10), 628.

Udomkun, P., Nimo, A., Nagle, M., Müller, J., Vanlauwe, B. & Bandyopadhyay, R. (2017a). A review: Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application. *Food Control*, 76, 127-138. doi: http://doi.org/10.1016/j.foodcont.2017.01.008

Udomkun, P., Wiredu, A. N., Nagle, M., Bandyopadhyay, R., Müller, J. & Vanlauwe, B. (2017b). Mycotoxins in Sub-Saharan Africa: Present situation, socio-economic impact, awareness, and outlook. *Food Control*, 72, 110-122.

Ulger, T. G., Uçar, A., Çakıroğlu, F. P. & Yilmaz, S. (2020). Genotoxic effects of mycotoxins. *Toxicon*, 5-18.

United State Department of Agriculture (USDA), Foreign Agricultural Service. (2014). *Livestock and Poultry*: *World Market and Trade*.

Valle-Algarra, F. M., Mateo, E. M., Medina, A., Mateo, F., Gimeno-Adelantado, J. V. & Jiménez, M. (2009). Changes in ochratoxin A and type B trichothecenes contained in wheat flour during dough fermentation and bread-baking. *Food Additives and Contaminants*, 26(6), 896-906.

Van Der Fels-Klerx, H. J., Klemsdal, S., Hietaniemi, V., Lindblad, M., Ioannou-Kakouri, E. & Van Asselt, E. D. (2012). Mycotoxin contamination of cereal grain commodities in relation to climate in North West Europe. *Food Additives & Contaminants*: Part A, 29(10), 1581-1592.

Van Dongen, P. W. & de Groot, A. N. (1995). History of ergot alkaloids from ergotism to ergometrine. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 60(2), 109-116.

Vanderfels-Klerx, H. J. & Stratakou, I. (2010). T-2 toxin and HT-2 toxin in grain and grain- based commodities in Europe: Occurrence, factors affecting occurrence, co-occurrence and toxicological effects. *World Mycotoxin Journal*, 3(4), 349-367.

Vekiru, E., Fruhauf, S., Hametner, C., Schatzmayr, G., Krska, R., Moll, W. D. & Schuhmacher, R. (2016). Isolation and characterisation of enzymatic zearalenone hydrolysis reaction products. *World Mycotoxin Journal*, 9(3), 353-363.

Vidal, A., Morales, H., Sanchis, V., Ramos, A. J. & Marin, S. (2014). Stability of DON and OTA during the bread making process and determination of process and performance criteria. *Food Control*, 40, 234-242.

Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G., Seifert, K.A., Varga, J., Yaguchi, T. & Samson, R. A. (2014). Identification and nomenclature of the genus *Penicillium*. *Studies in Mycology*, 78, 343-371.

Visagie, C. M., Renaud, J. B., Burgess, K. M. N., Malloch, D. W., Clark, D., Ketch, L., Urb, M., Louis-Seize, G., Assabgui, R., Sumarah, M. W. & Seifert, K. A. (2016). Fifteen new species of *Penicillium*. Persoonia: *Molecular Phylogeny and Evolution of Fungi*, 36, 247.

Voss, K. A., Smith, G. W. & Haschek, W. M. (2007). Fumonisins: Toxicokinetics, mechanism of action and toxicity. *Animal Feed Science and Technology*, 137(3-4), 299-325.

Wagacha, J. M. & Muthomi, J. W. (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*, 124, 1-12.

Waing, K. G., Abella, E. A., Kalaw, S. P., Waing, F. P. & Galvez, C. T. (2015). Studies on biodiversity of leaf litter fungi of Central Luzon State University and evaluation of their enzyme producing ability. *Current Research in Environmental & Applied Mycology*, 5(3), 269-276.

Wang, D. S., Liang, Y. X., Chau, N. T., Dien, L. D., Tanaka, T. & Ueno, Y. (1995). Natural co-occurrence of *Fusarium* toxins and aflatoxin B_1 in corn for feed in North Vietnam Nations. *Toxins*, 3, 445-449.

Waniska, R. D. (2000). Structure, phenolic compounds and antifungal proteins of sorghum caryopses. In technical and institutional options for sorghum grain mold management: *Proceedings of an International Consultation*, 18-19.

Warth, B., Parich, A., Atehnkeng, J., Bandyopadhyay, R., Schuhmacher, R., Sulyok, M. & Krska, R. (2012). Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/MS multitoxin method. *Journal of Agricultural and Food Chemistry*, 60(36), 9352-9363.

Wen, J., Mu, P. & Deng, Y. (2016). Mycotoxins: Cytotoxicity and biotransformation in animal cells. *Toxicology Research*, 5(2), 377-387.

Wild, C. P. & Gong, Y. Y. (2010). Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis*, 31(1), 71-82.

World Health Organisation (WHO) (2012). WHO Food Standards Programme. *Proceedings of the Sixth Session of CODEX Committee on Contaminants in Foods, Maastricht, The Netherlands*.

Woudenberg, J. H. C., Seidl, M. F., Groenewald, J. Z., De Vries, M., Stielow, J. B., Thomma, B. P. H. J. & Crous, P. W. (2015). *Alternaria* section *Alternaria*: Species, *formae speciales* or pathotypes?. *Studies in Mycology*, 82, 1-21.

Wu, F. (2007). Measuring the economic impacts of Fusarium toxins in animal feeds. *Animal Feed Science and Technology*, 137(3-4), 363-374.

Wu, F., Bhatnagar, D., Bui-Klimke, T., Carbone, I., Hellmich, R., Munkvold, G. & Takle, E. (2011). Climate change impacts on mycotoxin risks in US maize. *World Mycotoxin Journal*, 4(1), 79-93.

Wu, Q., Patocka, J. Nepovimova, E. & Kuca, K. (2018). A review on the synthesis and bioactivity aspects of beauvericin, a *Fusarium* mycotoxin. *Frontiers on Pharmacology*, 9, 1338. doi: 10.3389/fphar.2018.01338.

Wu, W., Flannery, B. M., Sugita-Konishi, Y., Watanabe, M., Zhang, H. & Pestka, J. J. (2012). Comparison of murine anorectic responses to the 8-ketotrichothecenes 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon X and nivalenol. *Food and Chemical Toxicology*, 50, 2056-2061.

Wu, Y., Li, X., Xiang, W., Zhu, C., Lin, Z., Wu, Y., Li, J., Pandravada, S., Ridder, D. D., Bai, G. & Wang, M. L. (2012). Presence of tannins in sorghum grains is conditioned by different natural alleles of Tannin1. *Proceedings of the National Academy of Sciences*, 109(26), 10281-10286.

Xing, F., Liu, X., Wang, L., Selvaraj, J. N., Jin, N., Wang, Y., Zhao, Y. & Liu, Y. (2017). Distribution and variation of fungi and major mycotoxins in pre-and post-nature drying maize in North China Plain. *Food control*, 80, 244-251.

Yakubu, A., Adamu, A. A. & Adaeze, E. (2018). Estimation of rainfall rate and rain attenuation for satellite communication in Imo, Osun and Niger State of Nigeria. *International Journal of Advanced Research*, 4(10), 157-166.

Yang, L., Yu, Z., Hou, J., Deng, Y., Zhou, Z., Zhao, Z. & Cui, J. (2016). Toxicity and oxidative stress induced by T-2 toxin and HT-2 toxin in broilers and broiler hepatocytes. *Food and Chemical Toxicology*, 87, 128-137.

Yazar, S. & Omurtag, G. Z. (2008). Fumonisins, trichothecenes and zearalenone in cereals. *International Journal of Molecular Science*, 9, 2062-2090.

Yazdanpanah, H., Zarghi, A., Shafaati, A. R., Foroutan, S. M., Aboul-Fathi, F., Khoddam, A. & Nazari, F. (2012). Exposure assessment of the Tehran population (Iran) to zearalenone mycotoxin. *Iranian Journal of Pharmaceutical Research: IJPR*, 11(1), 251.

Yılmaz, S. Ö. (2017). The contamination rate of aflatoxins in ground red peppers, dried figs, walnuts without shell and seedless black raisins commercialized in Sakarya City Center, Turkey. *Italian Journal of Food Science*, 29(4).

Yoshinari, T., Sakuda, S., Furihata, K., Furusawa, H., Ohnishi, T., Sugita-Konishi, Y., Ishizaki, N. & Terajima, J. (2014). Structural determination of a nivalenol glucoside and development of an analytical method for the simultaneous determination of nivalenol and deoxynivalenol and their glucosides, in wheat. *Journal of Agricultural and Food Chemistry*, 62 (5), 1174-1180. doi: 10.1021/jf4048644.

Yoshinari, T., Suzuki, Y., Sugita-Konishi, Y., Ohnishi, T. & Terajima, J. (2016). Occurrence of beauvericin and enniatins in wheat flour and corn grits on the Japanese market, and their co-contamination with type B trichothecene mycotoxins. *Food Additives & Contaminants*: Part A, 33(10), 1620-1626.

Zachetti, V. G., Cendoya, E., Nichea, M. J., Chulze, S. N. & Ramirez, M. L. (2019). Preliminary study on the use of chitosan as an eco-friendly alternative to control *Fusarium* growth and mycotoxin production on maize and wheat. *Pathogens*, 8(1), 29.

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, 15(2), 129-144.

Zhang, Z., Nie, D., Fan, K., Yang, J., Guo, W., Meng, J. & Han, Z. (2019). A systematic review of plant-conjugated masked mycotoxins: Occurrence, toxicology, and metabolism. *Critical Reviews in Food Science and Nutrition*, 1-15.

Zheng, W., Wang, B., Li, X., Wang, T., Zou, H., Gu, J., Yuan, Y., Liu, X., Bai, J., Bian, J. & Liu Z. (2018). Zearalenone promotes cell proliferation or causes cell death? *Toxins*, 10(5), 184.

Zilic, S., Milasinovic, M., Terzic, D., Barac, M. & Ignjatovic-Micic, D. (2011). Grain characteristics and composition of maize specialty hybrids. *Spanish Journal of Agricultural Research*, (1), 230-241.

Zinedine, A., Fernández-Franzón, M., Mañes, J. & Manyes, L. (2017). Multi-mycotoxin contamination of couscous semolina commercialized in Morocco. *Food Chemistry*, 214, 440-446.

Zinedine, A., Soriano, J. M., Molto, J. C. & Mañes, J. (2007). Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chemistry and Toxicology*, 45, 1-18.

APPENDIX A

Department of Biochemistry, School of Life Sciences

Federal University of Technology, Minna Niger State

Title: Exposure Studies and Risk Characterization of Human Exposure to Fusarium Mycotoxins from Maize grown in Niger State

Research Questionnaire

In this questionnaire, there is no wrong or correct answer. What is required is just your input on maize consumption rate in Niger state. The outcome will assist in assessing the exposure rate to mycotoxin in maize grown in the state. This research questionnaire is in partial fulfilments of the requirements for the award of Masters of Technology (M.Tech) in Biochemistry, Federal University of Technology, Minna, Niger State.

Section A: General Information/Biodata

Please kindly tick and answer appropriately the questions below

Date: _____

Name of Respondent: _____

Name of Local Government: _____

Name of Village: _____

Gender/Sex: Male ☐ Female ☐

Weight _____

Age: Below 20 ☐ between 20-40 ☐ between 41-60 ☐ Above 60 ☐

Level of Education: No education ☐ Primary education ☐ Secondary education ☐

Tertiary education ☐ Non-formal education ☐

Marital Status: Married ☐ Single ☐

Size of Household: _____

Section B

Click whichever is applicable in the boxes

1. Do you consume maize grain? Yes ☐ No ☐
2. Do you consume boiled maize Yes ☐ No ☐

- If yes, how many boiled maize do you consume per day (household) _____

- If yes, how many boiled maize do you consume per day (individual) _____

3. Do you consume roasted maize Yes ☐ No ☐

- If yes, how many roasted maize do you consume per day (household) _____
- If yes, how many roasted maize do you consume per day (individual) _____

4. I consume tuwo made from maize Yes ☐ No ☐

- If yes, how often do you consume it?

  Daily ☐ Weekly ☐ Monthly ☐ Occasionally ☐

- If weekly or monthly, how many times /week(or month) _____
- How many cups of maize make a meal size (household)? _____
- How many cups of maize make a meal size (individual)? _____

5. Do you take corn flakes/Golden morn? Yes ☐ No ☐

- If yes, how often do you consume it?

  Daily ☐ Weekly ☐ Monthly ☐ Occasionally ☐

- If weekly or monthly, how many times /week(or month) _____

6. Do you consume other maize products? Yes ☐ No ☐

- If yes, specify _____
- How often?

  Daily ☐ Weekly ☐ Monthly ☐ Occasionally ☐

7. Are you aware that maize grains can be contaminated with fungi? Yes ☐ No ☐

- If yes, how do you identify fungi contaminated maize _____

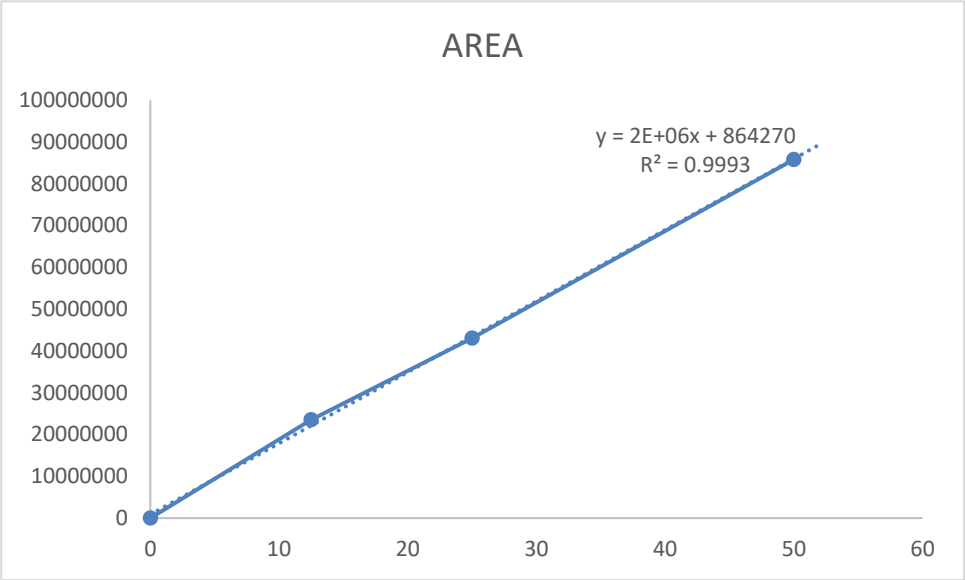
8. Are you aware of the effects/consequences of consuming fungi contaminated maize

   Yes ☐ No ☐

- If yes, what preventive measure can you recommend to prevent maize contamination by fungi

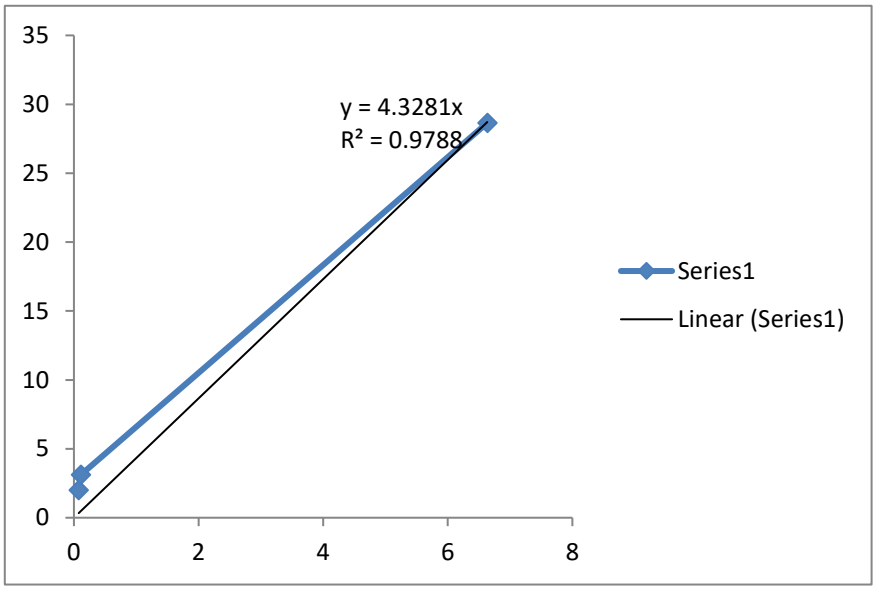
  _____

APPENDIX B



Caliberation of Nivalenol

APPENDIX C



Caliberation Curve of Deoxynivalenol