THE OCCURENCE OF OCHRATOXIN A AND CYCLOPIAZONIC ACID IN MAIZE AND SORGHUM GROWN IN MICROCLIMATIC ZONES OF NIGER STATE

ABSTRACT

Mycotoxigenic fungi and their associated mycotoxins remain a significant threat to growers, consumers, and food regulators. They damage the quality of agricultural crop commodities and impact negatively on food safety. The aim of the study was to determine the occurrence of *Penicillium* fungi and two mycotoxins in of maize and sorghum in Niger. Sixty-four (64) composite samples (CS) of different varieties of maize and sorghum were collected from stores and markets located in four microclimatic zones of Niger state. Standard procedure was used to isolate *Penicillium* spp. Ochratoxin A (OTA) and Cyclopiazonic acid (CPA) were extracted and quantified by Enzyme-Linked Immunosorbent Assay (ELISA) and High Performance Liquid Chromatography (HPLC) respectively. The risk assessment as a result of consumption was also determined. The fungi isolate from the samples were: *P. verrucosom*, *P. griseofulvum* and *P. chrysogenum*. The occurrence of *Penicillium spp* is highest in market maize samples (15.8 %) from wet zone and store samples in dry zone while sorghum had the highest prevalence (16.7 %) in stores sample from driest zone. The OTA was detected in 89.1 % of both maize and sorghum samples at level of 1.799– 75.462 μg/Kg and 0.297 – 49.344 μg/Kg respectively. The CPA was detected in 45.3 % of both maize and sorghum samples at levels of 1.002 – 419.248 μg/Kg and 0.205 – 79.981 μg/Kg respectively. The contamination of the samples collected with OTA had the highest concentration in yellow maize collected from Magama market with 75.462 μg/Kg while the lowest concentration was found in white sorghum collected in Suleja store with the concentration of 0.297 μg/Kg. The co-occurrence of OTA and CPA was high in Magama store with 75.0 % and lowest in Bosso market with 25.0 %. The Estimated Daily Intake (EDI) for male was 0.021 - 0.111 μg/Kg and was 0.020 - 0.423 μg/Kg for female and the Tolerable Daily Intake (TDI) for OTA was 0.014 μg/Kg. there is a health risk to consumers of maize and sorghum due to the contamination of the samples with OTA and CPA in these study areas. Hence, this study advise that consumers of maize and sorghum should ensure proper cooking to reduce the health risk posed by these contaminants.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Mycotoxins are ancillary extrolites of certain fungi with low molecular weight manufactured in agricultural products exposed to harsh climatic conditions and these substances are toxin of various degrees (Agriopoulou *et al.*, 2020). The production of toxin on crops by fungi is affected by atmospheric factors both in the field crops and stored crops. The contamination of mycotoxins in crops cannot be avoided and predicted, which present an exclusive challenge in the safety of food (Park and Stoloff, 1989; FAO, 1997). There is an increase in knowledge rate on mycotoxins and more discovery of mycotoxins co-contamination in food crops. Therefore, certain syndromes in animals are caused by mycotoxins which bears a strong relationship between the toxins and the resulted diseases (Gentles *et al.*, 1999). Even though specific evidences on the grounds of mycotoxins causing certain human diseases are inadequate, the potential risk of the dietary exposure in consumption of contaminated crops with mycotoxins should not be undermined. Ismaiel and Papenbrock (2015) reported that when the mycotoxins are present and there are no visible symptoms of a particularly disease, the plants physiology can still be slightly affected.

In 2017, the United Nation Population Division (UNPD) estimated a rise in world population by 2050 to be 9.8 billion people (Cumagun *et al.*, 2019). Though the available earth's resources, can basically cater for 10 billion people leading to swift diminution of such food supply leading to great shortage. Biotechnology's techniques employed in agriculture and crop sciences will help in upsurge of production of food crops through the refining of the varieties of crops which possess high-yielding, drought-tolerant, ability and crops that opposes

the infestation of pest and proliferation of diseases. Another approach in the reduction of food shortage is by circumvention of forfeiture of the yield of crop brought about by biotic and abiotic factors known to prevent the current food supply. In the framework of the safety and security of food crops, the worldwide deleterious effect of fungi that are toxigenic need to be taken seriously. The economics of such contaminated crops with these mycotoxins and also its negative health implication cannot be ignored. (Cumagun *et al.,* 2019). The present of these toxigenic fungi on cereals products have huge possible effect to the populace both economically and likewise in health (Milani, 2013). Fungi are plant pathogens that exist all around us which serve as a huge spoilage agent of foods and feedstuffs as indicated by Makun *et al*. (2010).

These toxins by fungi are produced by some genera of fungi such as *Penicillium*, Fusarium or Aspergillus that grow on cereals which include the following: wheat, maize, oat, sorghum, groundnuts, legumes likewise oilseeds. The production of mycotoxin by fungi are determined by varied conditions such as biotic factors, harvesting, storage and processing conditions and the most significant factors is the climate. The biosynthesis of toxins, particular on cereal crops, is extremely dependent on physical conditions (for instance humidity and suitable temperature) the crops are exposed to, before harvest and after harvest. Therefore, when alteration of weather conditions occurs, the generation of mycotoxins will be affected due to the fact that fungi are climate-dependent (Milani, 2013).

The fungi *Penicillium* is an enormous and most imperative genera of fungi among other microorganisms, with wide-reaching of more than 400 designated species. The name *Penicillium* is derived from "*Penicillus*" - Latin, and that denotes its conidiophores brush-like appearance with the likeness of a painter's brush (Yin *et al.,* 2017).

Penicillium is a very recognized and among the communal fungi found in a diverse range of habitats, which include the following: air, soil, environment within the house, food crops and vegetation. These fungi possess global distribution and a huge economic influence on the lives of human beings. Its main function in nature is the decomposition of organic materials, where species cause devastating rots as pre- and postharvest pathogens on food crops (Visagie *et al.*, 2014, Park *et al.*, 2019). The genus *Penicillium* is common in different environments such as soil, plant, air and food products. Some species can be used in cheese production, antibiotics production while others are pathogens for human. However, *Penicillium* is an important genus because of its commercial and industrial uses such as anti-tumoral, anti-fungal and anti-viral compounds, and in the production of enzymes (Kolanlarli *et al.*, 2019). They also synthesis a diversity of bioactive metabolites with suitable industrial application, which include antibacterial agents, mycotoxins, and enzymes (Park *et al.*, 2019).

Mycotoxins have triggered foremost epidemics among mankind and other animals in time past. The high ranking epidemics which include ergotism, which killed several thousands of people in Europe in the last millennium; alimentary toxic aleukia, which accounted for the death of not least than 100,000 people of Russia between 1942 - 1948; stachybotryotoxicosis in the USSR, lead to death of tens of thousands of horses in the 1930s; and aflatoxicosis in the United Kingdom (UK) , which lead to the fatality of 100,000 young turkeys in 1960 and also had resulted to casualties in other living organisms as well as man (Pitt, 2000). Ochratoxin A (OTA) is detected in grain crops, chiefly finely ground cereal grains. OTA is also detected in traded commodities such as solid food made from milk (cheese) and others animals' products which originated from the consumption of cereals contaminated with mycotoxins (Milani, 2013). Cyclopiazonic acid (CPA) occur naturally in groundnuts, maize, cheese, processed

products from tomato, and also animal products like poultry eggs, meat and milk of animals that were previously served by feeds contaminated with mycotoxins (Lavkor *et al.*, 2017). The co-occurrence of mycotoxin contamination of groundnut in Nigeria was reported to include CPA, moniliformin, aflatoxins (AFB1, AFB2, AFG1, AFG2 and AFM1), nivalenol, OTA and beauvericin. The samples with higher concentrations were detected with CPA above other mentioned mycotoxins (Oyedele *et al*., 2017 as cited by Ostry *et al.*, 2018).

Niger State has four microclimatic zone as follows: wettest zone (zone 1), wet zone (zone 2), dry zone (zone 3) and driest zone (zone 4) where cereal is cultivated. These climatic conditions can promote the infestation of fungi and lead to mycotoxins contamination in maize and sorghum commodities. Nigeria is the largest producer of Maize in Africa and Niger State is one of the five states leading in the maize production (Odusanya, 2018). On the other hand, Nigeria is the biggest producers of sorghum among the West Africa countries with about 71% and third-largest world producers of sorghum (OECD/FAO, 2019).

1.2 Statement of the Research Problem

The proliferation of mycotoxigenic fungi and the respective mycotoxins produce by such fungi stays a colossal hazard to growers, consumers, and regulators of food. They mutilate the essential property of crop products and badly effect the safety of food. The contamination of food crops and forage with mycotoxin is a problem that affect both Nigeria and much larger scale globally (Cumagun *et al.*, 2019).

Mycotoxins remain a crucial worldwide issue on food safety, even the Food Agriculture Organization (FAO) made a statement that crops products which are contaminated by fungi and mycotoxins with about 25 % will result to a lose in the economics of the crop amounting in one thousand million of dollars (FAO, 2004 as cited by Wolf and Schweigert, 2018).

Mycotoxins naturally occur in peanuts, sunflower seeds, Kodo millet, cheers and cereals like maize and sorghum. CPA is poisonous in diversity of living organisms with much consequences in human toxicity – Kodua poisoning (Ostryl *et al*., 2018). The aflatoxins and CPA co-occurred in agricultural products at the same time. For example, CPA was found to be present in aflatoxicosis, even though it might may be concealed, for instance, the peanut meal that caused Turkey "X" disease were isolated both with aflatoxins and CPA which had led to the loss of above 100,000 turkeys. It is the basis for tissues death in vital organs involving in metabolic process like the kidney and the liver (Lavkor *et al*., 2017).

Ochratoxin A (OTA) rank among the highly lethal to warm blooded vertebrate animals like mammals, providing a grounds for diverse lethal effects including toxicity on kidney (hepatotoxicity), agent affecting embryo or fetus (teratogenicity), and agents that cause mutation (mutagenicity), resulting in specific health disorder such as inflammation of liver, excessive bleeding, excessive fluid in body tissues, suppression of a healthy immune response, malignant tumor in the liver, carcinoma in the throat passage, and failure in the function of kidney (Santos *et al*., 2001 as cited by Reddy *et al*., 2010; Milani, 2013). The contact of people and other animals to food contaminated with mycotoxins is predominantly a tropical region problem (Reddy and Raghavender, 2008). To scrutinize the lethal consequences of OTA which is an acute toxin and CPA which is chronic toxins on human beings and other organisms, it is vital to appreciate the level of these mycotoxin contaminating foods globally so as to ascertain and carry out the needed supervision strategies. For that reason, the level of contaminations of OTA and CPA detected in the diversity of food crops nationally and globally require level of review, along with health implications in human health, together with a specific emphasis on tropical regions like Nigeria (Reddy *et al*., 2010). The synthesis of

OTA and CPA by *Penicillium spp* activities, results to crops contamination with such toxins in the course of storage within humid conditions. As long as the field crop turn out to be infested with the growth of fungal this growing situation will be maintained if the humidity and temperature favors the proliferation during storage of crops and processing of food (Reddy *et al*., 2010).

1.3 Justification for the Study

Presently, the account on the bid to measure the extent of CPA contamination on food crops are solely minimal. (Ostry *et al.,* 2018). There are no enough statistical reports on CPA occurrences in maize and sorghum grains in Niger. The problems of cereal contamination with mycotoxin by fungi can be controlled and monitored by careful commodity screening and provision of improved storage conditions (Lavkor *et al*., 2017). The cultivation of maize and sorghum in Niger state is highly favored among others cereal grains, this therefore prompt the need for the choice of these cereals for monitoring of mycotoxins contamination. Also, the literatures on the co-occurrences of OTA and CPA in maize as well as sorghum samples each is considered to be very low since *Penicillium spp* can produce these mycotoxins (Makun *et al*., 2010). The samples collected from stored and market are prompt to be infested by storage fungi, such as *Penicillium* and aspergillus and therefore the mycotoxins are produced (Adam and Moss, 1999). Therefore, co-occurrences of OTA and CPA will result to multiple health problems in the animals and human beings that feed on such contaminated cereals. The risk assessment on human being on the possibility of been at higher risk by multi-mycotoxins is not fully carried out due to inadequate food consumption data, and this study is providing such data for Niger state (Viegas *et al*., 2011).

1.4 Aim and Objectives

1.4.1 Aim

The aim of this study includes determining presence of *Penicillium* fungi, ochratoxin A and cyclopiazonic acid as detected in maize and sorghum from the four microclimatic regions of Niger State.

1.4.2 Objectives of the study

These objectives include, to:

i. Isolate and identify *Penicillium* species both morphologically and microscopically from the varieties of maize and sorghum grains.

ii. Analyse the naturally occurring OTA and CPA extracted from maize and sorghum using Enzyme-Linked Immunosorbent Assay (ELISA) and High Pressure Liquid Chromatography (HPLC).

iii. Assess the dietary exposure risk from the consumption of maize and sorghum from the. four microclimatic zones in Niger State.

iv. Determine the co-occurrence of OTA and CPA in maize and sorghum varieties from the study area.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fungi

Fungi are associated with an assorted collection of living things that differ to other plants and animals in the method of obtaining food through absorbing nourishment from outside the fungi organism. This organism ranges from miniature, single-celled organisms' invisible to the naked eye to some of the largest multicellular organisms. Fungi work a serious job in the biodegradable and recycling of minerals and carbon. The value mold to human race is immeasurable, however it is not the entire fungi that are useful, a number of them cause destruction of agricultural products, causing infection in living organisms and produce substances that are poisonous as detected in food substances. The toxins produce by fungi are called mycotoxins. The progressive growth of generally found filamentous fungi as detected in foods products may consequently lead to mycotoxins production (Cysewski, 1973; Pitt, 2000).

2.2 Toxigenic Fungi

The mycotoxins producing fungi are referred as toxigenic fungi, the growth of these fungi is control by substances that are worked upon in biochemical reaction stature (crop granule destruction and nutritional constituents), environment condition (humidity and varied degree of heat) and natural conditions (insect pests as well as microorganism) (Mannaa and Kim, 2017). Mycotoxigenic fungi importantly bring about the effect on the quality and quantity of agricultural commodities. These fungi play a crucial role on food safety and security on a global scale and therefore a major concern worldwide (Makun *et al.,* 2011a, Anjorin *et al.*, 2013, Balendres *et al*., 2019). Fungi are group according to the different phases of their

occurrence, which include: (i) field fungi – hygrophilic example – *Alternaria*, *Fusarium*, *Epicaccum*; (ii) intermediate fungi – mesophile example *Verticillium species*, *Gladosporium species*, *Geotricum species*; (iii) storage fungi – xerophile example *Aspergillus*, *Eurotium*, Penicillium species (Mannaa and Kim, 2016)

2.3 Storage Fungi

The two storage fungi genera that produce mycotoxins of significant importance include *Aspergillus* and *Penicillium* genus.

2.3.1 *Penicillium*

Penicillium genus is common in different environments such as soil, plant, air and food products. Some species can be used in the cheese production, also some of them are pathogens for human (Mahomed *et al.*, 2016); in addition, some species can use for antibiotics production. Besides, *Penicillium* is an important genus because of commercial and industrial uses such as production of anti-tumoral, anti-fungal and anti-viral compounds, and using in production of extra cellular enzymes (Houbraken *et al*., 2014).

The classification of the species of *Penicillium*, concentrate on their rate of growth as well as colonies size on standardized media; color and texture of colonies; conidiophore structure, together with divergence pattern and shapes, dimensions, and ornamentations of the various section of the conidiophore; and the synthesis of particular metabolites like enzymes, antibiotics, mycotoxins and pigments (Martinez-Blanco *et al.,* 1992, Houbraken *et al*., 2014; Visagie *et al.,* 2014; Refai *et al.,* 2015; Guohua *et al.,* 2017; Yin *et al*., 2017). Below is the microscopy result of *Penicillium* spp from the grain samples:

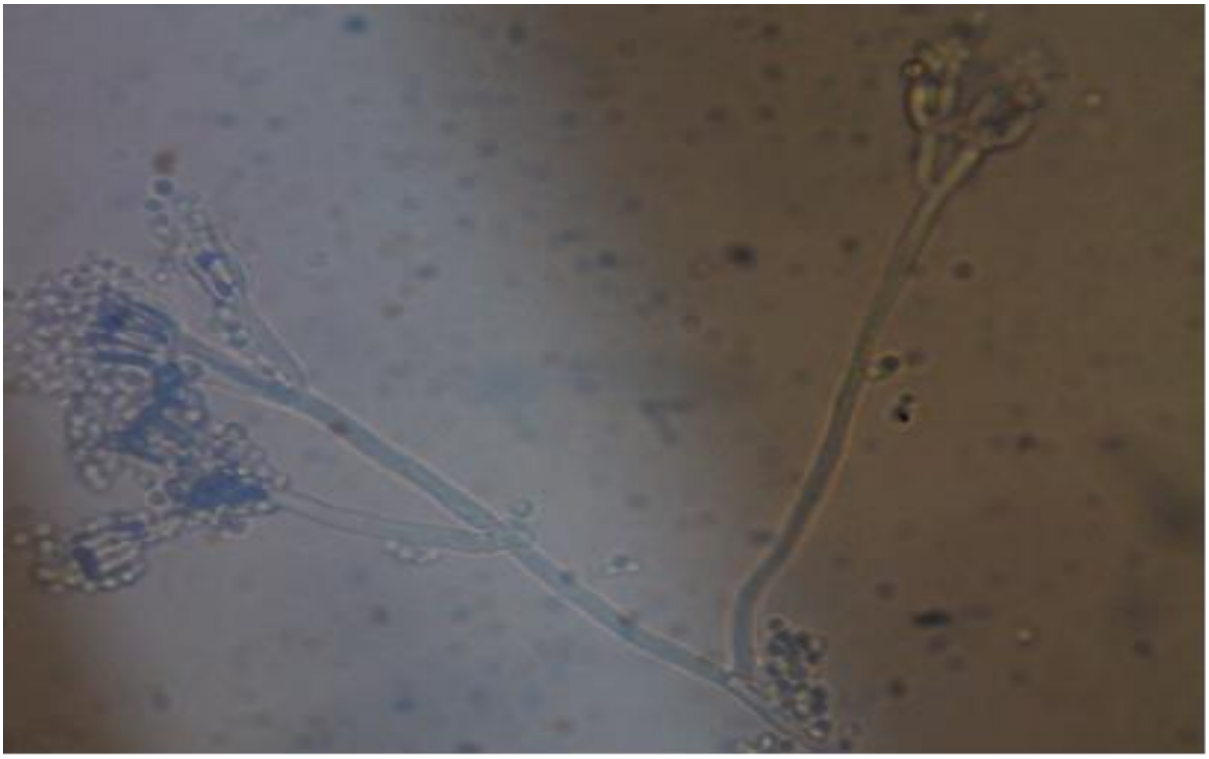


Plate 1.1: *Penicillium* spp microscopy of sample

Source: Martinez-Blanco *et al.* (1992)

The *Penicillium* species are responsible for the synthesis of ochratoxin A and cyclopiazonic acid. They produces quite a number of mycotoxins which include Ochratoxin A and Cyclopiazonic acid, others include gliotoxin, patulin, secalonic acid D, citrinin, citroviridin, verraculogen and emodin (Makun *et al*., 2009, Refai *et al*., 2015, Tugba *et al.,* 2019). Ochratoxin A is produced by *Penicillium verrucosom* and *Penicillium nordicum* while Cyclopiazonic acid is produce by *P. palitans, P. camemberti, P. dipodomyicola, P. commune* and *P. griseofulvum* (Refai *et al*., 2015, Tugba *et al.,* 2019)

2.3.2 Aspergillus

Aspergilli are the most common fungal species that can produce mycotoxins in seeds, food and feedstuffs. Several outbreaks of mycotoxicoses diseases in humans and animals caused by various mycotoxins have been reported after the consumption of mycotoxin contaminated food and feed (Anjorin *et al.,* 2013). In the time since its discovery many of the fungi in the genera *Aspergillus* and *Penicillium* have been found to produce this OTA and CPA which are frequently produced by some of the same fungi that produce the better known, and hepatocarcinogenic, aflatoxins (Maragos, 2018). These two large groups of mycotoxins that have come into the focus of scientific interest for their demonstrated or suspected direct and/or indirect harmful effects on human health: aflatoxins (G1, B1, and M1) and ochratoxins (A, B, and C) are all synthesis by *Aspergillus* spp (Puntaric *et al.,* 2001). Stored maize is associated with a large number of fungal species belonging to the genders of *Aspergillus* and *Penicillium*. Concern about mycotoxins occurrence in maize are usually associated with *Aspergillus flavus* for production of aflatoxins and *Aspergillus ochraceus* for the production of ochratoxin A (Soares *et al.,* 2013).

2.4 Factors Affecting the Development of Fungi in Association to Foods

These factors affecting the development of fungi in association to food include: intrinsic conditions and extrinsic conditions.

2.4.1 Intrinsic conditions (substrate limitation)

The following conditions affect the development of Penicillium fungi on food substrate, which include:

2.4.1.1 Dietary composition

Microbes consume solid nourishment as sources of nutrients as well as energy. These organisms derived the chemical component that make up their biomass in ecosystem, those molecules are vital for the growth and development of the microbes however, they cannot synthesis such molecules. The degree of microorganisms' development is influences by concentration of key nutrients to a large extend (Javis, 1971; Adams and Moss, 1999).

2.4.1.2 Measure of acidity and alkalinity (pH) and buffering capacity

The extend of acid or alkali in a natural world lead to a great influence on the action and steadiness of macromolecules for example enzymes, therefore the aforementioned is not surprising since the growth and metabolism of microorganisms are influenced greatly by degree of acidity and alkalinity. In a wide range, bacteria quickly develop naturally in the pH range of 6.0 – 8.0, while yeast grow in pH of 4.5 – 6.0 and filamentous fungi grow in pH of 3.5 – 4.0. Most foods are at least slightly acidic (Javis, 1971; Adams and Moss, 1999).

2.4.1.3 Oxidation-reduction (Redox) potential (Eh)

The transfer of electrons involving several atoms and molecules encompasses oxidation and reduction process simultaneously. Active cells in an organism portrayed harmonious sequences mutual reactions of electron and hydrogen transfer which together is vital characteristic of the following biochemical processes such as oxidative phosphorylation for energy production and electron transport chain. The propensity of an agent to give or to take electron, to oxidize and reduce is expressed as redox potential. Oxygen, which existent is about 21 % level by volume in the component of air, normally produce an effect on redox potential in the system of food crop. Redox potential put forth a principal electrons' consequence in the micro flora of a foodstuff. Although microbial growth can occur over a

wide spectrum of redox potential, individual microorganisms are conveniently classified into one of several physiological groups on the basis of redox range over which they can grow and their response to oxygen**.** The intrinsic factors of reduction and oxidation potential is complicatedly connected with the external condition of storage. Redox potential can be increased through the upsurge of the admittance of air to a foodstuff by chopping, mincing or grinding (Adams and Moss, 1999).

2.4.1.4 Water activity (a_w)

The water activity of a medium is really suitably defined as the proportion of the partial pressure of water in the atmosphere in equilibrium with pure water at the same temperature, Po. Equilibrium relative humidity (ERH) is equal to water activity statistically expressed not as percentage but in fraction: $a_w = P/Po = 1/100$ ERH

The activity of water is a colligative behavior, to be precise, be contingent on the numeral of molecules or ions existing in the solution, other than their size. For instance, common table salt can be effective in reducing the water activity than sucrose because it has high hygroscopic nature. A parameter related to water is osmotic pressure which can be thought of the force per unit area required to stop the net flow of water molecules from a region of high to a region of low water activity. As water activity is diminishing, or there is upsurge of osmotic pressure in the surrounding it is fundamental that the a_w of the cytoplasm is similarly lower or its osmotic pressure at the same time higher. The decline of the activity of water in the surrounding of various groups of microorganisms reduces their growth. The minimal a_w at which active growth can take place in a number of microbes comprise: most filamentous fungi (0.80), most yeast (0.88) and xerophilic fungi (0.61). The range of a_w 0.85 – 0.60 match up approximately to a humidity substance of 15 % – 50 %. When spoilage happen, the aforementioned is as a

result of incorrect storage in an elevated relative humidity of the environment. The limiting value of a_w in the emerging and growing of several microbes is approximately 0.6 and under the aforementioned unit the decomposition of foodstuff is no longer activity of microbes nonetheless may perhaps be owing to pest injury or chemical oxidation. High moisture content during storage of cereal will encourage the growth of harmful fungi (Adams and Moss, 1999; Sautour *et al*., 2002; Abiose and Ikujenlola, 2014, Perrone *et al.,* 2014).

2.4.2 Extrinsic factors (environmental limitation)

The following environmental limitation affect the development of fungi on food substrate, which include:

2.4.2.1 Relative humidity

Relative humidity and water activity are interconnected; thus, relative humidity is fundamental a quantity of the activity of water of the air stage. As soon as food products bearing the low activity of water are stocked in a climate of high relative humidity, moistness will be moved from the air stage to the food grains. Consequently, the conversion of gas to liquid may possibly happen on the superficial of the grains leading to confine zone of extreme activity of water. As long as microbes have commenced growth and turn out to be physiologically active, they normally give off water as a finish product of internal respiration. Consequently, the microbes escalate the activity of water of their particular immediate environment so that finally microbes necessitating a soaring activity of water are capable to produce and damage foodstuff which originally are stable for the activity of microbes (Adams and Moss, 1999).

To be precise, a circumstance can take place in stored grain crops in Silos or in tank or in large-scale storage units, especially in grain crops Silos due to the relative humidity of air is

actually delicate to degree of heat. When one surface of a Silo become hotter in the course of the day owing to contact to the Sun at that time the relative humidity on that surface diminish and at hand a clear relocation of several molecules of water starting at cooler surface to re-equilibrate the relative humidity. If that similar surface depressed in coolness once more the relative humidity rises and, even though water moved rearmost once more, the momentary upsurge in relative humidity possibly will be adequate to be basis for regional conversion of vapor to liquid upon the cereal crops with a localized rise in water activity enough to permit incubation of fungi reproduction structure which will ensue degeneration of the cereal crops. This kind of event can frequently explain the reason behind the localized congealing of cereals which before now obviously been stock in much safer container free of water (Adams and Moss, 1999; Leggieri *et al.,* 2016).

2.4.2.2 Temperature

The growth of microscopic organisms can take place keeping pace with a temperature range beginning at – 08 °c to as high as 100 °c by atmospheric pressure. Not single living things can accomplish its growth using the entire range of temperature listed below (Table 2.1). Every single living thing exhibit a lowest, optimal and supreme temperature at which growth can occur. Though all the below listed cardinal temperature are controlled by additional dynamics in atmosphere namely nutrient readiness, activity of water and pH. Microorganism have the following classes, grouped according to the functions of organisms with their fundamental temperature as follows:

Table 2.1: **The Classification of Microorganism Base on Physiological Groups and Temperature**

Groups	Temperature °c		
	Minimum	Optimum	Maximum
Mesophiles	5 – 15	30 – 40	40 – 47
Thermophiles	40 – 45	55 – 75	60 – 90
Psychrotrophs (facultative psychrophiles)	-5 – +5	25 – 30	30 – 35
Psychropiles (obligate psychrophiles)	-5 – +5	12 – 15	15 – 20

Source: Adams and Moss, 1999

At low temperature there is slowing and eventual cessation of microbial growth while as the degree of hotness increase over the optimal, the growing process regress considerable quickly by way of uncontrollable damage of proteins structure and the collapse of cell's plasma membrane due to involvement of much heat (Adams and Moss, 1999; Sautour *et al*., 2002, Leggieri *et al.*, 2016).

2.4.2.3 Gaseous atmosphere

Oxygen constitutes the atmosphere of the earth with extreme significant among the other gases which interact with foodstuff within regular situation. Its existence and its influences on the potential of redox are crucial contributing factors of the microscopic organisms' association that breed and their degree of growing process. Carbon dioxide (CO_2) have the ability to impede the growing process of microbes and the effect is not uniform on microorganism (Adams and Moss, 1999).

Mold are sensitive while some yeasts show substantial acceptance above average of CO_2 level and govern the putrefaction of micro flora of carbonated beverages. The reservation for growing process is typically grander in condition that require oxygen for biochemical process

than the one that does not require oxygen and decline in temperature will lead to increase in the inhibitory effect. Some microorganisms are destroyed by protracted contact to CO_2 nevertheless as a rule its consequence is inhibiting bacteria growth. The system of reaction of inhibition by CO_2 is the synergy of numerous courses of action which take account of the upshot of CO_2 on pH since when dissolve in water (H_2O) it will produce trioxocarbonate (IV) acid (H_2CO_3) which can separate into bicarbonate (HCO_3^-) and proton (H^+). Additional related aspects comprise of adjustment in the physical properties of the plasma membrane of the body fluid of cell unpleasantly altering the transport of solute, hindering of vital biocatalyst chiefly those reaction concerning carboxylation and decarboxylation in which CO_2 is a reactant, and reaction with protein of amino acid group leading to vicissitudes in their properties and activity (Adams and Moss, 1999).

2.5 Mycotoxin

Mycotoxins are poisonous (toxic) secondary metabolites yielded through numerous filamentous fungi affiliated to the Ascomycota phylum (Alshannaq and Yu, 2017). These molecules seem to bear not one function in the conventional metabolism concerning growing processes of the fungus. There are a lot of weird molecules, together with ranging configuration from single heterocyclic ring having about 50 molecular weights, to collection of membered rings organized involving six or eight associated rings having over 500 molecular weights (Pitt, 2000; Dawlatana *et al*., 2008). The growing process of *Penicillium* in addition to the mycotoxins production is highly favored as found in maize flowering period and storage period depends on the humidity and temperature (Adebajo *et al*., 1994; Domijan *et al.,* 2005). Fungi develop naturally pleasingly on a lofty substrate of complex natural compounds like carbohydrate and protein in hot and humid regions (Shah *et al*., 2010).

2.5.1 Ochratoxin A (OTA)

Grains and food/feed source from grains are the foremost source of OTA consumption in humans and other animals, meanwhile it is stable under normal regular food processing conditions thereby persisting in processed product even after raw food had undergone processing (Puntaric *et al*., 2001; Soares *et al*., 2013). Ochratoxin A was firstly isolated in 1965 from contaminated maize-based products (Darsanaki *et al*., 2015). Ochratoxin A is the most important naturally occurring mycotoxin of that is composed of a dihydrocoumarin one of two parts connected with β-phenylalanine molecule (Palencia *et al.*, 2014). The group of ochratoxin comprise of 3 members which include Ochratoxin B (OTB), Ochratoxin C (OTC) and Ochratoxin A (OTA) which differ structurally one from another. The OTA toxicity is due to the chlorinated structure, while OTB not as much as toxic when put side by side to OTA, owing to substitute in isocumarin moiety of chloride with the hydrogen atom as (Figure 2.1) (Majeed *et al.*, 2017; Darsanaki *et al*., 2015).

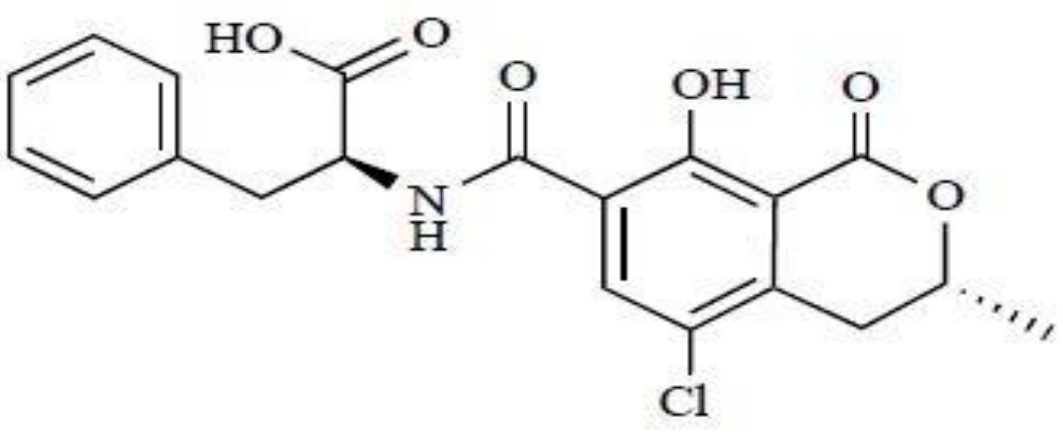


Figure 2.1: Structural Molecular Formula of Ochratoxin A (OTA) Source: Majeed *et al.*, 2017.

The synthesis of Ochratoxin A (OTA) primarily is exclusive by the fungi genus: *Aspergillus* and *Penicillium*, where *A. ochraceus* and *P. verrucosom* have being the major prolific species responsible for the above-mentioned mycotoxin (Sekiyama *et al.*, 2005; Akman *et al*., 2012).

Moisture content and temperature are the extremely significant variables which serve as a contributing factor in the growing process and rate of production of mycotoxin by mold in stockpile cereal crops ecosystems. The species *P. verrucosum* is an archetypal xerophilic storage variety that is capable to grow well at pretty short supply of activity of water and temperate climate (Czaban *et al.*, 2006; Manna and Kim 2017). Ochratoxin A (OTA) is an agent that can cause genetic mutation, agent that can interrupt the development of embryo and also affect the cells in the kidney which had necessitated causes of Endemic Nephropathy in Balkan (Sekiyama *et al*., 2005; Scudamore, 2005; Ostry *et al.*, 2016, Majeed *et al*., 2017).

Ochratoxin A is measured using thin layer chromatography (Puntaric *et al*., 2001; Palencia *et al*., 2014; Nalle *et al.*, 2019), liquid chromatography–tandem mass spectrometry (LC-MS) analysis (Palencia *et al*., 2014), method based on Immune Affinity Column (IAC) clean-up followed by liquid chromatography coupled with fluorescence detection as reported by Majeed *et al*., 2017, High Performance Liquid Chromatography (HPLC) equipped by means of a 474 fluorescence detector/UV detector (Czaban *et al.*, 2006; Ifeji *et al*., 2014), liquid chromatography-coupled triple-quadrupole mass spectrometry (LC-MS/MS) as report by Park *et al*., 2018. Gas chromatography (GC) linked with ECD, MS or FID detection; Ultra Performance Liquid Chromatography (UPLC); enzyme-linked immunosorbent test (ELISA); and rapid strip screening assay are other methods for analysis (Alshannaq and Yu, 2017).

2.5.2 Cyclopiazonic acid (CPA)

In 1968, Holzapfel was the first to isolated Cyclopiazonic acid from the fungus *P. cyclopium* Westling (appropriate name: *P. griseofulvum* Dierckx) and characterizes it chemically (figure 2.2). CPA is an indole (C_8H_7N) tetramic acidic polymers and monobasic acid having chemical

attraction for lipids with a low molecular weight compound (Maragos, 2018; Ostry *et al*., 2005; Ostry *et al*., 2018).

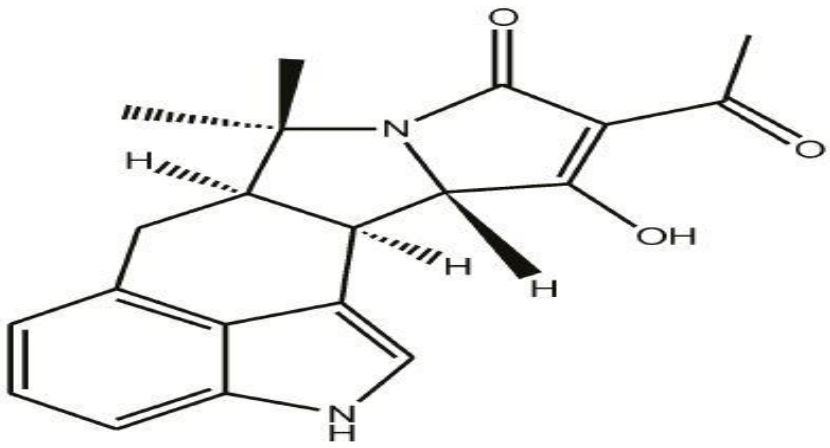


Figure 2.2: Cyclopiazonic Acid Structural Molecular Formula

Source: Ostryl *et al*. (2018)

The configuration of CPA-type mycotoxins chemistry consist of the indole (C_8H_7N) derivatives (bis-secodehydrocyclopiazonic acid – β CPA, Cyclopiazonic acid – α CPA and Cyclopiazonic acid imine – α CPA imine) and oxindole derivatives (speradines, aspergillines, cyclopiamides). CPA is a tetramic indole (C_8H_7N) acid and its biosynthesis involved two acetate molecules, tryptophan and the mevalonate pathway. CPA possess optical activity, colorless, odorless, a metabolite with definite shape. When use in chemical analysis, it is soluble in the following solvent: chloroform ($CHCl_3$), dichloromethane, methanol (CH_3OH), sodium bicarbonate and acetonitrile (CH_3CN), though the dissolution capacity of CPA in the aforementioned diluent is around 20 mg/ml. CPA does not dissolve in water but thinly dissolve in buffers mostly made up of water (Burdock and Flamm, 2000; Ostryl *et al*., 2018). CPA is unstable in the company of oxygen and formic acid (HCOOH) which easily adsorbs the above mentioned to form polypropylene (Ostryl *et al.,* 2018).

Cyclopiazonic acid is a strong, definite, and rescindable substance that can slow chemical reaction in the sarcoplasmic and endoplasmic reticulum Ca^{2+} - activated ATPase (Burdock and Flamm, 2000). Cyclopiazonic acid (CPA) is a substances toxic to nerves, it takes action during the course of slowing down reaction of the sarco (endo) plasmic reticulum Ca^{2+}-ATPase (SERCA) it impedes the calcium access channel of the enzymes (Maragos, 2018). The net consequences of CPA in the fiber of muscle tissues is to generate a damaging contraction and relaxation of the muscles outcome that results to a visible symptom at hand as CPA toxicity (Burdock and Flamm, 2000). CPA can originate quite different form of warning signs and these can differ from one species to another. These warning signs and symptoms possibly will comprise of loss of body mass, diarrhea, deterioration and tissue death of the internal organs and muscles, leading to convulsions which can result to death. The foremost organs of focus include the following: spleen, liver, lymphoid tissue, kidneys alimentary tract, myocardium and skeletal muscle (Maragos, 2018). Since CPA has high oral LD_{50} (30,000–70,000 μg/kg) it is not considered to be a strong and severe rather it is chronic toxin (Oyedele *et al*., 2017) nonetheless it can engender harsh medical consequences as mentioned above, in the biological systems when expose to it at very extraordinary degrees or amount. According to a hitherto proposed no-observable-effect-level of 1000 μg/kg and the endorsed highest acceptable daily intake of 10 μg/kg/day (Burdock and Flamm, 2000 as cited by Oyedele *et al.,* 2017),

The biosynthesis of CPA is primarily carried out by *Penicillium species (P. dipodomyicola, P. griseofulvum, P. commune, and P. camemberti)* and *Aspergillus species* (*A. flavus, A. tamarii* and *A. oryzae*) (Perng-Kuang *et al.,* 2009, Chang *et al*., 2009, Ostryl *et al*., 2018). At present, endeavors to measure the quantity of CPA in foodstuffs are relatively fewer in

available reports. CPA is found in more than a few items for consumption of plant sources, which include maize, figs, rice, groats, tomato paste and puree, kodo millet, sunflower seeds, peanuts and wheat as reviewed by Ostryl *et al*. (2018). While other foodstuff of animal sources, CPA had been detected in milk, cheese as well as salami as reviewed by Ostryl *et al*., 2018. Maragos (2018) had also reported chicken meat detected with CPA.

In the detection and quantification of CPA in a cultured fungus and in contaminated food, there are arrays of analytical techniques which including capillary electrophoresis, immunochemical methods (immunoaffinity columns (IAC) and ELISA) and chromatographic methods (HPLC, TLC and LC-MS/MS) (Ostryl *et al*., 2018).

2.6 Cereal production

Cereals like Sorghum, Millets, Wheat, Maize and Rice are major staple foods of the most population. These cereals are grown over an area of 98.6 m ha producing 162 m tons (Table2.2).

Table 2.2: The Area and Production of Selected Cereal Crops

Crop	**Africa (2012)**	
	Area (ha)	**Production (t)**
Maize	34,075,972	70,076,591
Sorghum	23,142,595	23,350,064
Rice, paddy	11,206,813	28,798,202
Millet	19,998,008	16,008,838
Wheat	10,224,952	24,704,201
Total	**98,226,080**	**162,422,507**

Source: Macauley, 2015

Global agriculture is facing the probable impact of global warming. Recent studies suggest that the production of major commodities has declined since 1980 due to global warming (Macauley, 2015). There has been a fluctuating trend in Maize production over the last decade, which threatens household food security and income sources (Ibitola *et al.*, 2019). Cereals and cereal-based products represent one of the most important dietary items in the world. Cereals are very susceptible to fungal attacks on the farm and during storage (Darsanaki and Takrami, 2017; Reza and Saied, 2017)

2.6.1 Maize

Maize (*Zea mays L*.) is one of the most widely known and utilized species from the family of grasses (Poaceae) which is an important cereal grain in the world providing nutrients for human and animals (Abiose and Ikujenlola, 2014; Iken and Amusa, 2014; Qamar *et al*., 2017). Maize is a chief steady food grains that is grown up in various cultivation system and agro-ecological regions, and feed on by human beings involving variable preference for food and also changeable socio-economic context in South of Saharan Africa (SSA). Maize contributes one-fifth in quality of the source of protein and energy feed on in West Africa. An estimated 208 million people in SSA including Nigeria depend on maize as a source of food security and socio-economic stability and wellbeing (Macauley, 2015; Ibitola *et al*., 2019).

2.6.1.1 Varieties of maize

There about 50 maize species in existence with different colors of germplasm (blackish, bluish-grey, purple, green, red, orange, white and yellow - white and yellow are the main colour and they are bicolor), textures and grains shape and size. The maize kernel is the largest in seed cereal weighing 250 – 300 mg each (Abiose and Ikujenlola, 2014). Difference between

white and yellow maize is in present or absent of carotenoids. Yellow maize is high β-carotenoids, which is a sources of vitamin A know as retinol while white maize does not have carotenoids. Orange and yellow maize have varying level of carotenoids and lutein (Iken and Amusa, 2004; Beta *et al*., 2014).

2.6.1.2 Nutritional composition of maize

The composition of maize has wide discrepancy between its different species and subspecies which depends upon the various environmental and topographical conditions (Qamar *et al*., 2017). Starch with 72 -73 %, of the kernel weight, low in fat, primary it composed of starch 62 %, protein and fibers 19 %, water 15 % and oil 4 % and other tracers Phosphorus, Magnesium, Manganese, Zinc, Copper, Iron, Selenium, Potassium, Calcium. Most of the nutritionally important minerals are higher in yellow maize flour as compared to available white maize flour as reported by Beta *et al. (*2014); Qamar *et al*. (2017). Maize is used as human food in the form of *waina*, porridge, popcorn and barbecues and as forage and silage for animals. It is rated high as a commercial crop as raw materials for industries purposes. Therefore, it is recognized as a major source of food and cash income in Nigeria (Iken and Amusa, 2004; Abiose and Ikujenlola, 2014; Ammani, 2015).

2.6.2 Sorghum

Sorghum (*sorghum bicolor L moench*) is the second most important cereal after maize with 22 % of total cereal area, followed by millets (pearl and finger) with 19 % of the total cereal land coverage. The continuing demand for these two crops is reflected in the trend for increasing area under sorghum and millets in Africa over the last fifty years. In Africa, Nigeria and Sudan are leading producers of sorghum. Nigeria accounts for up to 40 % of total sorghum production in Africa (70 % in West Africa) and is the third largest producer after the United

States and India. Sorghum can thrive in both temperate and tropical climates as it has a high photosynthetic efficiency. Sorghum is one of the most drought tolerant cereal crops currently under cultivation (Aduba *et al*., 2013; Macauley, 2015; Palavecino *et al*., 2016; Mundia *et al*. 2019). Sorghum grains are often contaminated by moulds, when poorly dried and stored therefore serving as ideal substrates for mould growth. Mould infections in stored grain limit the allowable storage time (Kange *et al*., 2015). Sorghum is one of the most important staple food crops in Nigeria. The cereal crops are employed basically in feeding farm animals, also very valuable industrial crop for brewing alcoholic and non-alcoholic drinks as well as in the baking and confectionery industry in Nigeria (Baiyegunhi and Fraser, 2009).

2.6.2.1 Varieties of sorghum

Sorghum exist in widespread hereditarily sundry crop mutually in natural and wild species. The five Sorghum races are identified as *bicolor*, *guinea*, *caudatum*, *kafir* and *durra*. In Africa, *durra* is found in the region from the Horn of Africa/East Sahel to the West. Guinea sorghum (Guinea corn) is widely adapted in the wetter West Africa (western Nigeria to Senegal) and the *caudatum* race is associated with the Chari-Nile speaking Africans of the eastern savannah and largely extends from eastern Nigeria, Chad and western Sudan. *Kafir* is mainly grown in areas from Tanzania to South Africa. (Sani *et al*., 2013; Freitas *et al*., 2014; Mundia *et al*., 2019). Here in Nigeria, the most common land races of sorghum are: *Kaura*, *Fara-fara* and Guinea. The great diversity as seen in sorghum is manifest in the reality that more 18 varieties exist at certain period as identified by researchers and grain color is one of the features that is used to differentiate them (Sani *et al*., 2013). Sorghum is highly diverse in genetics, and a wide range of sorghum varieties are available in various colors, sizes, structure, and shapes. The color is closely related to the phenolic profile of the grain. The colour of sorghum varieties

can be white, yellow, red, brown and black. White sorghum contains minimal quantity of aggregate phenolic matter while the black ranks with elevated quantity of aggregate phenolic substances (Mejia and Lewis, 1999; Xiong *et al*., 2019).

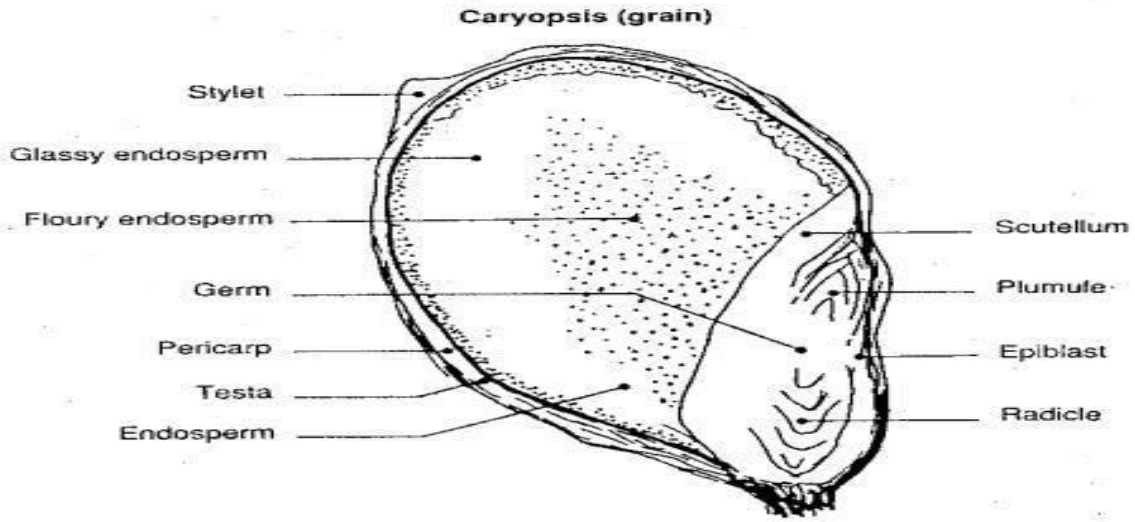


Figure 2.3: The Structure of Sorghum grain (Source: Xiong *et al*., 2019)

2.6.2.2 Nutritional composition of sorghum

A number sorghum sub-species abounds with so much agent that deter oxidation and the entire sorghum sub-species are unburden with gluten, it is a rich in resistant starch, fibers, unsaturated fatty acid, minerals, protein and polyphenolic compounds like flavonoids, benzoic acid, cinnamic acid, 3-Deoxyanthocyanidin, proanthocyanidin and condensed tannins (which can complex with protein and carbohydrate) in the testa (Figure 2.3). The proximate of sorghum in Argentina shows 0.68 % ash, 3.67 % fat, 12.21 % protein, 83.45 % total carbohydrates, 79.77 % starch (amylose 26.6 %) and 34.9 mg of tannic acid per 100 g of flour (Mejia and Lewis, 1999; Aduba *et al*., 2013; Freitas *et al*., 2014; Palavecino *et al*., 2016; Xiong *et al*., 2019). Sorghum and maize have comparable nutritive composition, though sorghum grain constitute anti-nutritional agents (like phytate and oxalate) than maize which make it less palatable and digestible than maize thereby reducing the availability of these

nutrients and minerals. Sorghum's starch-protein matrix resists the penetration of enzyme and moisture more than other cereal grains (Legodimo and Madibela, 2013). The red grain sorghum types are reported to contain high amount of tannins than the white sorghum. (Mejia and Lewis, 1999; Legodimo and Madibela, 2013).

2.7 The Risk Analysis Framework for Safety of Food

Risk analysis of food safety is the process of identifying and analyzing potential issues that could negatively impact on food safety in order to help avoid those risks. The component of risk analysis (Figure 2.4) include risk assessment (hazard identification, hazard characteristics, exposure assessment, and risk characterization), risk management (acceptable risks, prevention, option, cost/benefits and regulation) and risk communication (compassion, interaction, prioritization and education).

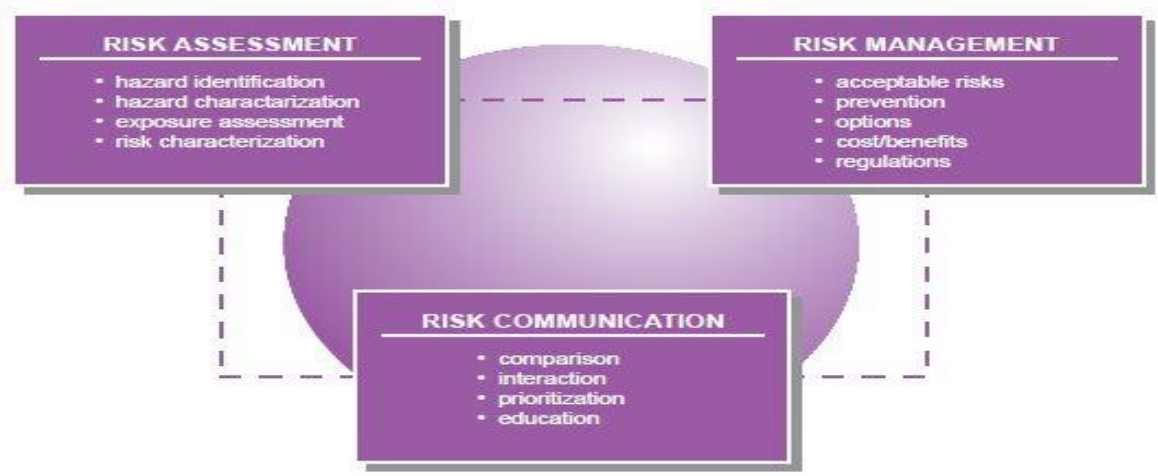


Figure 2.4: The Components of Risk Analysis on Food Safety
Source: Kuiper-Goodman, 1999.

2.7.1 Risk assessment

Risk assessment for humans potentially exposed to multi-mycotoxins suffers very much from the lack of adequate food consumption data (Viegas *et al*., 2011). In the assessment of the risk of mycotoxins, among others measure the utmost significant framework is to establish the extent of human exposure to the aforementioned toxins (Luz *et al*., 2018).

2.7.1.1 Hazard identification

Hazard identification of important mycotoxins are obtained through the carcinogenic properties they are exacting on many organs of the body. Other effects, include: birth defects, effect on the immune system, neurotoxicity, skin itchiness, trouble of the gastrointestinal tract (GIT), and hematological outcome are well noted. The above finding typically establish experimentally the "no-detect-hostile-effect point" (NOAEL), and this will be look upon as a "threshold". At this level connecting risk assessment, information obtained from epidemiological studies of exposed humans can be useful (Kuiper-Goodman, 1999).

2.7.1.2 Hazard characterization

Hazard characterization is the conclusion stage of risk assessment. Its goal is to invent a prognostic characterization of the hazard to humans being, depending on result obtained from studies of animals (extrapolate from species) and below minimal contact state (extrapolate beginning with extreme to minimal dose). The point of no return of hazard characteristic is the approximation of a "safe dose" which include provisional tolerable daily intake (PTDI) or its likes. The term "tolerable" point to the fact, of non-benefit of mycotoxins to humans being. According to wider view, (P)TDIs are verify simply as soon as there is possibility to obtain the starting point in dose-effect relationship, depending on information of the technicalities and manner of activities (Assunção *et al*., 2016).

In order to originate TDI for human beings, the regular approach is to divide up the NOAEL through a safety value of 100 which is estimated from other experimental animals to human beings. This includes contemplation of another value of 10 to accommodate differences in interspecies as well as yet another value of 10 for intra-species (this only involve within human) disparity. Supplementary ambiguity factors aside the once mentioned above may as well be considered if fundamental permanent effects for which starting point have been determined (especially for non-genotoxic cancer causing agent) or scarce factual information, Occasionally the establishment of a (P)TDI is delayed while waiting for the availability of adequate factual information - data. The TDI can be seen to be an inherent possession of mycotoxins, thereby encompass the reflection mutually of the strength of the effects determined and also the biological conditions concerning the severity, relevance and significance of the effects to humans being (Kuiper-Goodman, 1999; Assunção *et al*., 2016).

2.7.1.3 Exposure assessment

The exposure to mycotoxins depends on the level of these substances in different foods and on the intake of those foods (Figure 2.5) (Baert, 2017). The intake of food, national and regional differ largely, therefore contact evaluation differ from one country to another thereby posing a setback on harmonization.

Figure 2.5: The Connection of Exposure Assessment to Chemical Occurrence and Food Consumption (Source: Baert, 2017)

Consequently, the eating experiences of specific group of people regarding mycotoxins as found in food sample can be computed by means of Estimated Daily Intake (EDI) as portrayed below:

EDI (ng/kg bw/day) = Mean Conc. of Mycotoxin (ng/kg) **X** Sample consumption (g/kg bw/day) (Adetunji *et al*., 2017; Luz *et al*., 2018).

In the place of mycotoxins, the monitoring data for foodstuff items of interest sampled throughout quite a lot of years frequently granted the feedback for facts on concentrations. Approximation possibly will require refining by additional bearing in mind adjustment to the mycotoxins concentration, in the foodstuff that is usually fed on, through counting on evidence on industrial and fact on processes put together via home and industry set up. These estimations may possibly be focus on discrete stage or target groups of people, subject to the specific situation being examined (i.e. severe intake versus long-lasting intake). Contact to toxins bears divergence in harmony to difference ages, where juvenile, usually, being endanger with abundantly advanced proportion in terms of their body mass for each food items namely milk substance (up to seven times) and peanut butter (up to four times) which may perhaps comprise of Ochratoxin A and Cyclopiazonic acid as well as the byproducts their metabolism (Adetunji *et al*., 2017).

The evaluation of contact to toxins also can occasionally depend on quantification of biological markers in humans being (with toxins like aflatoxins, ochratoxin A), and the consumption can then be appraised with the starting point of pharmacokinetic relationships. This is likewise required to tackle the conceivable threat to the health of human being which come about by the contamination of food products with mycotoxins. When the concentration is extreme in feed, mycotoxins can result to malfunction of health likewise developing of

animal toxicoses which can lead to death of farm animals. Extensive studies in farm animals on the effect major mycotoxins had been enquired, on the other hand additional fact about accessibility in humans' system of the whole toxins and their metabolites, which include metabolites that are bound (for instance conjugates bound protein), is in demand. At minimal concentration in foodstuff, these mycotoxins display little of no obvious consequences on farm animals' production, nevertheless the remains and associated molecules usually persist in the food chain. This incoherent consumption of mycotoxins and linked compound obtained from dietary intake of foodstuff for animals can stance human with health implication and hazard. In a foregoing appraisal it was establish that the hazard to human beings linked with unintended contact from food products obtained through animal origin is known to elicit a much lower effect as compared to foodstuff contaminated with mycotoxins and is consume (Kuiper-Goodman, 1999).

Meanwhile mycotoxins contaminated basically common foods, contact in market sourced commodities, which is the combination from various sources mixed together, so present minimal exposure with chronic exposure. On the other hand, food crops from farming communities, can result to extreme exposure with occurrence of shorter duration. Not much have been revealed on the differences linking extended-term threat among the two kind of contact. Dietary exposure assessment comprises of deterministically or probabilistically food intake data with incidence of a specified chemical compound in various sort of food. Probabilistic exposure assessments have lately been launch and assist to imagine the allocation of contact beneath varied situation. There is a pivotal need for fitting parameter for establishment of International agreement (Kuiper-Goodman 1999, Assunção *et al*., 2016; Oyedele *et al*., 2017).

The deterministic approach comprises of the determination of the probable daily intake (PDI) of mycotoxins. To approximate the PDI (ng/kg bw/day) for mycotoxin as entity, the amount of toxin in the food samples with the multiplication via the mean of consumption rate of samples in Nigeria and thereafter divided it with the body mass for the diverse groups of people [toddlers (0 – 3 years), youngster (5 – 12 years) and grown-up (18 – 65 years) (Oyedele *et al*., 2017).

2.7.1.4 Risk characterization

Risk characterization is the appraisal relating to quality and/or quantity, also involving the associated uncertainties, of the severity and probable existence or nonappearance of acknowledge and conceivable hostile physical condition outcome on population that are endanger. As shown below in (Figure 2.6), Risk characterization comprises of hazard identification, hazard characterization and exposure assessment (Kuiper-Goodman, 1999; Assunção *et al.*, 2016; Baert, 2017)

Figure 2.6: The Relationship of all the Components of Risk Assessment (Source: Baert, 2017)

The health risk characterization of each mycotoxin will be carried out by dividing the previously calculated EDI by the Tolerable Daily Intake (TDI) (ng/kg bw/day) of the corresponding mycotoxin (when available), as indicated in the following equation (Adetunji *et al*., 2017; Luz *et al*., 2018): %TDI = (EDI/TDI) X 100.

Risk characterization can likewise be the formation of amount of everyday contact in which the risk is inconsequential throughout a life expectancy (i.e. contact is required to be below the TDI or other degree of harmless dosage). This second meaning may perhaps be more pertinent, keeping in mind the connection of the uncertainties which had been previously have already been deliberated and hash out. Taken into consideration of substance in which the decision for its TDI had not been established, the security border joining human exposure and adverse effects observe in experimental animal variety may work as a vital suggestion of the possibility of under the weather effects happening in humans being, then this may possibly be employed in risk management. This was the case in the recent evaluation of fumonisins (Kuiper-Goodman, 1999).

In addition, bearing in mind the mean of the group of people, the risk characterization likewise must think through any vulnerable group which might had been exposed, for instance children (due to smaller body mass), and additional groups whereby their other factors such as variance in bio-accessibility, biochemical activity or hereditary nature, for example those advanced in years. Judging from the above stated fact, the sufficiency of a tenfold security dynamics to dispatch variance in human being vulnerability evolving starting at human being changeability require to be scrutinized. Comprehensive risk assessments had been accomplished for merely a limited number of mycotoxins (deoxynivalenol, fumonisins, zearalenone, ochratoxin A, aflatoxins) (as reviewed by Kuiper-Goodman, 1999) and these assessments essentially

requires verification over and over again to accommodate up to date obtained knowledge in association to toxin exposure and basic toxicology along with upgraded knowledge on the mechanism of action (Kuiper-Goodman, 1999). The difficulty presented by the risk assessment of manifold mycotoxins was published by Assunção *et al*. (2016) and Figure 2.7 point out the interconnection between the diverse risk assessments of the steps including the noted difficulties for each step.

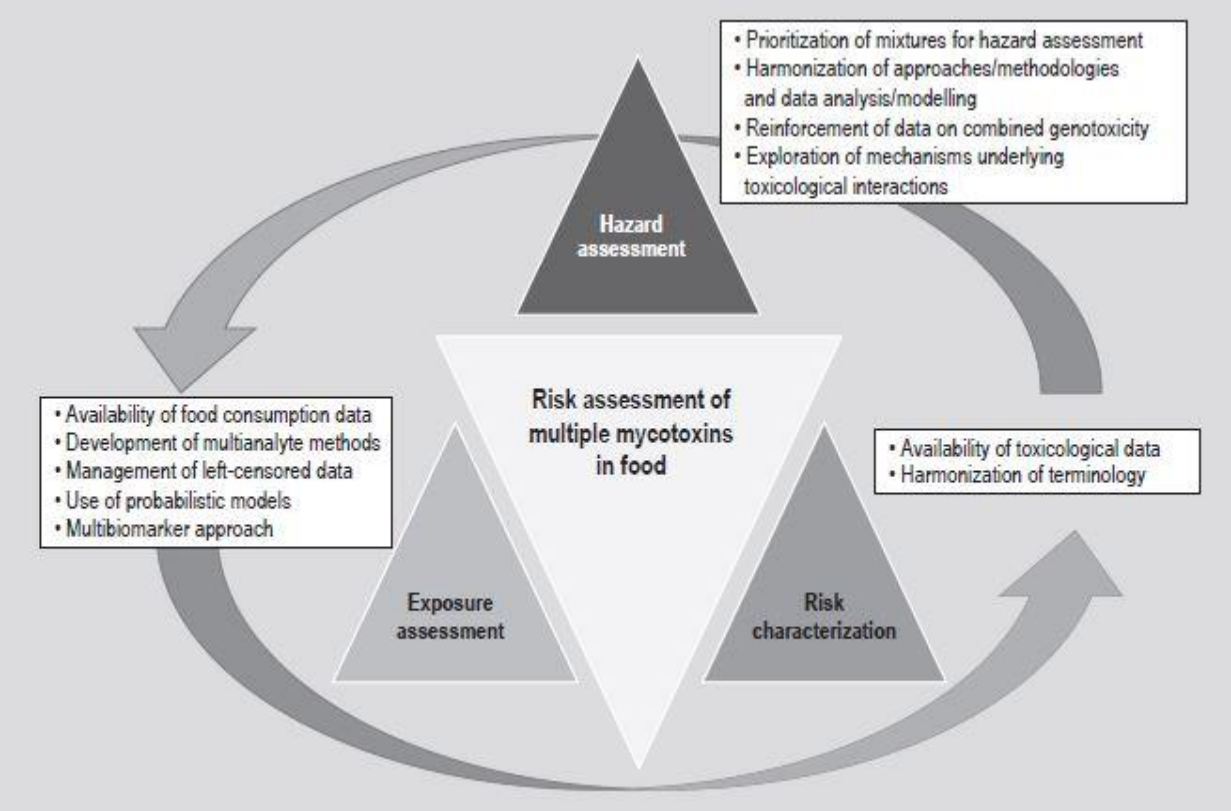


Figure 2.7: Overview of the Interconnection between the diverse steps of Manifold Mycotoxins Health Risk Assessment and their Corresponding Difficulties (Source: Assuncao *et al.*, 2016).

2.7.2 Risk management

The risk management of mycotoxins have variety of routes that will support to safeguard security in the free access in sources of food. These extend from stoppage of the growth of

fungi then establishing of controlling threshold, follows by digression into every other usage. Massive economic charges are connecting by means of all the above out listed choices. Which is a unique obstruction to synchronicity in business connected to the establishing of maximum residue limits (MRLs) for foodstuff contaminated with mycotoxins. MRLs are presently by and large depend on the finding of researchers. These finding assist in ensuring such limit is not beyond business products, nevertheless the concentration of mycotoxins to certain degree beyond these concentrations can be allow on the basis of a minimal occurrence. A great proportion of prevalence of the frequencies of mycotoxin are fortunately lower than the MRL. The selling of foodstuff items abroad is a constant activity, therefore there is no likelihood to restrict permissible concentration on the measured mean of the exact concentration, as regulated many food items, otherwise resort to modify the permissible concentration for a variety food items on annual ground. Conversely, in the ultimate risk assessments, exposure assessments for mycotoxins can likewise be founded solely upon exact residue concentration (not only on MRL) as a least good circumstance. The concern of regulators is that, if a higher MRL were to be allowed, it would become the acceptable level for industry and blending upwards to this level would occur, thus increasing exposure. For this reason, blending is not allowed in many jurisdictions (Kuiper-Goodman, 1999; Assunção *et al*., 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The materials used in this study include:

3.1.1 Chemicals and reagents

The chemicals used are all of analytical grade and they include: Cyclopiazonic acid 98 % - 1 mg/ml (Sigma-Aldrich), acetonitrile (CH_3CN) -99.9 % (LiChrosolv®, Merck KGaA Germany), dichloromethane (CH_2Cl_2) - 99.9 (Merck KGaA Germany), orchratoxin A ELISA kit (Cusabio), anhydrous sodium tetraoxosulphate (VI) (Na_2SO_4), distilled water (H_2O), methanol (CH_3OH) - 99.9 %, (LiChrosolv®, Merck KGaA Germany), nitrogen gas (N_2), iso-octane, disodium sulphate salt (Na_2SO_4), tetraoxosulphate (VI) acid (H_2SO_4), disodiumhydrogencarbonate (Na_2HCO_3) and acetic acid (CH_3COOH).

3.1.2 Equipment

The list of equipment used and their models include: High Performance Liquid Chromatography (HPLC) - LC98II, Magnetic stirrer hot plate - 78 – 1, Chromatographic column - Microsphere c-18 (150*4.6 mm), Centrifuge - 80-2, Microscope - Olumpus DHB, Water bath - DK-420, Orbital Shaker - Celtech KJ-2018D, Laminar air flow hood - LAFC-VI LKH00652G, Centrifuge - Adventurer Pro Model: AV313, Electrothermal Oven – DHG, Thermostat Oven - DHG—9053A, Weighing balance - JA103P / 1704017, Mixer grinder - VTCL-Smart Leaf 154250, Microplate reader - Diatek DR-200Bs, Vortex Mixer - XH-C and Micropippete – Huawei Dragon – YE5A530682.

3.1.3 Study area

The samples were collected in the four microclimatic zones in Niger State. These zones are listed as follows: wettest zone, wet zone, dry zone as well as driest zone with one local government area in each zone that is Suleja, Magama, Bosso and Mariga, respectively. The major activity in Niger state is agricultural especially in the rural areas. Most people in Niger state derived their livelihoods from farming, fishing and cattle rearing. Others economic activities include banking, trading, transportation and local arts and craft (Sallawu *et al.*, 2016).

The following are quick climate information about Niger state: hottest Month is March (33 °C avg), the Month with much cold is August (26 °C avg), May is the month with much wetness (33.2 mm avg), December is the month with much wind (14 km/h avg), yearly precipitation is 122.2 mm (annually). There is fluctuation on the present weather conditions (Akpodiogaga-A & Odjugo, 2010; Simon *et al.*, 2010; Odjugo, 2011)

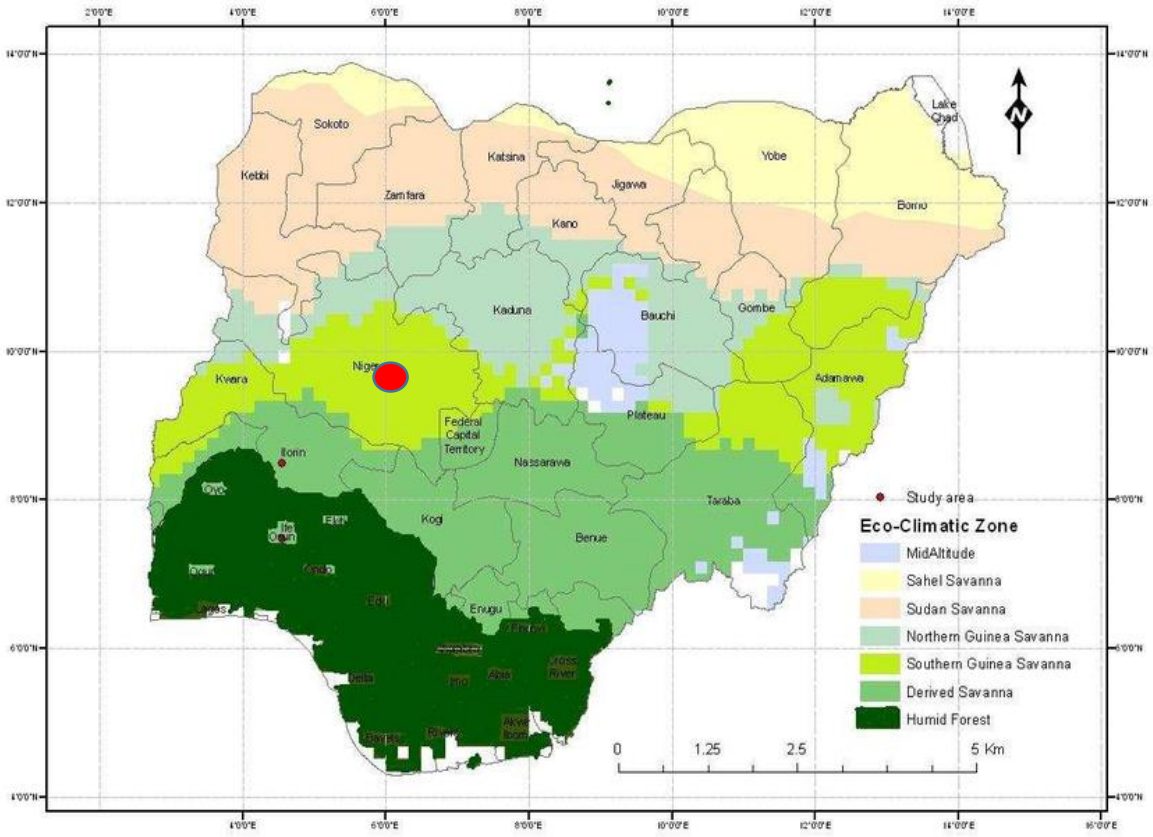


Figure 3.1: Nigerian Map Indicating the Eco-Climatic Zone with Focus on Niger State where Maize and Sorghum Sample Collected (Source: Ananymous)

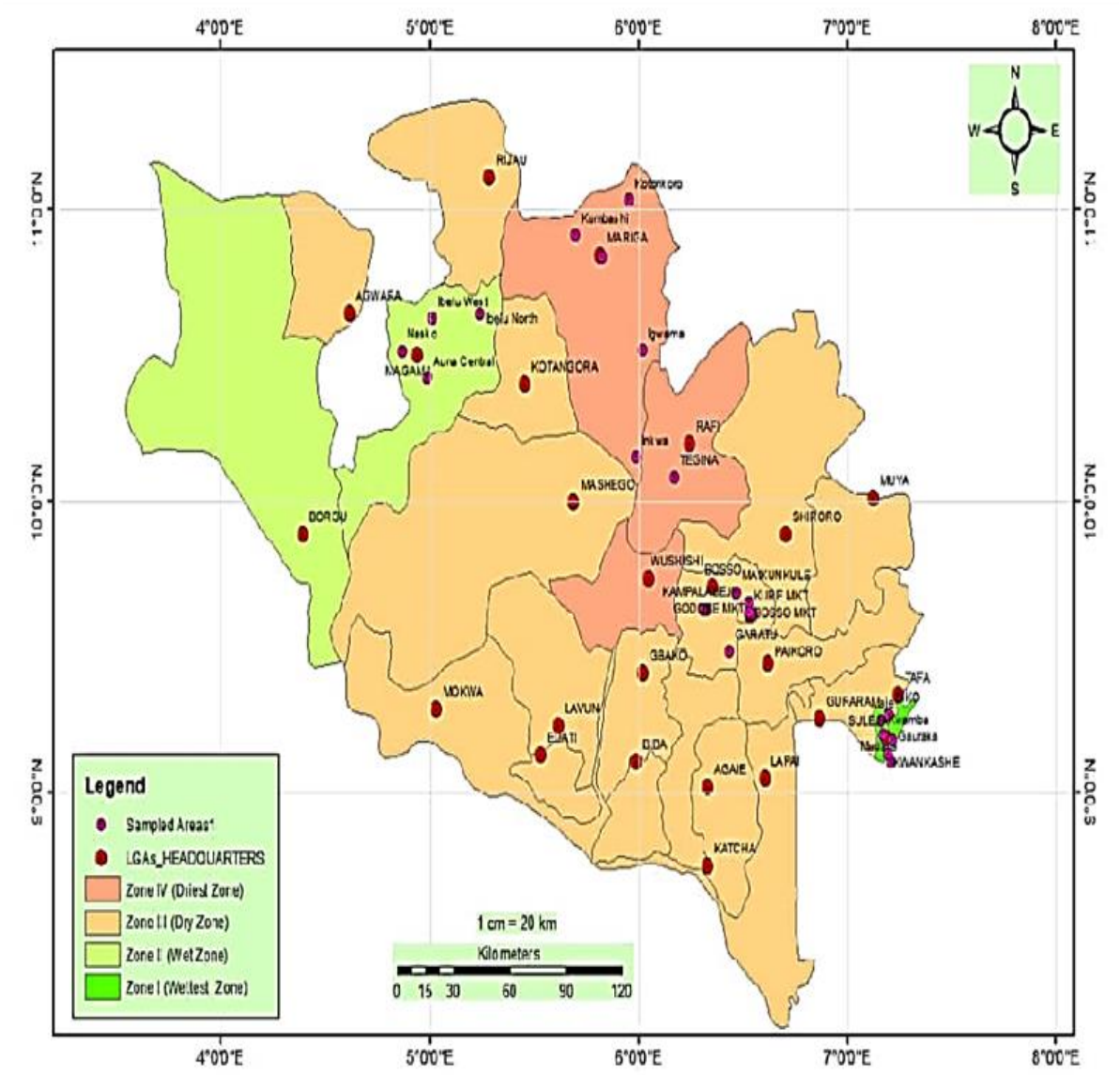


Figure 3.2: Maize and Sorghum Samples Collection According to Microclimatic Zone of Niger State

Source: Ananymous

Table 3.1: The Locations where the Sample were Collected

Microclimatic Zones	LGA	Districts	Rainfall range (mm)
Wettest	Suleja		>1400
Wet	Magama		1200 - 1400
Dry	Bosso		1000 - 1200
Driest	Mariga		<1000

3.1.4 Sample collection and preparation

The samples of maize and sorghum were obtained in agreement to Commission Regulation/ European Union (EU) No 178/2010 (2010); by means of 4 (properly) progressively samples amounting to 100 g cumulative sample for every single maize and sorghum samples.

The mean body weight of an adult consumers of maize and sorghum was estimated as 63.03 Kg for male and 64.29 Kg for female while the average weight of total population was 61.58 Kg. The average daily consumption of maize across the zones is 331.96 g and that of sorghum is 328.79 g. A total of four hundred and eighty (480) samples of stores and markets maize (with white and yellow maize) and sorghum (white and red sorghum) were collected comprising of 120 each of stored maize (white and yellow maize with 60 samples each), market maize (white and yellow maize with 60 samples each), store sorghum (white and red sorghum with 60 samples each) and market sorghum (white and red sorghum with 60 samples) samples. This collection was from the 5 communities each in 20 districts of the 4 Local Government Area (LGA) of the microclimatic zone of Niger state and compounded into 64 composite samples which comprises of 32 each of maize and sorghum composite samples.

The mixer grinder was employed to grind the maize and sorghum samples each, then thereafter 100 g of every single was separated for the different investigation.

3.2 Methods

The method includes fungal isolation and identification, the quantification of ochratoxin A and Cyclopiazonic acid in the collected samples of maize and sorghum.

3.2.1 Fungal isolation and identification

The method adopted by Njobeh *et al*. (2009) was employed in the fungal isolation and identification. The samples (1 g) macerated was measure out then suspended in solution (9 ml). This was blended for a period of 2 minutes with the aid of vortex mixer. Successive dilutions in three consecutive times were performed in order to get hold of 10^1 dilutions and 10^2 dilutions. The 10^2 dilution was plated. One milliliter of the diluent was cultured on potato dextrose agar (PDA). The cultured plates were carried out in incubation condition of 28 ± 2 ^oC. The growing process was watch after then stopped from 3^{rd} to 5^{th} days. The counting of the plate was carried out then separate isolates were further cultivated on Potato Dextrose Agar (PDA). The identification and naming were carried out by scrutinizing mutually macroscopic and microscopic characteristics of the fungi species for *Penicillium spp.* as it thrived on different suitable media.

3.2.2 Quantification of multiple mycotoxin

Except it is previously mentioned differently, all investigation was carried out at the "Central Research and Laboratory Diagnostics Ltd Tanke, Ilorin, Kwara State." The Nitrogen gas and the facility in Central Research Laboratory University of Ilorin, Kwara State were coupled to attempt the dryness of the sample extract. During the extraction of toxin from the samples as

found in sorghum and maize. The extraction of CPA with the initial method whereas the second method was employed for OTA extraction.

3.2.2.1 Extraction cyclopiazonic acid from maize and sorghum samples

Milled samples of all maize and sorghum were weighed (10 g every single one) into corresponding marked conical flasks. The samples in the conical flasks were treated in the present of 20 ml of the mixture of methanol (60 %): water (40 %) and mixed on a mechanical shaker lasting up to 2 hours. The mixed content was filtered through a Whatmann No. 2 filter paper. The micro filter (5µm pore size) which was preconditioned with mixture of methanol: water (3:1 v/v) was employed to further purify the obtained filtrate. Thereafter the purified filtrate was kept under suitable temperature of for 4^{o} C for subsequent analysis (Njobeh *et al*., 2009).

3.2.2.2 Extraction of Ochratoxin A from maize and sorghum

Each Maize and Sorghum samples (12.5 g) were measured into a conical flask to it added homogenous solution of 50 ml Acetonitrile 90 %: Water 10 % as the diluent. The conical flask containing the sample to be analysis and diluent was positioned on the mechanical shaker and let for 60 minutes to proceed. From that time on, the agitated substances were filtered by means of a Whatmann No 2. Filter paper and collected into a separating funnel. Subsequently, the collected filtrate and 12 ml of iso-octane were mixed together, were moved swirled carefully and the separated portion of liquid on the surface were thrown away. The technique employ will be done again in two more occasions in order to free the samples of fat substances.

The extract was restoring into the separating funnel for further extraction again and then put in 15 ml concentrated disodium hydrogen carbonate (Na_2HCO_3). The extraction process continues three more times in the present of 12 ml of Dichloromethane (DCM), in every single

extraction process, the collected layers of DCM is to be pooled into sole composite. Furthermore, the layer of DCM and layer of saturated disodium hydrogen carbonate layer (the aqueous layer) were collected separately with interest in the aqueous layer for separate mycotoxin analysis. Tetraoxosulphate (VI) acid (H_2SO_4) was measured (15 ml) into the aqueous layer shake up for 10 minutes, after a while it got settled and the effervescence gas ($CO_{2)}$ present finally evolved. Then 10 ml DCM was added further two more times to complete the extraction process. And the obtained extract is allowed to go pass through a bed of Na_2SO_4 salt which is collected in a round bottom flask (as acid fraction). The acid fraction was dried, and 2 ml DCM was used to reconstitute it, afterward poured into a screw cap vial and placed on the plate which is hot at 50^o C. Therefore, the OTA analysis is carried out from acid fraction portion (Njobeh *et al*., 2009).

3.2.3 Validation of mycotoxins method of HPLC computation

The consideration of linearity (quantification), precision (% retrieval) and responsiveness (limit of detection (LOD) and limit of quantification (LOQ)) were assessed for all the mycotoxins analyzed according to method of Abia *et al*. (2013). For quantification purposes, external calibration curves were established based on serial dilutions of the mycotoxin standard solutions as presented as follows: % retrieval is 96; calibration level (µg/kg) are 0.001, 0.01, 1.0; equation of straight line is y = 4E+07x – 27468 and r2 is 0.9998. Linear calibration curves created for the mycotoxin standards were taken to be acceptable after their correlation coefficients (r^2) are above 0.99. Recovery analysis was performed in multiply of three primarily on 3 smallest samples that are contaminated via spiking 5 g of each with 100 µl of standard concentration used in calibration. Finally, in order to ascertain the stability

between samples matrix and the mycotoxins, these were blended then kept for 60 hours in a fume cupboard at room temperature of 27 ^{o}C.

Thereafter 20 µL of the obtained extract using every single spiked sample, was introduced into the HPLC for analysis. The peak area of chromatogram of every single mycotoxin standard and the detected analyte was measured up to determine the quantity of the concentration of present mycotoxins. The Table 3.2 contain the % the recovery obtained are in line with the allowable limits of the recovery recommended by Codex Alimentarius or Association of Official Analytical Chemists (Patricia and AOAC, 1995). The suggestion of retrieval rate of mycotoxins according to Codex is 60 % – 120 % while AOAC is 70 – 125 %.

% Recovery = Concentration equivalent to the peak area computed from the spiked sample **X** 100 **/** Every single amount of toxin used for samples that are spiked

LOQ (0.06) as well as LOD (0.02) for CPA toxins in maize and sorghum samples were assessed treating the minimal amount in the samples that were spiked are determined as signal-to-noise ratio (S/N) of 3:1 and 10:1 respectively. HPLC specification for quantification of CPA include: column - C-18, Column Temperature (^{o}C) – 37, Wavelength - 284 nm, Injection Volume - 20 µl, Pressure Maximum - 420 Pa, Flow rate -0.6 ml/ml and Mobile phase (30: 70) - $ZnSO_4$(aq) (0.4mM): Methanol

3.2.4 The Enzymes Linked Immunosorbent Assay (ELISA) for ochratoxin A

The enzymes linked immunosorbent assay (ELISA) procedures was employed to measure OTA. This technique was used strictly on the guide and instruction of the producers of the testing kits. The validation parameters for enzyme-linked immunosorbent assay method

include: limit of detection is 2 µg / kg, Recovery (%) is 70 – 100 and Standard concentration (µg / kg) are 0, 0.15, 0.45, 1.35, 4.05 (Cusabio, 2015).

All reacting chemicals and the respective samples are allowed to attained suitable temperature in advance of the analysis proper. The analysis was carried out in replica of two of all samples, standards and controls. In each well introduce 50 µL of standard then followed by the respective samples. Further drop in each well 50 µL of HRP-conjugate and 50 µL of Antibody. Wrap the microtiter plate with a new adhesive strip and blend well, then allow the chemical kinetics to complete in a period of 30 minutes at temperature of 25 °C.

Remove the liquid in every single well and clean up, replicate the procedure four times. Add wash buffer once to every single well with 250 µL in order to further clean up and give it 30 seconds to stay, to achieved an excellence result then each step require holistic taken away of liquid. Through the means of aspiration or decantation the residue of wash buffer should be done away with. Turn the plate upside down and wipe out by the aid of clean hand towels.

Insert 100 µL substrate of TMB to every single well, mix well. Then allow the reaction to complete in 15 minutes at temperature 25 °C. Keep light from reaching the well at this step.

Introduce in every single well 50 µL of Solution to halt the reaction, mildly hit the plate in order to achieve homogeneousness of the content.

Then establish the optical density of every single well around 5 minutes, through the instrument microplate reader with wavelength of 450 nm (The suggested wavelength for Optical Density is: 450/630 nm not more than 0.083 hour), Cusabio (2015).

3.3 Data Analyses

The IBM SPSS Version 20, MS Excel 2016 and Origin 7.0 were used for data analyses. Results of the Ochratoxin and Cyclopiazonic acid in maize and sorghum samples were

reported on charts and tables after testing for significance using Analysis of Variance coupled with post – hoc Duncan analysis for evaluation of means of mycotoxins across the cereal types, varieties of cereal samples, location of the samples and among the microclimatic zones. The level of significance was accepted at $p < 0.05$. Percentage occurrence of each fungi species and colony forming units were determined using MS Excel (Table) and Origin application (Charts).

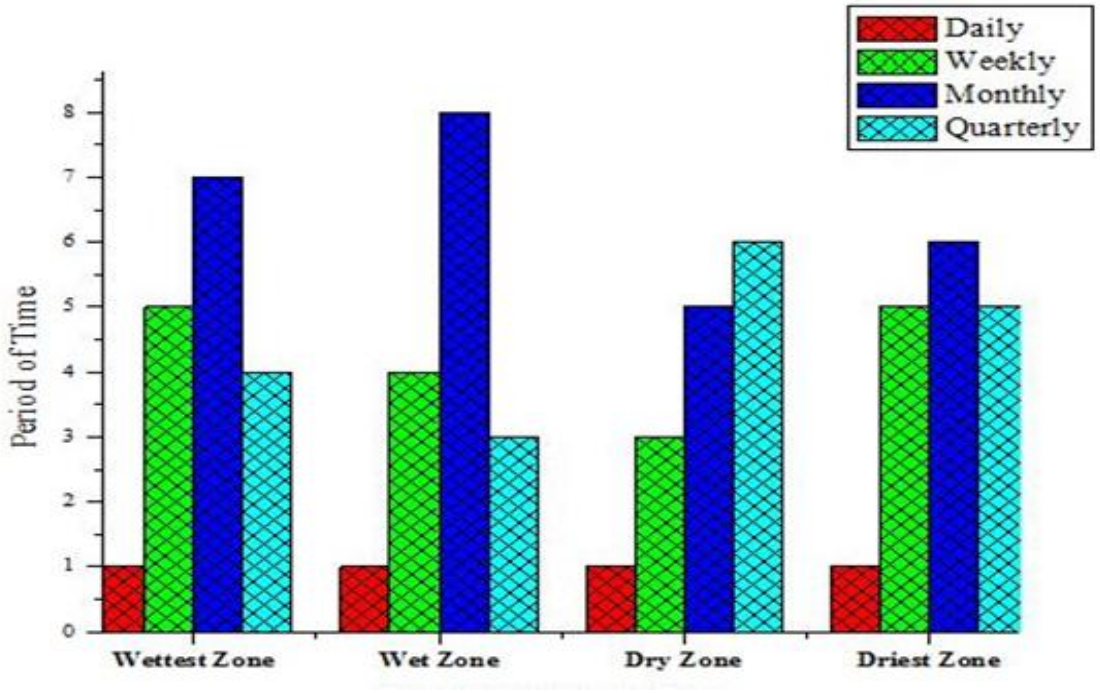
CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 The average number of time people consume maize and percentage frequency of maize consumption

The average of number of times of maize consumption in the four microclimatic zone is represented in Figure 4.1 as follows: the daily consumption of maize (once a day) is the same across the zones while weekly consumption (5 times a week) was highest in wettest and driest zones. The monthly consumption is highest in wet zone (8 times a month) and for quarterly consumption the highest consumption is in dry zone (6 times in 3 months). Therefore, the highest consumption rate is once daily in all the zones (Figure 4.1).



Key: Period of time is in days

Figure 4.1: The Average Number of Time People Consume Maize within the four Microclimatic Zones of Niger state

The frequency (%) of maize consumption in the different zones include : the decreasing order of frequency of maize consumption in wettest zone include - weekly (45 %) > daily (25 %) > monthly (15 %) > quarterly (10 %) > not at all (05 %), in wet zone - it include weekly (45 %) > daily (25 %) > monthly (20 %) > quarterly (10 %), in dry zone it include weekly (55 %) > monthly (15 %) > not at all (15 %) > daily (10 %) > quarterly (05 %) and in driest zone it include daily (45 %) > monthly (25 %) > weekly (20 %) > quarterly (10 %) > not at all (0 %). The frequencies of maize consumption are highest in dry zone as weekly (55 %), in wettest zone as weekly (45 %), in wet zone as weekly (45 %), and in driest zone as daily (45 %). Therefore, the highest frequency (%) of maize consumption is daily (45 %) in driest zone. The details are contained in Figure 4.2

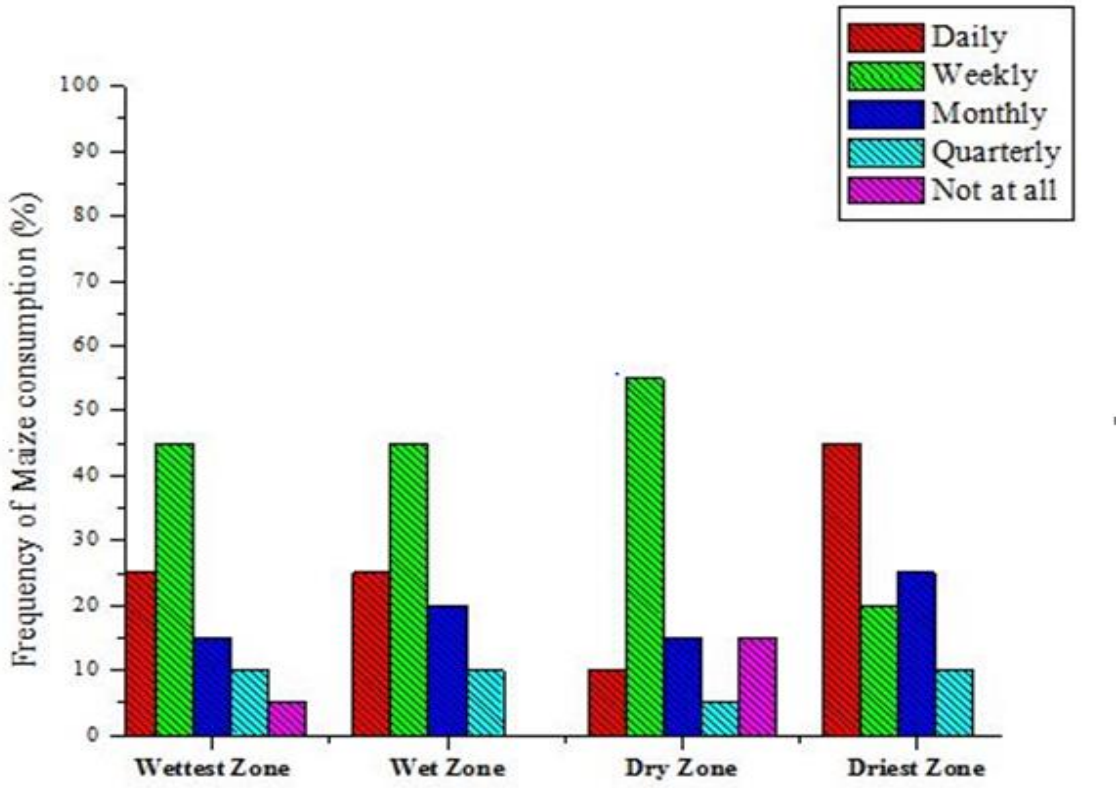
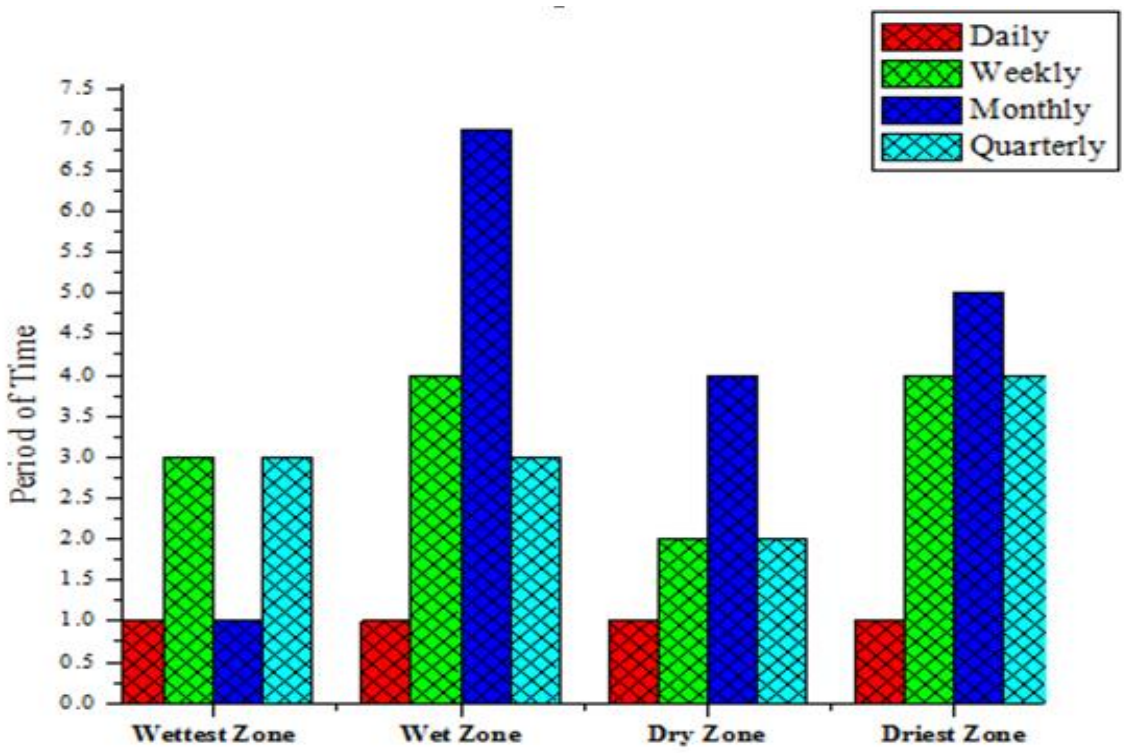


Figure 4.2: **Frequency (%) of Maize Consumption within the four Microclimatic Zones of Niger state**

4.1.2 The average number of times people consume sorghum and percentage frequency of sorghum consumption

The average number of times of sorghum consumption in the four microclimatic zone is represented in Figure 4.3 as follows: the daily consumption rate of sorghum is the same (once in a day) across the zones, for weekly consumption rate both wet and driest zones have the same number of time (4 times a week), monthly consumption rate have the highest consumption in wet zone (7 times a month) and quarterly consumption rate is highest in driest zones (4 times in 3 months). Therefore, the highest consumption rate is once daily in all the zones.



Key: Period of time is in days

Figure 4.3: The Average Number of Times People Consume Sorghum within the four Microclimatic Zones of Niger state

The frequency (%) of sorghum consumption in the wettest zone, wet zone, dry zone and driest zones include: the decreasing order of sorghum consumption in wettest zone include – weekly (40 %) > monthly (25 %) > not at all (15 %) > daily (10 %) > quarterly (10 %), it include wet zone – weekly (55 %) > daily (20 %) > monthly (15 %) > quarterly (05 %) > not at all (05 %), it include dry zone – weekly (35 %) > quarterly (25 %) > not at all (20 %) > monthly (15 %) > daily (05 %) and in driest zone it include – weekly (45 %) > daily (25 %) > quarterly (15 %) > not at all (10 %) > monthly (05 %). The frequency of sorghum consumption is highest in wet zone as weekly (55 %), in dry zone as weekly (45 %), in wettest zone as weekly (40 %) and in driest zone as weekly (35 %). Therefore, the highest frequency (%) of sorghum consumption is daily (25 %) in driest zone (Figure 4.4).

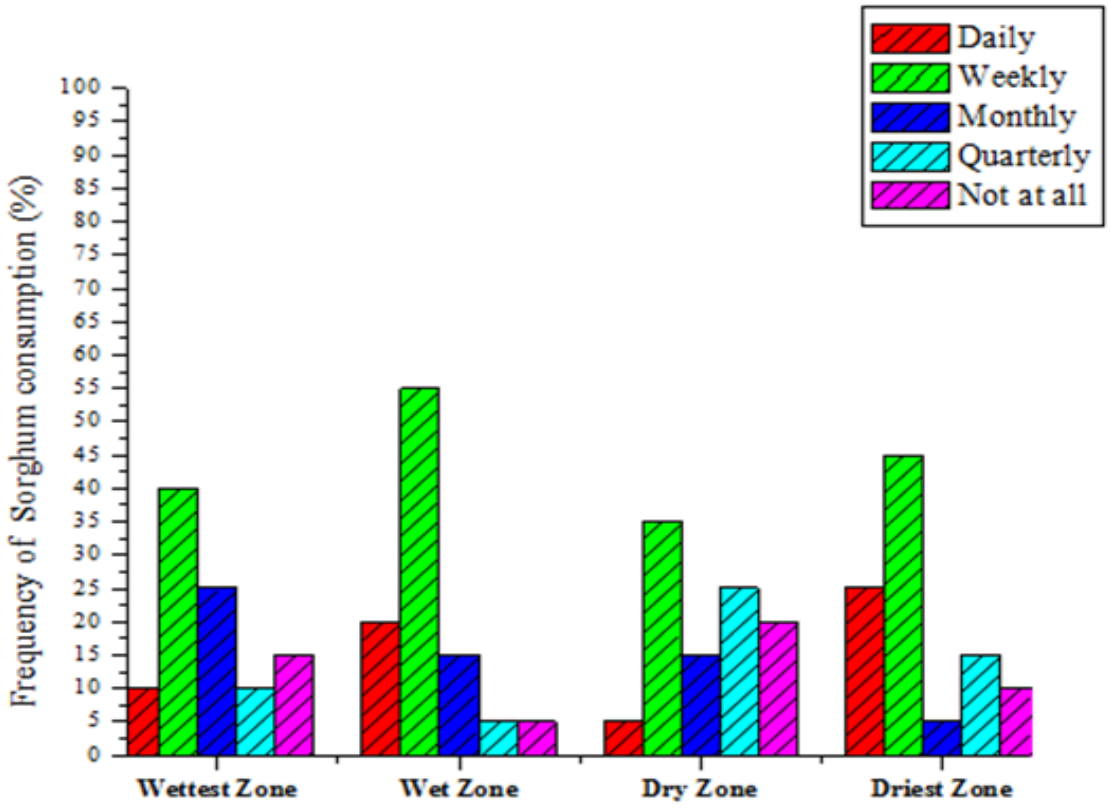
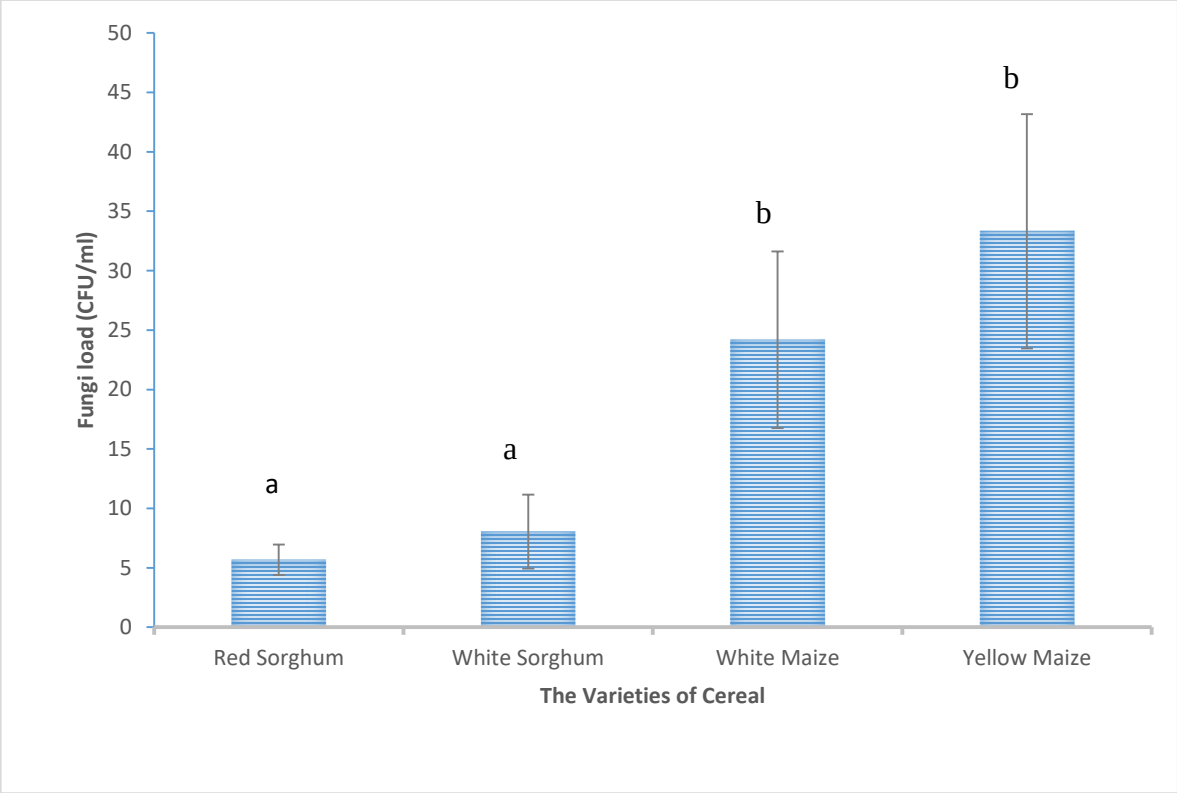


Figure 4.4: Frequency of Sorghum Consumption within the microclimatic zones of Niger state

4.1.3 The Fungal load in colony forming unit (CFU/ml) of varieties of cereals

The fungal load in colony in red sorghum, white sorghum, white maize and yellow maize are represented below (Figure 4.5). The fungal load in CFU/ml bears a meaningful dissimilarity ($p \leq 0.05$) between maize and the sorghum cereals, but no significant difference between white and red sorghum and also between white maize and yellow maize sample. The Mean±SEM value fungi load is highest in yellow maize samples as 33.31±9.86 X 10^6 CFU/ml and the lowest is in red sorghum as 5.69±1.28 X 10^6 CFU/ml.

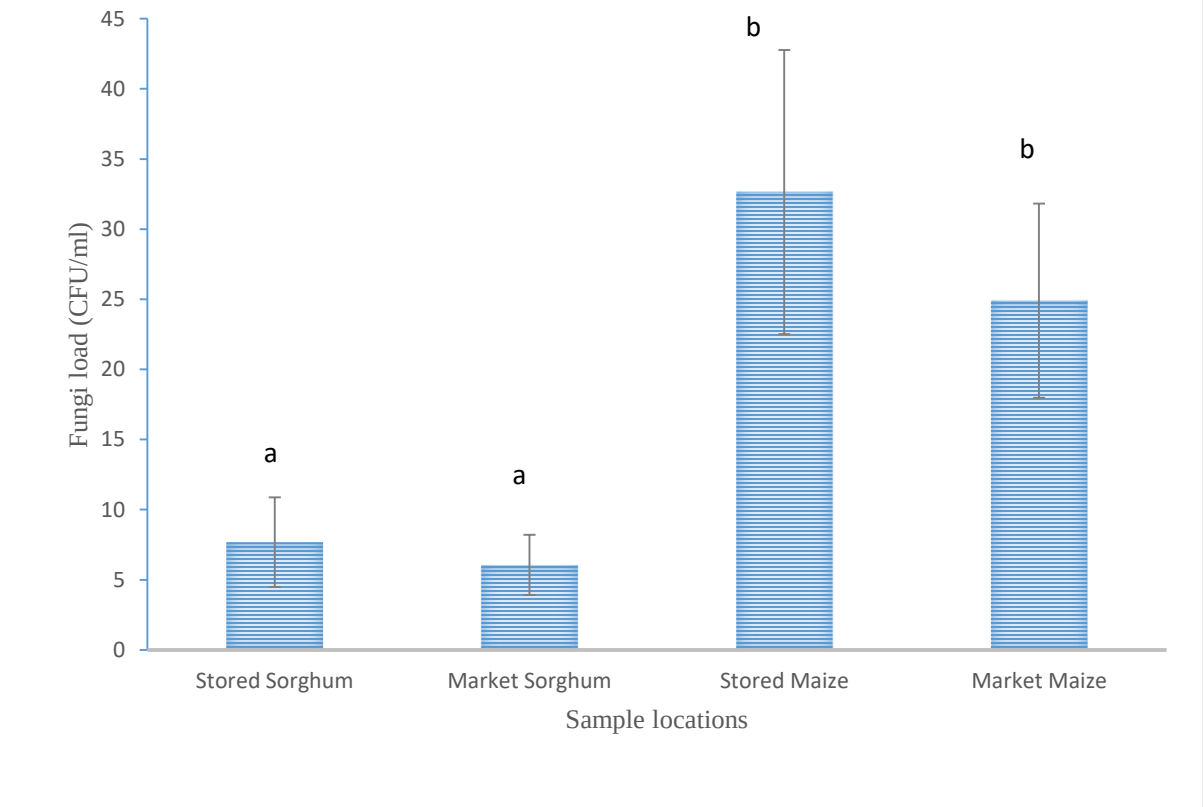


Key: Values with different letters on sorghum and maize bars each across the bars are significantly different ($p \leq 0.05$).

Figure 4.5: The Fungal Load (CFU/ml) of Varieties of Cereal Samples

4.1.4 The Fungal Load in colony forming unit (CFU/ml) of stored and market samples

The fungal load in colony in store sorghum, maize sorghum, store maize and market maize are represented below (Figure 4.6). There is a significant difference ($p \leq 0.05$) in the fungi colony forming unit in the cereal samples between stored sorghum and stored maize and likewise between market sorghum and market maize samples. The stored maize has the highest Mean±SEM value of 32.63±10.13 X 10^6 CFU/ml while the lowest Mean±SEM is in market sorghum as 6.06±2.15 X 10^6 CFU/ml.

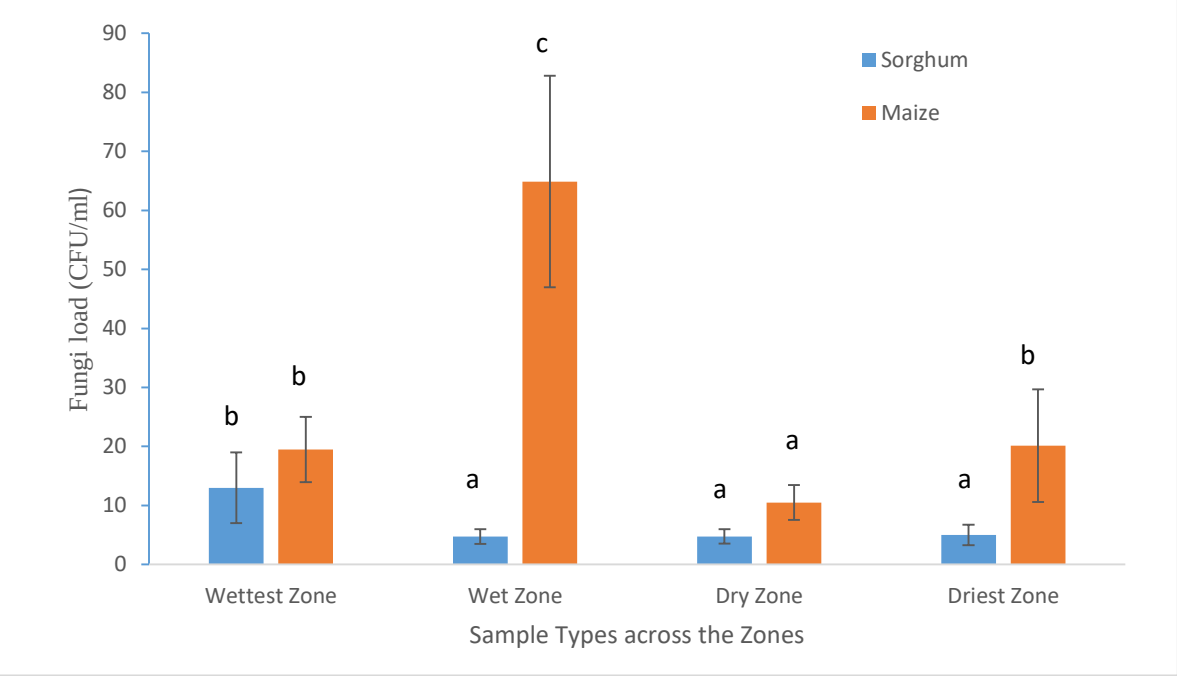


Key: Values with different letters on sorghum and maize bars each across the bars are significantly different ($p \leq 0.05$).

Figure 4.6: The Fungal Load (CFU/ml) of Stored and Market Cereal Samples

4.1.5 The Fungal load in colony forming unit (CFU/ml) of the cereals across the zones

The fungal load in colony in sorghum and maize from wettest zone, wet zone, dry zone and driest zone are represented below (Figure 4.7). In sorghum sample implicative differences is not present ($p \leq 0.05$) in the Mean±SEM value from one zone to another apart from wettest zone while in maize sample there is a significant difference ($p \leq 0.05$) Mean±SEM except between wettest zone and wet zone. From Figure 4.7 the highest value of the fungi load (CFU/ml) Mean±SEM of sorghum is 13.00±5.98 X 10^6 CFU/ml in wettest zone and the lowest is 4.75±1.25 X 10^6 CFU/ml in each of wet and dry zones. In maize, the highest Mean±SEM value is 64.88±17.91 X 10^6 CFU/ml in wet zone and lowest is 10.50±2.96 X 10^6 CFU/ml in dry zone.



Key: Values with different letters on sorghum and maize bars each across the bars are significantly different ($p \leq 0.05$).

Figure 4.7: The Fungal Load (CFU/ml) of the Cereals across the Zones

4.1.6 *Penicillium* species in maize samples across the zones

The occurrence of *Penicillium spp* in maize sample samples in wettest zone, wet zone, dry zone and driest zone is represented in Table 4.1. The total occurrence of each *Penicillium species* in maize samples across the zones are as follows: *P. verrucosom* 25 (32), *P. griseofulvum* 06 (32), and *P. chrysogenum* 07 (32). The highest occurrence of *P. verrucosom* is 07 (08) in wettest zone, *P. griseofulvum* is 02(08) in wettest and driest zone each, and *P. chrysogenum* is 03 (08) in wettest and dry zone each. Therefore *P. verrucosom* had the highest incidence of 21.9 % in wet zone and the lowest incidence is in driest zone with *P. chrysogenum* of 0 %.

Table 4.1: Occurrence of *Penicillium spp* across the Zones for Maize Sample.

***Penicillium* species**	**Wettest Zone N=08**	**Wet Zone N=08**	**Dry Zone N=08**	**Driest Zone N=08**	**Total Occurrence N=32**	**% Freq. of Wettest Zone**	**% Freq. of Wet Zone**	**% Freq. of Dry Zone**	**% Freq. of Driest Zone**
P. verrucosom	6 (54.5)	7 (77.8)	6 (60)	6 (75)	25	18.8	21.9	18.8	18.8
P. griseofulvum	2 (18.2)	1 (11.1)	1 (1.0)	2 (25)	06	6.3	3.1	3.1	6.3
P. chrysogenum	3 (27.3)	1 (11.1)	3 (30.0)	0 (0)	07	9.4	3.1	9.4	0
T. Occurrence	11	09	10	08	38	34.5	28.1	31.3	25.1

Key: Freq.= frequency, %= percentage, T = Total, N= number of samples, () = the number in the bracket is % of total occurrence of *Penicillium spp* in each zone while the number outside the bracket is number of positive samples with the *Penicillium spp.*

4.1.7 *Penicillium* species in store and market maize samples

The occurrence of *Penicillium spp* in store and market maize samples are represented in Table 4.2. The total occurrence of each *Penicillium species* in store and market maize samples are as follows: *P. verrucosom* 25 (32), *P. griseofulvum* 06 (32), and *P. chrysogenum* 07 (32). The highest occurrence of *P. verrucosom* is 14(16) in market samples, *P. griseofulvum* is 04(16) in market samples, and *P. chrysogenum* is 05(16) in store samples. Therefore, the highest incidence is 44 % of *P. verrucosum* in market maize samples while the lowest incidence is 6 % each of *P. griseofulvum* and *P. chrysogenum* in store and market samples respectively.

Table 4.2: Occurrence of *Penicillium* in Store and Market Maize Sample.

***Penicillium* species**	**SM samples N=16**	**MM samples N=16**	**Total Occurrence N=32**	**% Freq. of SM samples**	**% Freq. of MM samples**
P. verrucosom	11 (61)	14 (70)	25	34	44
P. griseofulvum	02 (11)	04 (20)	06	06	13
P. chrysogenum	05 (28)	02 (10)	07	16	06
T. Occurrence	18	20	38	118.76	63

Key: Freq.= frequency, %= percentage, T = Total, N= number of samples, SM= Store maize, MM= Market maize, () = the number in the bracket is % of total occurrence of *Penicillium spp* in each of store and market maize while the number outside the bracket is number of positive samples with the *Penicillium spp.*

4.1.8 *Penicillium* species in white and yellow maize samples

The occurrence of *Penicillium spp* in white maize and yellow maize samples are represented in Table 4.3. The total occurrence of each *Penicillium species* in white and yellow maize samples are as follows: *P. verrucosom* 25 (32), *P. griseofulvum* 06 (32), and *P. chrysogenum* 07 (32). The highest occurrence of *P. verrucosom* is in white maize as 13 (16), *P. griseofulvum* is in both white and yellow maize as 03 (16) each, and *P. chrysogenum* is in yellow maize as 05 (16). Therefore, the highest incidence of *P. verrucosom* is 40.63 % in white maize, of *P. chrysogenum* is 15.63 in yellow maize and of *P. griseofulvum* is 9.37 % in both white and yellow maize.

Table 4.3: Occurrence of *Penicillium spp* for Varieties of Maize Sample.

Penicillium **species**	**WM N=16**	**YM N=16**	**Total Occurrence N=32**	**Frequency % Occurrence of WM**	**Frequency % Occurrence of YM**
P. verrucosom	13 (72.2)	12 (60)	25	40.63	37.5
P. griseofulvum	03 (16.67)	03 (15)	06	9.37	9.37
P. chrysogenum	02 (11.11)	05 (25)	07	6.25	15.63
Total Occurrence	18	20	38	56.25	62.5

Key: WM= White maize, YM= Yellow maize, %= percentage, *P.*= *Penicillium*, () = the number in the bracket is % of total occurrence of *Penicillium spp* in each white and yellow maize sample while the number outside the bracket is number of positive samples with the *Penicillium spp.*

4.1.9 *Penicillium* species in Sorghum Samples across the zone

The occurrence of *Penicillium spp* in sorghum samples from wettest zone, wet zone, dry zone and driest zone are represented in Table 4.4. The total occurrence of *Penicillium species* is as follows: *P. verrucosom* as 24 (32), *P. griseofulvum* as 04 (32), and *P. chrysogenum* as 05 (32). The highest occurrence of *P. verrucosom* is 07 (08) at driest zone, *P. griseofulvum* is 02(08) at wet zone, and *P. chrysogenum* is 02 (08) at wet and dry zone each (Table 4.4). Therefore, the highest incidence is in *P. verrucosum* (21.9 %) in driest zone while incidence of 0 % occurred in dry and driest zone with *P. griseofulvum* and *P. chrysogenum* respectively.

Table 4.4: The Occurrence of *Penicillium spp* across the zones for Sorghum Sample

***Penicillium* species**	**Wettest Zone N=08**	**Wet Zone N=08**	**Dry Zone N=08**	**Driest Zone N=08**	**Total Occurrence N=32**	**% Freq. of Wettest zone**	**% Freq. of Wet zone**	**% Freq. of Dry zone**	**% Freq. of Driest zone**
P. verrucosom	05 (71.4)	06 (60.0)	06 (75)	07 (87.5)	24	15.6	18.8	18.8	21.9
P. griseofulvum	01 (3.1)	02 (20.0)	0 (0.0)	01 (12.5)	04	3.1	6.3	0	3.1
P. chrysogenum	01 (3.1)	2 (20.0)	02 (25.0)	0 (0.0)	05	3.1	6.3	6.3	0
T. Occurrence	07	10	08	08	33	21.9	31.25	25	25

Key: Freq. = frequency, N= number of samples, %= percentage, T.= Total, () = the number in the bracket is % of total occurrence of *Penicillium spp* in each zone while the number outside the bracket is number of positive samples with the *Penicillium spp.*

4.1.10 *Penicillium* species in store and market sorghum samples

The occurrence of *Penicillium spp* in store and market sorghum samples are represented in Table 4.5. The highest occurrence of *P. verrucosom* is in market samples 14 (16), *P. griseofulvum* is 02 (16) in each of store and market samples and *P. chrysogenum* is 03 (16) in market samples. Therefore, the highest incidence is 43.8 % of *P. verrucosum* in market maize samples while the lowest incidence is 6.3 % each of *P. griseofulvum* (in both store and market samples) and *P. chrysogenum* in store samples.

Table 4.5: **The Occurrence of *Penicillium spp* in Store and Market Sorghum Samples**

***Penicillium* species**	**Store samples N=16**	**Market samples N=16**	**Total occurrence N=32**	**% Freq. of Store sample**	**% Freq. of Market sample**
P. verrucosom	10 (71.4)	14 (73.7)	24	31.3	43.8
P. griseofulvum	02 (14.3)	02(10.5)	04	6.3	6.3
P. chrysogenum	02 (14.3)	03 (15.8)	05	6.3	9.4
Total Occurrence	14	19	33	43.9	59.5

Key: Freq. = frequency, %= percentage, N= number of samples, *P*. = *Penicillium*, () = the number in the bracket is % of total occurrence of *Penicillium spp* in each store and market sorghum while the number outside the bracket is number of positive samples with the *Penicillium spp.*

4.1.11 *Penicillium* species in white and red sorghum samples

The occurrence of *Penicillium spp* in white sorghum and red sorghum samples are represented in Table 4.6. The highest occurrence of *P. verrucosom* is in white sorghum 13 (16), *P. griseofulvum* is 02 (16) in red sorghum, and *P. chrysogenum* is in red sorghum 03 (16). (See details in Table 4.6). Therefore, the highest incidence of *P. verrucosom* is 40.6 % in white sorghum, of *P. chrysogenum* is 9.4 % in red sorghum and of *P. griseofulvum* is 6.3 % in red sorghum.

Table 4.6: Occurrence of *Penicillium* spp in Varieties of Sorghum Samples

***Penicillium* species**	**RS N=16**	**WS N=16**	**Total occurrence N=32**	**% Freq. in RS sample**	**% Freq. in WS sample**
P. verrucosom	11 (68.80)	13 (76.47)	24	34.40	40.6
P. griseofulvum	02 (12.50)	02 (11.76)	04	6.30	6.30
P. chrysogenum	03 (18.80)	02 (11.76)	05	9.40	6.30
T. Occurrence	16	17	32	50.10	53.20

Key: Freq. = frequency, %=percentage, N= number of samples, *P.* = *Penicillium*, T. = Total, WS= White sorghum, RS= Red sorghum, () = the number in the bracket is % of total occurrence of *Penicillium spp* in each red and white sorghum while the number outside the bracket is number of positive samples with the *Penicillium spp.*

4.1.12 Ochratoxin A (OTA) concentration detected in the zones

The Table 4.7 represent the summary details of ochratoxin A: the ranges of concentration, the figure of samples (N), the quantities of samples detected to be containing OTA (n) and % incidences of samples detected with OTA in μg/kg. The highest % incidence of OTA production in wettest zone is 100 % in store maize, market maize and store sorghum; in wet zone is 100 % in store maize, market maize and market sorghum; in driest zone is 100 % only store sorghum and market sorghum. The widest range values of market sample are 0.88 – 75.46 μg/kg in wet zone, store maize is 3.48 – 47.84 μg/kg in wettest zone, market sorghum is 5.97 – 49.34 μg/kg and in store sorghum zone is 0.66 – 9.78 μg/kg in dry zone.

Table 4.7: Ochratoxin A Concentration, % Incidence, Ranges and Numbers of Samples

Food Samples	Wettest Region – Suleja				Wet Region-Magama				Dry Region - Bosso				Driest Region - Mariga			
	N	n	% Incidence	Range in µg/kg	N	N	% Incidence	Range in µg/kg	N	n	% Incidence	Range in µg/kg	N	n	% Incidence	Range in µg/kg
SS	4	4	100	0.30-7.30	4	3	75	0.92-5.17	4	3	75	0.66-9.78	4	4	100	7.55-10.52
MS	4	3	75	8.39-22.53	4	4	100	2.28-37.51	4	3	75	5.97-49.34	4	4	100	2.28-6.93
SM	4	4	100	3.48-47.84	4	4	100	5.43-7.07	4	3	75	4.91-7.99	4	3	75	3.52-6.67
MM	4	4	100	4.29-5.72	4	4	100	0.88-75.46	4	4	75	1.80-8.28	4	3	75	4.11-23.23

Key: SS= Store sorghum, MS= Market sorghum, SM= Store maize, MM= Market maize, N= number of samples, n= number of positive samples, %= percentage

4.1.13 Ochratoxin A (OTA) concentration in stored and market samples

The Table 4.8 represent the mean concentration of stored sorghum, market sorghum, store maize and market maize detected with OTA in μg/kg. Stored sorghum values are significantly difference ($p \leq 0.05$) except wettest zone and dry zone. Market sorghum estimate are implicatively dissimilar in all the zones. Stored maize estimates are implicatively dissimilar in all the zone as well. And market maize estimates are implicatively dissimilar in all the zones except wettest and dry zones. In the zones, there is a significant difference among the sample locations in wettest zone; wet zone and driest zone while in dry zone there is significant difference between stored sorghum and market sorghum only. The OTA was detected highest Mean±SEM in market maize as 24.381±17.185 μg/kg in wet zone, market sorghum Mean±SEM is 16.138±11.233 μg/kg in dry zone, stored maize as 15.663 ± 10.762 μg/kg in wettest zone, and the OTA in stored sorghum as 8.447± 0.703 μg/kg in driest zone. While in each zone, the highest concentration of OTA in wettest zone is stored maize as 15.663 ± 10.762 μg/kg, in wet zone is market maize as 24.381±17.185 μg/kg, dry zone is market sorghum as 16.138± 11.233 μg/kg and driest zone is market maize as 10.140± 5.164 μg/kg.

Table 4.8: Ochratoxin A (OTA) Concentration in Stored and Market Cereals in each Zones

Food Samples (µg/kg)	Wettest zone - Suleja OTA	Wet zone - Magama OTA	Dry zone - Bosso OTA	Driest zone – Mariga OTA
SS	$4.006 \pm 1.448^{b}_{a}$	$2.594 \pm 1.250^{a}_{a}$	$4.215 \pm 2.349^{b}_{a}$	$8.447 \pm 0.703^{c}_{c}$
MS	$10.094 \pm 4.654^{b}_{c}$	$12.861 \pm 8.271^{c}_{c}$	$16.138 \pm 11.233^{d}_{c}$	$3.969 \pm 1.084^{a}_{b}$
SM	$15.663 \pm 10.762^{d}_{d}$	$6.286 \pm 0.337^{c}_{b}$	$4.663 \pm 1.665^{b}_{b}$	$3.574 \pm 1.374^{a}_{a}$
MM	$4.995 \pm 0.298^{a}_{b}$	$24.381 \pm 17.185^{c}_{d}$	$4.663 \pm 1.665^{a}_{b}$	$10.140 \pm 5.164^{b}_{d}$

Key: SS= Store sorghum, MS= Market sorghum, SM= Store maize, MM= Market maize, N= number of samples, n= number of positive samples, %= percentage, Values with different superscripts across the column and subscripts down the row are significantly different ($p \leq 0.05$).

4.1.14 Ochratoxin A (OTA) concentration in varieties of sorghum and maize samples

The Table 4.9 represent the mean concentration of red sorghum, white sorghum, white maize and yellow maize detected with OTA in μg/kg. Red sorghum values are significantly difference ($p \leq 0.05$) in all zones except in dry and driest zones. White sorghum values are significantly different in all except wettest and wet zones. White maize is not significantly different between wettest and dry zones, and between wet zone and driest zones. Yellow maize values are significantly different in all the zones except dry and driest zones. In wettest zone there is no significant difference ($p \leq 0.05$) between white sorghum and white maize. In wet zone and driest zone, there is no significant in among the varieties of cereals. While in dry, only white sorghum and white maize have significant difference. The highest OTA detected was in yellow maize as 22.439±17.727 μg/kg in wet zone, white sorghum as 14.105±11.834 μg/kg in dry zone, red sorghum as 10.177±9.124 μg/kg in wet zone and in white maize as 8.501±5.097 μg/kg in driest zone (Table 4.9). While in each zone, the highest concentration of OTA in wettest zone is yellow maize as 16.313±10.529 μg/kg, wet zone is yellow maize as 22.439±17.727 μg/kg, in dry zone is white sorghum as 14.105±11.834 μg/kg and in driest zone is white maize as 8.501±5.097 μg/kg.

Table 4.9: Ochratoxin A (OTA) Concentration in Varieties of Cereals in each Zone

Food sample (µg/kg)	Wettest zone - Suleja OTA	Wet zone – Magama OTA	Dry zone – Bosso OTA	Driest zone – Mariga OTA
RS	$8.618\pm4.880^{c}_{b}$	$10.177\pm9.124^{d}_{c}$	$6.248\pm2.246^{b}_{b}$	$5.096\pm1.591^{a}_{a}$
WS	$5.489\pm2.120^{a}_{a}$	$5.279\pm0.566^{a}_{a}$	$14.105\pm11.834^{c}_{c}$	$7.320\pm1.286^{b}_{b}$
WM	$4.345\pm0.385^{a}_{a}$	$8.227\pm1.438^{b}_{b}$	$4.994\pm1.254^{a}_{a}$	$8.501\pm5.097^{b}_{c}$
YM	$16.313\pm10.529^{b}_{c}$	$22.439\pm17.727^{c}_{d}$	$5.589\pm1.926^{a}_{b}$	$5.213\pm2.821^{a}_{a}$

Key: RS= Red sorghum, WS= White sorghum, WM= White maize, YM= Yellow maize, Values with different superscripts across the column and subscripts down the row are significantly different ($p\leq0.05$).

4.1.15 Cyclopiazonic acid (CPA) concentration detected in the zones

The result in Table 4.10 represents the summary details of Cyclopiazonic acid concentration, the ranges of concentration, the figure of samples (N), quantities of samples detected to be containing CPA (n) and % incidences of samples detected with CPA in μg/kg. The highest % incidence of CPA production in wettest zone is 75 % in store maize; in wet zone is 75 % in store maize, market maize and store sorghum; in dry zone is 75 % in store maize and in driest zone is 75 % in store sorghum. The widest range values of market maize sample are 0.0 - 83.6 μg/kg in dry zone, store maize is 39.3 – 417.2 μg/kg in dry zone (had the highest value), market sorghum is 17.2 – 79.9 μg/kg and in store sorghum zone is 2.5 – 54.4 μg/kg in wet dry zone.

Table 4.10: Cyclopiazonic Acid Concentration, % Incidence, Ranges and Numbers of Samples

Food Samples	Wettest zone – Suleja				Wet zone – Magama				Dry zone - Bosso				Driest zone - Mariga			
	N	n	% Incidence	Range in µg/g	N	n	% Incidence	Range in µg/kg	N	n	% Incidence	Range in µg/kg	N	n	% Incidence	Range in µg/kg
SS	3	0	0	-	4	3	75	2.5 – 54.4	4	1	25	0.0– 1.2	4	3	75	0.2– 27.3
MS	4	2	50	17.2-79.9	3	1	33.3	0.0 – 13.1	3	1	33.3	0.0– 36.8	4	1	25	0.0– 13.1
SM	4	3	75	19.7-77.8	4	3	75	2.7 – 33.8	4	3	75	39.3-417.2	3	0	0	-
MM	4	1	25	0.0 -1.0	4	3	75	1.6– 61.4	4	1	25	0.0-83.6	4	2	50	45.7– 75.3

Key: SS= Store sorghum, MS= Market sorghum, SM= Store maize, MM= Market maize, N= number of samples, n= number of positive samples, %= percentage, Values with different superscripts across the column and subscripts down the row are significantly different ($p \leq 0.05$).

4.1.16 Cyclopiazonic acid (CPA) concentration in stored and market samples

The Table 4.11 represent the mean concentration of stored sorghum, market sorghum, store maize and market maize detected with CPA in μg/kg. Stored sorghum values are all significantly difference ($p \leq 0.05$) in all the zones. Market sorghum values are having significantly different except in wet and dry zones. Stored maize values are all significantly different from each other. Market maize values bears significant different in all zones. There is significant difference ($p \leq 0.05$) in CPA concentration of stored and market cereals in wettest zone, wet zone dry zone and driest zone. The highest CPA concentration was detected in stored maize as 135.921± 95.450 μg/kg in dry zone, market maize as 33.994±18.843 μg/kg in wet zone, market sorghum as 24.297±18.999 μg/kg in wettest zone and in stored sorghum as 16.991±12.682 μg/kg. While in each zone, the highest concentration of CPA in wettest zone is stored maize as 36.805±17.070 μg/kg, in wet zone is market maize as 33.994±18.843 μg/kg, in dry zone is stored maize as135.921± 95.450 μg/kg and in driest zone is market maize as 30.256 ± 18.482 in μg/kg.

Table 4.11: Cyclopiazonic (CPA) Concentration in Stored and Market Cereals in each Zones

Food Samples (μg/kg)	Wettest Région - Suleja CPA	Wet Region – Magama CPA	Dry Region - Bosso CPA	Driest Region – Mariga CPA
SS	$0.000 \pm 0.000^{a}{}_{a}$	$16.991 \pm 12.682^{d}{}_{c}$	$0.311 \pm 0.311^{b}{}_{a}$	$9.609 \pm 6.426^{c}{}_{c}$
MS	$24.297 \pm 18.999^{c}{}_{c}$	$9.236 \pm 6.114^{b}{}_{a}$	$9.207 \pm 9.207^{b}{}_{b}$	$3.295 \pm 3.295^{a}{}_{b}$
SM	$36.805 \pm 17.070^{c}{}_{d}$	$12.444 \pm 7.664^{b}{}_{b}$	$135.921 \pm 95.450^{c}{}_{d}$	$0.000 \pm 0.000^{a}{}_{a}$
MM	$0.251 \pm 0.251^{a}{}_{b}$	$33.994 \pm 18.843^{d}{}_{d}$	$20.903 \pm 20.903^{b}{}_{c}$	$30.256 \pm 18.482^{c}{}_{d}$

Key: SS= Store sorghum, MS= Market sorghum, SM= Store maize, MM= Market maize, Values with different superscripts across the column and subscripts down the row are significantly different ($p \leq 0.05$).

4.1.17 Cyclopiazonic acid (CPA) concentration in varieties of sorghum and maize samples

The Table 4.12 represent the mean concentration of red sorghum, white sorghum, white maize and yellow maize detected with CPA in μg/kg. Red sorghum values bear significant difference ($p \leq 0.05$) only in dry and driest zones. The values of white sorghum, white maize and yellow maize showed significant difference in all the zones. In each zone, there is significant difference ($p \leq 0.05$) among the varieties of cereals. The highest CPA concentration was detected in white maize as 114.154±101.457 μg/kg in dry zone, yellow maize as 27.108±13.376 μg/kg in wet zone, red sorghum as 20.041±12.957 μg/kg in wet zone and white sorghum as 19.995±19.995 μg/kg in wettest zone. While in each zone, the highest concentration of CPA in wettest zone is white maize as 31.868±19.285 μg/kg, in wet zone is yellow maize as 27.108±13.376 μg/kg, in dry zone is white maize as 114.154±101.457 μg/kg and in driest zone is white maize as 30.256±18.482 μg/kg (Table 4.12).

Table 4.12: Cyclopiazonic (CPA) Concentration of Varieties of Cereals in the Zones

Food Samples	Wettest zone - Suleja CPA in μg/kg	Wet zone - Magama CPA in μg/kg	Dry zone - Bosso CPA in μg/kg	Driest zone – Mariga CPA in μg/kg
RS	$4.302 \pm 4.302^{a}{}_{a}$	$20.041 \pm 12.957^{d}{}_{c}$	$9.518 \pm 9.108^{b}{}_{b}$	$10.171 \pm 6.489^{c}{}_{c}$
WS	$19.995 \pm 19.995^{d}{}_{c}$	$6.186 \pm 2.889^{c}{}_{a}$	$0.000 \pm 0.000^{a}{}_{a}$	$2.732 \pm 2.732^{b}{}_{b}$
WM	$31.868 \pm 19.285^{c}{}_{d}$	$18.643 \pm 17.190^{a}{}_{b}$	$114.154 \pm 101.457^{d}{}_{d}$	$30.256 \pm 18.482^{b}{}_{d}$
YM	$5.187 \pm 4.859^{b}{}_{b}$	$27.108 \pm 13.376^{c}{}_{d}$	$42.671 \pm 24.646^{d}{}_{c}$	$0.000 \pm 0.000^{a}{}_{a}$

Key: RS= Red sorghum, WS= White sorghum, WM= White maize, YM= Yellow maize, Values with different superscripts across the column and subscripts down the row are significantly different ($p \leq 0.05$).

4.1.18 Co-occurrence of OTA and CPA in store and market samples

The co-occurrence of OTA and CPA in store and market samples are represented in Table 4.13. The collected samples have co-occurrence of OTA/CPA as follows: stored maize samples as 9 (16), 56.25 %; stored sorghum samples as 7(16), 43.75 %; each of market maize and market sorghum samples as 6 (16), 37.50 %.

Table 4.13: The Co-occurrence of OTA and CPA in Store and Market Samples

Food Samples	**OTA / CPA N=16**	**% Co-occurrence per sample**
Stored Sorghum	07	43.75
Market Sorghum	06	37.50
Stored Maize	09	56.25
Market Maize	06	37.50

Key: N= number of samples, %= percentage, OTA= ochratoxin A, CPA= cyclopiazonic acid

4.1.19 Co-occurrence of OTA and CPA in sorghum and maize sample

The co-occurrence of OTA and CPA in red sorghum, white sorghum, white maize and yellow maize samples are represented in Table 4.14. The collected samples have existence OTA/CPA together as follow: white maize samples as 9 (16), 56.25 %; red sorghum samples as 8(16), 50.00 %, yellow maize samples as 7(16), 43.75 and white sorghum samples as 5 (16), 31.25 %.

Table 4.14: Co-occurrence of OTA and CPA in Maize and Sorghum Samples

Food Samples	OTA/CPA N=16	% Co-occurrence per sample
Red Sorghum	08	50.00
White Sorghum	05	31.25
White Maize	09	56.25
Yellow Maize	07	43.75

Key: N= number of samples, %= percentage, OTA= ochratoxin A, CPA= cyclopiazonic acid

4.1.20 Co-occurrence of OTA and CPA in all the zones

The co-occurrence of OTA and CPA in wettest zone, wet zone, dry zone and driest zone are represented in Table 4.15. The mycotoxins OTA and CPA are produced in the sorghum and maize samples in the different zones co-occurred is presented in the appendices. The highest existence of CPA and OTA together in all the zones in maize items is 6(8), 75 % in wet zone and in sorghum samples is 5 (8), 62.5 % in wet zone.

Table 4.15: Co-occurrence of OTA/CPA in Maize and Sorghum samples in each Zones

Food Samples	Wettest Region - Suleja OTA/CPA N=08		Wet Region - Magama OTA/CPA N=08		Dry Region - Bosso OTA/CPA N=08		Driest Region – Mariga OTA/CPA N=08	
	Co-occurrence	% of Co-occurrence	Co-occurrence	% of Co-occurrence	Co-occurrence	% of Co-occurrence	Co-occurrence	% of Co-occurrence
Sorghum	02	25 %	05	62.5 %	02	25 %	04	50 %
Maize	04	50 %	06	75 %	04	50 %	02	25 %

Key: N=number of samples, % = percentage, OTA= ochratoxin A, CPA= cyclopiazonic acid

4.1.21 Exposure assessment of male and female to OTA in maize and sorghum samples

Exposure assessment of male and female to OTA in maize and sorghum samples represented in Table 4.16. The estimated daily intake (EDI) of OTA in the cereal's samples showed the highest value in white maize in female population of dry zone (EDI=423.099 μg/kg bw/day respectively), yellow maize in male population of wet zone (EDI=110.803 μg/kg bw/day respectively) and yellow maize in female population of wet zone (EDI=104.013 μ/kg bw/day respectively).

Table 4.16: The Estimated Daily Intake of OTA in Maize and Sorghum Samples

Food Samples	EDI of OTA in Wettest Zone– Suleja ($x10^{-3}$)		EDI of OTA in Wet Zone– Magama ($x10^{-3}$)		EDI of OTA in Dry Zone– Bosso ($x10^{-3}$)		EDI of OTA in Driest Zone – Mariga ($x10^{-3}$)	
	Male	Female	Male	Female	Male	Female	Male	Female
Red Sorghum	42.162	39.800	49.789	46.737	30.567	28.694	24.931	23.403
White Sorghum	26.699	25.203	25.678	24.417	68.608	64.403	35.605	33.423
White Maize	21.409	20.209	40.537	38.053	24.606	423.099	41.887	39.320
Yellow Maize	80.336	75.413	110.803	104.013	27.517	25.831	25.664	24.091

Key: EDI= estimated daily intake, OTA= ochratoxin A

4.1.22 Exposure assessment of male and female to CPA in varieties of maize and sorghum samples

Exposure assessment of male and female to CPA in varieties of maize and sorghum sample are represented in Table 4.17. The estimated daily intake of the cereal indicted higher exposure of CPA to male and female in each of wettest zone (EDI=157.023 µg/kg bw/day, 148.226 µg/kg bw/day respectively), of dry zone (EDI=562.471 µg/kg bw/day, 528.002 µg/kg bw/day respectively) and of driest zone (EDI=149.080 µg/kg bw/day, 139.945 µg/kg bw/day respectively) with white maize consumption and also male and female in each of wet zone (EDI=133.859 µg/kg bw/day, 126.360 µg/kg bw/day respectively) and dry zone (210.076 µg/kg bw/day, 197.202 µg/kg bw/day respectively) with yellow maize consumption.

Table 4.17: The Estimated Daily Intake of CPA in Maize and Sorghum Samples

Food Samples	EDI of CPA in Wettest Zone – Suleja X 10^{-3}		EDI of CPA in Wet Zone – Magama X 10^{-3}		EDI of CPA in Dry Zone – Bosso X 10^{-3}		EDI of CPA in Driest Zone – Mariga X 10^{-3}	
	Men	**Women**	**Men**	**Women**	**Men**	**Women**	**Men**	**Women**
Red Sorghum	21.047	19.868	98.046	92.553	46.565	43.446	49.759	46.710
White Sorghum	97.258	91.809	30.089	28.245	0.000	0.000	13.289	12.474
White Maize	157.023	148.226	91.860	84.737	562.471	528.002	149.080	139.945
Yellow Maize	25.536	23.971	133.859	126.360	210.076	197.202	0.000	0.000

Key: EDI= estimated daily intake, CPA= cyclopiazonic acid, X= Multiplication

4.1.23 Health risk characterization of OTA

The potential health risk characteristics of OTA in the population of the study is represented below (Table 4.18). The tolerable daily intake OTA is 0.014 μg/kg and therefore the %TDI revealed a very higher risked of the consumption of maize and sorghum to both male and female population in wettest zone, wet zone, dry zone and driest zone. The highest risk is in the consumption of yellow maize among male population (79.1450 μg/kg) of wet zone.

Table 4.18: Health Risk Characteristics of OTA in the Population of the study

Food Samples	%TDI of OTA in Wettest Zone – Suleja		%TDI of OTA in Wet Zone – Magama		%TDI of OTA in Dry Zone – Bosso		%TDI of OTA in Driest Zone – Mariga	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Red Sorghum	30.1157	28.4287	35.5636	33.3836	21.8336	20.4929	17.8079	16.7164
White Sorghum	19.2786	18.0021	18.3414	17.4407	49.0057	46.0021	25.4321	23.8736
White Maize	15.2921	14.4350	28.9550	27.1807	17.5757	302.2136	29.9193	28.0857
Yellow Maize	57.3829	53.8664	79.1450	74.2950	19.6550	18.4507	18.3314	17.2079

Key: TDI= tolerable daily intake, TDI = 0.014 µg/kg, **%**= percentage

4.2 Discussion

The study provides average consumption rate, evidence of the presence of three fungal species belonging to *Penicillium* in stored and marketed grains of the white and red sorghum varieties from the four microclimatic zones of Niger State. It also records the natural occurrence, concentrations and associated risks of dietary exposure to OTA and CPA in the studied grain. Most importantly, the data generated for CPA in sorghum samples is the first of its kind.

The daily consumption of sorghum and maize is the same rate in all zones, this is due to the fact the consumers cereals are also the producers therefore having maize and sorghum as a stable food. These producers are able to store these crops for a longer use since they consume it daily and in different cereal product like *Tuwo, Akamu, Kunu*, Cornflakes, Golden morn and Malt. For the percentage frequency of consumption of the cereals, daily maize and sorghum consumption had the highest frequency in the driest zone (Sallawu *et al.*, 2016).

The yellow maize had highest colonies of fungi form due to its susceptibility to fungi infestation than white maize under suitable condition. Red sorghum had the lowest colony forming unit because of high present of phenolic compounds and condensed tannin which are antifungal agent and therefore reduces the growth of fungi. Stored maize had highest fungi load this may be due to the storage condition like suitable temperature and sufficient moisture content of the cereals and the storage environment which promote fungi growth. While the lowest fungi load (CFU/ml) is in stored sorghum which is not implicatively dissimilar from market sorghum at $p \leq 0.05$. The highest fungi load in the zone is maize grains in the wet zone while Sorghum grains had highest fungal load (CFU/ml) in wettest zone which condition renders high water activity and moderate

The incidence of each *P. species* in the maize samples are very unique and does not follows a similar trend. Lack of proper storage facilities had promoted low maize production in the Nigeria (Ibitola *et al*., 2019). The northern and middle belts of Nigeria where Niger state belong to have adequate sunshine and moderate rainfall which are condition that the storage of grains can be achieved with low damage from insect which can reduced the infestation of toxigenic fungi in the grains (Iken *et al*., 2004). In the zones, the highest occurrence of *P. species* includes *P. verrucosum* in wet zone as 7 (25), *P. chrysogenum* in wettest and dry zone as 2 (6) each and *P. griseofulvum* in wettest and driest zones as 3 (7) each. Here we see the activities of these *P. species* is much predominate in wettest zone for *P. chrysogenum* and *P. griseofulvum*. The ability of fungi growth on food is determined by moisture content as well as temperature where the effect of moisture content is higher than temperature (Sautour *et al*., 2002).

The growth of *Penicillium spp* and the production of OTA depends on the humidity and temperature in maize (Domijan *et al*., 2005). In the study by Domijan *et al*. (2005) the contamination of maize samples with *Penicillium spp* was high and that with aspergilli spp low, since both fungi can produce both OTA and CPA each.

Occurrence of *Penicillium spp* in stored sorghum sample from storage facilities of farmers was reported as 12/88 (13.64 %) in Kenya (Kange *et al*., 2015) which is contrast in this study given a higher occurrence of *Penicillium spp* in store sorghum samples as 14/32 (42.42 %). The highest occurrence of *Penicillium spp* across the zones in sorghum samples include the following: *P. verrucosom* is highest in driest zone as 7/24, *P. griseofulvum* is highest in wet zone as 2/4 and *P. chrysogenum* is highest in both wet and dry zones as 2/5 each. Therefore,

wet zone had highest frequency of *P. griseofulvum* and *P. chrysogenum* in sorghum samples. The growth of fungal is governed by substrate stature (damage on grains and constituents' nutrients), environment features (degree of hotness/coldness and humidity) and biological feature (fly pest as well as other microbes) (Mannaa and Kim, 2017). The different zones will provide varies temperature conditions and moisture content which is a combination of decreasing temperature and increasing moisture or vice versa

The main OTA concentration in maize is 6.65 μg/kg and sorghum is 58.29 μg/kg as accounted through Makun *et al*. (2007) work. The result through Sekiyama *et al. (*2005) show one sample of corn flour indicated 64 mg/kg of OTA which surpass the highest permissible limits in Brazil regulation and legislature. The Temporary Tolerable Week Intake (TWI) is 0.1 μg per kg bw per Kg, and the mean amount of OTA tested was detected to be 0.5289 μg per Kg, therefore the mean possible week consumption (PWI) amounted to 0.0022 μg per kg bw per week Sekiyama *et al. (*2005). The corn grains from farmers and traditional markets in Indonesia were not contaminated with OTA due to good storage facilities like metal drum or *huma* made from *gebang* tree (Nalle *et al*., 2019). Field maize sample collected from the Southeastern and Mideastern United State were not detected with OTA (Palencia *et al*., 2014). In Pakistan, OTA level in market corn flour samples detected 30 out of 40 samples above the maximum level by European Union regulation (5.0 μg/kg) Majeed *et al.,* 2017. Shah *et al.* (2010) reported contamination of OTA in maize grain collected at Swat valley of Pakistan. Using LC – MS/MS. Ochratoxin A contamination level in corn and corn-derived products was 0.12 – 50.5 μg/kg Park *et al*. (2018). This present study recorded lower contamination of OTA in market maize collected from wet zone as compared to Bangladesh which is a tropical and rainfall

country where the incidence rate was higher and shows higher level of OTA contamination. (Dawlatana *et al*., 2008).

Natural incidence of OTA in stored groundnut in Niger State collected in May – October show high incidences of 30/32, with lower level of contamination of the mean±SE of as (Ifeji *et al*., 2014). These OTA occurrences were reviewed by Darsanaki *et al*. (2015) as follows: maize in Nigeria through ELSA detected 0.14 μg/kg in 2009, maize in Croatia through ELISA detected 12.7 μg/kg in 2007, maize in Hungry through HPLC detected 0.4 μg/kg in 2001, sorghum in Ethiopia through HPLC detected 174.8 μg/kg in 1999 and sorghum in Tunisia through HPLC detected 117 μg/kg in 2007.

The Balkan peninsula where the Balkan endemic nephropathy revealed corn contamination to be 20.0±14.8 μg/kg (Puntaric *et al*., 2001). The occurrence of OTA contamination had been reported with highest concentration in cereal grains than in fruits and vegetable (Ciconova *et al*., 2010). Poor storage of cereal grain will promote the growth of OTA – toxigenic fungi (Scudamore, 2005). Mycotoxin's concentrations are greatly influenced by storage conditions as observed at the different location of Borno state of Nigeria rather than cereal type (Gwary *et al.*, 2012). According to the result for maize samples location and maize varieties with highest concentration, market maize as location has a higher level of detection than yellow maize as cereal type.

Market sorghum as well as maize within Niger state of the country Nigeria, show array of OTA inside maize as 0 - 139.2 μg per kg and sorghum as 0 - 29.5 μg/kg in which 15(17) of maize samples with OTA and 12(17) of sorghum samples OTA exceeded the EU limits (Makun *et al*., 2013, Mohammad and Olurunmowaju, 2014). The report by Sibanda indicated

high level of OTA detected in maize from northern Nigeria as cited by Adetunji *et al*. (2017). In Ethiopia, the maximum level of OTA recorded is considerable superior put side by side to account in other location like central/east Europe in which detected OTA is renowned in predominance. The OTA that was quantified within sorghum analytes collected from covert storage holes showed influences in the preferential synthesis of OTA by fungi. This is because there is a stable upsurge of water activity in stored sorghum grain in holes (Ayalew *et al*., 2006). In this study market sorghum have higher level of OTA in dry zone than stored sorghum while white sorghum is higher in dry zone than red sorghum. While the incidence of OTA in samples of sorghum was about 38 % as compared to all analysed samples of Tunisian cereals as reported by Zaied *et al*. (2009).

The stored maize bears a high concentration of CPA due to the fact that grains from the field are kept in store before moving to the market as seen in Argentina for the synthesis of CPA on corn (Resnik *et al*., 1996). Mice that are pregnant, with only one oral dosage of CPA (15 – 50 μg per kg) this will reduce the gain of body weight and also importantly the frequency of pregnancy (Chang *et al*., 2009b). The report by Lansden and Davidson (1983) shows that Peanut in Southeastern United State were contaminated with CPA. Cyclopiazonic acid is not well thought out as to be an effectively severe mycotoxin due to LD_{50} span with 30 – 70 mg/kg (Burdoch and Flamm, 2000). CPA was detected at a more extremely high level in Czech camembert type cheeses as report by Ostry *et al*. (2005) as compared to this level quantified in this study. There is paucity of reports on prevalence of Cyclopiazonic acid in sorghum. In a comprehensive review, Burdock and Flamm (2000) and Ostry *et al*. (2018) documented its presence in peanut, maize, figs, rice, tomato paste and puree, millet, sunflower seeds, wheat, cheese and milk over a period of 50years without a mention of sorghum. CPA was not detected

in 40 samples of sorghum from Somalia (Wielogorska *et al*. (2019). However, there are reports of the occurrence of CPA producing fungi in the grain (del Palacio and Pan, 2020). Nafuka *et al*. (2019) found high incidence, mean (range) concentrations in sorghum malt use for preparation of two Namibian traditional fermented beverages, Omalodu (69 %, 456.15 (55.17-2070 µg/kg) and Otombo (39 %, 122 (60.4-486 µg/kg). The two reports on the occurrence of CPA in Nigeria are in peanut at concentration between 9.6 – 114 µg/kg (Onyeke, 2020) and maize at levels between 0 – 417.5 µg/kg (Ogara *et al.*, 2017). The current study seems to report for the first-time prevalence of CPA in sorghum in Nigeria at 44.79 % occurrence and an average concentration of 21.43 µg/kg in the 64 composite samples analysed. Although there are no legislated maximum levels and TDI for the mycotoxin. De Waal (2002) proposed a TDI of 0.1 µg/kg bw/day CPA, and 10 µg/kg/day or 700 µg/day was suggested as the maximum acceptable daily intake (ADI) dose by Burdock and Flamm, (2000). Using the most recent and stringent TDI proposed by De Waal (2002), our data suggests that Nigerians are not at risk of CPA poisoning due to sorghum consumption while that of Ogara *et al*. (2017) suggest that Nigerians are at risk of CPA poisoning from consumption of maize. CPA is calcium uptake disrupters which results in increased muscle contraction (Chang *et al.*, 2009a). The same review article reports that it has been implicated in the 'kodua poisoning' toxic syndrome in cattle and man in Northern India, and the symptoms of Kodua poisoning in the affected people are characterized by nausea, vomiting, delirium, depression, intoxication, and unconsciousness (Chang *et al.*, 2009a). Bazlur (1960) reported that the poisoning of cattle is characterized by symptoms of nervousness, lack of muscular coordination, staggering gait, depression and spasms. The cattle and man usually recover after 3 days but cases of death of cattle due to poisoning have also been reported.

Several mycotoxins normally found together within food therefore widely effecting global food markets (Makun *et al.,* 2011b, Assunção *et al.,* 2016). The singular and mutual consequences of CPA (34 mg per kg) and 2.5 mg per kg of OTA to young chicken, show as additive interaction of OTA together with CPA (Gentles *et al*., 1999, Chang *et al*., 2009b). However, aflatoxin and OTA interaction have been observed to be synergistic (Gentles *et al.,* 1999). Kumar and Balanchandran (2009) reported that the occurrence of aflatoxin and CPA result to cumulative effect. The interaction of aflatoxin and OTA have been observed to be synergistic as indicated by Kumar and Balanchandran (2009) likewise Oliveira *et al.* (2008).

Products that are contaminated with Ochratoxin A pose a high risk to its consumer which is nephrotoxic, teratogenic and immunosuppressive compounds. The particular average nationwide PDI and also %TDI for kids (193.1 and 2061.7) and immature adults (140.0 and 824.7) among the populace indicated that infant and youth (IYC) bear an alarming danger in the intake of maize crops in the country Nigeria (Adetunji *et al*., 2017). This present study also show a high risk of the consumption of both white and yellow maize for OTA and CPA. Male in wet zone and female in dry region bears the superior risk exposure of OTA with yellow maize and white maize respectively. Likewise, male and female in wettest and wet zone have high risk of the consumption of white maize and in consumption of yellow maize in wet and dry zone. Report from a lot of research indicated that in Africa, high danger of mycotoxins contact is superior as compare to other countries of the world and this is due to elevated tendencies to the ingestion of food basically sourced from maize, particularly the vulnerable groups like toddlers as food for weaning or free foods (Adetunji *et al*., 2017).

The OTA %TDI of the female in the dry zone show high risk in white maize consumption and male in wet zone show high risk in yellow maize consumption. Luz *et al.* (2018) reported health risk assessment of the consumption of Spanish breadcrumb to be below the %TDI value. Coronel *et al.* (2012) evaluated the OTA contamination in compounded items of food for toddlers obtained from cereal and morning food from grains were less than the recent temporary acceptable daily consumption (PTDIs) of 17 and 14 ng/kg/bw/day established by the European Food Safety Authority (EFSA) correspondingly, of the range 1 % - 2 % as PTDIs estimate in youths and kids, to 3 % - 11 % in adults as well as toddlers. The other risk in the consumption of these cereals is the co-occurrence of OTA and CPA in these food samples (Baert, 2017).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study revealed that *penicillium species* was isolated from different varieties of cereal including yellow maize, white maize, white sorghum and red sorghum samples collected. This caused some level of cyclopiazonic acid and ochratoxin A contamination in the samples. The contamination was through the growth of *Penicillium species* like *P. griseofulvum, P. verrucosum, P. chrysogenum* which were above the regulatory safe limit by CODEX Alimentarius. Therefore, from the result obtained from the risk assessment of these mycotoxins (CPA and OTA) in the different varieties of the cereals, the male and female population are at risk of consuming contaminated food samples and thereby affecting the health of the populace. The children will be affected immensely because of the lower body weight as compared to the adult male and female who are already at risk. The need for regular evaluation and monitoring of cereal grains should be undertaken by quality control units of government agencies to ensure food safety standards are maintained.

5.2.1 Recommendation

The research carried out in the evaluation of contamination of the maize and sorghum with ochratoxin A and cyclopiazonic acid, prompted the following recommendations:

i. Maize and sorghum full-grown with the aimed of consumption by human in regions with climatic conditions that mostly support attack from fungi and the synthesis of mycotoxin within the farmland should be analysed for contamination of mycotoxin before consumption by regulatory body like National Agency for Food and Drugs Administration Control (NAFDAC)

ii. Further researches are required to advance the capacity to forecast the period and the location of an environmental conditions that can enhance the possible contamination of mycotoxin and to develop the means to spread cautionary measure to farmers as well as the processors of such farm produces. Also, the varieties of sorghum and maize cultivars which are resistant to drought stress, infestation by insect's pest and also growth of fungi is require to be originated then given out to farmers. Research should be anchored by Mycotoxin Society of Nigeria.

iii. If spoilage in the storage and market is apparent and environmental factors are suitable for synthesis of mycotoxin either within the field or at storage, precaution should be embarked upon to lessen origins of substance that trigger inoculation then also to diminish the stress in plant as well as damage by insects. As soon as possible contamination of food crops is established from the field, engage precaution measures to lower the fungal growth subsequently as harvest is carried out as well as during stowage. Plans should be put in place for fumigation of stores and sorting out those crops that are already contaminated with mycotoxins to be kept from human, animals and other sensitive species within the food chain by the Federal and State Ministry of Agriculture

REFERENCES

Abia, W. A., Simo, G. N., Warth, B., Sulyok, M., Krska, R., Tchana, A. & Moundipa, P. F. (2013). Determination of multiple mycotoxins levels in poultry feeds from Cameroon - Review. *Japanese Journal of Veterinary Research*, 61, 33 – 39.

Abiose, S. H., & Ikujenlola, A. V. (2014). Comparison of chemical composition, functional properties and amino acids composition of quality protein maize and common maize (Zea may L). *African Journal of Food Science and Technology*, 5(3), 81 – 89.

Adams, M. A. & Moss, M. O. (1999). *Food Microbiology* 2nd edition, Royal society of Chemistry. London, U.K., Cambridge University Press, 390 - 392

Adebajo, I.O., Idowu, A. A., & Adesanya, O.O. (1994). Mycoflora, and mycotoxins production in Nigerian corn and corn-based snacks. *Mycopathologia*, 126, 183-192.

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 (58), 1 – 10.

Aduba, J. J., Mohammed, B. T., Jilasaya, I., & Ozumba, I. C. (2013). An Economic Analysis of Sorghum Production among Sorghum Farmers in Kwara State Nigeria. *Global Journal of Current Research*, 1 (4), 116-123.

Agriopoulou, S., Stamatelopoulou E., and Varzakas T. (2020). Review - Advances in occurrence, importance and mycotoxin control strategies: prevention and detoxification in foods. *Food*, 9 (137), 1 - 48.

Akman, S. A., Adams, M., Case, D., Park, G., & Manderville, R. A. (2012). Mutagenicity of ochratoxin A and its hydroquinone metabolite in the SupF gene of the mutation reporter plasmid Ps189. *Toxins*, 4, 267-280.

Akpodiogaga-A, P., & Odjugo, O. (2010). General Overview of Climate Change Impacts in Nigeria. *Journal of Human Ecology*, 29(1), 47-55.

Alshannaq, A., & Yu, J. (2017). Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. *International Journal of Environmental Research and Public Health*, 14(6), 632-637.

Ammani, A. A. (2015). Trend analysis of maize production and Productivity in Nigeria. *Journal of Basic and Applied Research International*, 2(3), 95-103.

Anjorin, T. S., Salako, E. A., & Makun, H. A. (2013). Control of toxigenic fungi and mycotoxins with phytochemicals: *potentials and challenges.* licensee InTech. Chapter 8, page181 – 202.

Assunção, R., Silva, M. J. & Alvito, P. (2016). Challenges in risk assessment of multiple mycotoxins in food. *World Mycotoxin Journal*, 9 (5), 791-811.

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

Baert, K. (2017). Risk assessment of mycotoxins: the European Food Safety Authority Approach. International Conference the Burden of Mycotoxins on Animal and Human Health, Rome. http://www.efsa.europa.eu/

Baiyegunhi, L.J.S., & Fraser, G.C.G. (2009). Profitability in sorghum production in three villages of Kaduna state Nigeria. *Journal of Applied Sciences Research*, 5(10), 1685-1691.

Balendres M. A. O., Karlovsky P., & Cumagun C. J. R. (2019). Mycotogenic Fungi and mycotoxins in Agricultural Crop Commodities in the Philippines: A Review. *Foods*, 8 (249), 1-12.

Bazlur, M. (1960). Probable mona grass (*P. commersoni*) poisoning. *Indian Veterinary Journal*, 37, 31-34.

Beta, T., Arntfield, S., Noren, N., Bezner-Kerr, R., Katundu, M., Ndolo, V. & Shumba, L. (2014). Proximate composition and carotenoid content of white, orange and yellow maize cereals grown in central and northern Malawi – baseline information. Conference paper, University of Manitoba, Winnipeg, MB, Canada, Faculty of agricultural and Food Sciences.

Burdock, G. A., & Flamm, W. G. (2000). Review Article: Safety assessment of the mycotoxin cyclopiazonic acid. *International Journal of Toxicology*, 19(3), 195-218.

Chang, C. H., Yu, F. Y., Wang, L. T., Lin, Y. S., & Liu, B. H. (2009a). Activation of ERK and JNK signaling pathways by mycotoxin citrinin in human cells. *Toxicology and applied pharmacology*, 237(3), 281-287.

Chang P. K., Ehrlick K. C., & Fujii I. (2009b). Cyclopiazonic acid biosynthesis of *Aspergillus flavus* and Aspergellius oryzae - Review. *Toxin*, 1, 74 – 99.

Cicoňová, P., Laciaková, A., & Máté, D. (2010). Prevention of achratoxin A contamination of food and ochratoxin A detoxification by microorganisms– A Review. *Czech Journal Food Science*, 28 (6), 465–474.

Coronel, M. B., Marín, S., Cano-Sancho, G., Ramos, A. J., & Sanchis, V. (2012). Exposure assessment to ochratoxin A in Catalonia (Spain) based on the consumption of cereals, nuts, coffee, wine, and beer. *Food Additive Contaminationa Part A Chem Anal Control Expo Risk Assess*, 29 (6), 979-993.

Cumagun, C. J. R., Balendres, M. A. O., & Karlovsky, P. (2019). Mycotogenic Fungi and mycotoxins in Agricultural Crop Commodities in the Philippines: A Review. *Foods*, 8 (249), 1-12.

Cusabio (2015). Ochrtoxin A ELISA kits for testing cereals, product code – WSK-E19C. www.cusabio.com.

Cysewski, S. J. (1973). A tremorgenic mycotoxin from *Penicillium* puberulum, its isolation and neuropathic effects. Iowa State University Capstones, Retrospective Theses and Dissertations. https://lib.dr.iastate.edu/rtd/6143

Czaban, J., Wróblewska, B., Stochmal, A., & Janda, B. (2006). Growth of *penicillium* verrucosom and production of ochratoxin A on nonsterilized wheat grain incubated at different temperatures and cater Content. *Polish Journal of Microbiology*, 55 (4), 321 – 331.

Darsanaki, R. K., Rahbar, S., & Tajehmiri, A. (2015). Occurrence of zearalenone and ochratoxin A in cereals and cereal based products. *Journal of Chemical Health Risks*, 5(4), 301–311.

Darsanaki, R. K., & Takrami, S. R. (2017). Occurrence of T-2 toxin and aflatoxin B1 in cereals and cereal based products: A short review. *Journal of Chemical Health Risks*, 7(4), 2.

Dawlatanaa, M., Shahidaa, S., Rahima M. & Hassana, M. T. (2008). Investigation on the occurrence of ochratoxin A in maize in Bangladesh. *Bangladesh Journal of Scientific and Industrial Research*, 43(4), 495-500.

del Palacio, A., & Pan, D. (2020). Occurrence and toxigenic potential of *Aspergillus* section Flavi on wheat and sorghum silages in Uruguay. *Mycology*, 11(2), 147-157.

De Waal, E. J. (2002). Letter to the Editor—Safety assessment of cyclopiazonic acid. *International journal of toxicology*, 21(5), 425-427.

Domijan, A., Peraica, M., Cvjetkovi, B., Turcin, S., Jurjevi, E., & Ivi, D. (2005). Mould contamination and co-occurrence of mycotoxins in maize grain in Croatia. *Acta Pharmacy*, 55, 349–356.

Freitas, E. R., Raquel, D. L., Nascimento, A. J. N., Watanabe, P. H., & Lopes, I. R. V. (2014). Complete replacement of corn by white or red sorghum in Japanese Quail feeds. *Brazilian Journal of Poultry Science*, 16 (3), 333-336.

Gentles, A., Smith, E. E., Kubena, L. F., Duffus, E., Johnson, P., Thompson, J., Harvey, R. B., & Edrington, T.S. (1999). Toxicological evaluations of cyclopiazonic acid and ochratoxin A in Broilers. *Poultry Science*, 78, 1380–1387.

Guohua, Y., Yuliang, Z., Kayla, K. P., Guangxi, W., Sui, S. T. H., Jiujiang, Y., Wayne, M. J., Anping, G., & Joan, W. B. (2017). Characterization of blue mold *penicillium* species isolated from stored fruits using multiple highly conserved loci. *Journal of Fungi*, 3(12), 1-10.

Gwary, O. M., Hati, S. S., Dimari, G. A. & Ameh, J. A. (2012). Assessment of Mycotoxins (Total Aflatoxins and Ochratoxin-A) Contamination of Staple Cereals. *International Journal of Chemical and Biochemical Sciences*, 2, 1-6.

Houbraken, J., Visagie, C.M., Meijer, M., Frisvad, J. C., Busby, P.E., Pitt, J. I., Seifert, K. A., Louis-Seize, G., Demire, R., Yilmaz, N., Jacobs, K., Christensen, M. & Samson, R. A. (2014). A taxonomic and phylogenetic revision of *Penicillium* section Aspergilloides. *Studies in Mycology*, 78, 373–451.

Ibitola, O.R., Fasakin, I.J., Popoola, O.O. & Olajide, O.O. (2019). Determinants of maize famers' productivity among small holder farmers in Oyo state, Nigeria. *Greener Journal of Agricultural Sciences,* 9(2), 189-198.

Ifeji, E. I., Makun, H. A., Mohammed, H. L., Adeyemi, R. Y. H., Mailafiya, S. C., Iken, J.E., & Amusa, N.A. (2014). Maize research and production in Nigeria. *African Journal of Biotechnology*, 3 (6), 302-307.

Iken, J.E., & Amusa, N.A. (2004). Maize research and production in Nigeria. *African Journal of Biotechnology*, 3 (6), 302 – 307.

Ismaiel, A. A., & Papenbrock, J. (2015). Mycotoxins: Producing Fungi and Mechanisms of Phytotoxicity – Review. *Agriculture*, 5, 492-537.

Javis, B. (1971). Factors Affecting the Production of Mycotoxins. *Journal of applied Bacteriology*, 34 (1), 199 – 213.

Joint Food and Agriculture Organization / World Health Organization Expert Committee on Food additives (2007). Evaluation of certain food additives and contaminants: sixty-eight report of the joint FAO/WHO Expert Committee of Food additives. Rome, Italy, World Health Organization.

Joint Food and Agriculture Organization / World Health Organization Expert Committee on Food additives (1997). Evaluation of certain food additives and contaminants: forty-ninth report of the joint FAO/WHO Expert Committee of Food additives. Rome, Italy, World Health Organization.

Kange, A. M., Cheruiyot, E. K., Ogendo, J. O., & Arama, P. F. (2015). Effect of sorghum (*Sorghum bicolor L. Moench*) grain conditions on occurrence of mycotoxin-producing fungi. *Agriculture and Food Security*, 4 (15), 1 – 8.

Kolanlarli, T. K., Asan, A., Sen, B., & Okten, S. (2019). Biodiversity of *Penicillium* species isolated from Edirne Söğütlük Forest soil (Turkey). *The Journal of Fungus*, 10(1), 26-39

Kuiper-Goodman, T. (1999). Approaches to the risk analysis of mycotoxins in the food supply. The Toxicological Evaluation Section, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health Canada, Ottawa. FNA/ANA 23.

Kumar, R., & Balachandran, C. (2009). Histopathological changes in boiler chickens fed aflatoxins and cyclopiazonic acid. *Veterinarski Arhiv*, 79 (1), 31-40.

Lansden, J. A., & Davidson, J. I. (1983). Occurrence of Cyclopiazonic Acid in Peanuts. Applied and Environmental Microbiology, 45 (3), 766-769

Lavkor, I., Var, I., Oztemiz, S., & AlMatar, M. (2017). First Report of Mycotoxins in Second Peanuts Crop in Adana and Osmaniye at Harvest, Drying, Prestorage and Storage Periods. *Intechopen Science*.

Leggieri, M. C., Decontardi, S., Bertuzzi, T., Pietri, A., & Battilani, P. (2016). Modeling Growth and Toxin Production of Toxigenic Fungi Signaled in Cheese under Different Temperature and Water Activity Regimes. *Toxins*, 9 (4), 1 -17.

Legodimo, M. D., & Madibela, O. R. (2013). Effect of sorghum variety on chemical composition and in vitro digestibility of malted grains from Botswana. *Botswana Journal of Agricultural Applied Sciences*, 9 (2), 104-108.

Luz, C., Saldino, F., Manes, J. & Meca, G. (2018). Risk assessment of mycotoxin intake through the consumption of Spanish breadcrumbs. *Revista de Toxicología*, 35 (2), 11 – 15.

Macauley, H. (2015). Background paper - Cereal Crops: Rice, Maize, Millet, Sorghum, Wheat. Feeding Africa, Abdou Diouf International Center Dakar, Senegal.

Mahomed, S., Baijnath, P., & Mlisana, K. (2016). *Penicillium* Species: is it a Contaminant or Pathogen? Delayed Diagnosis Ina Case of Pneumonia Caused by *Penicillium* Chrysogenum in a Systemic Lupus Erythematosus Patient. *International Journal of Tropical Medicine and Public Health*, 6 (1), 14-16.

Majeed, M., Khaneghah, A. M., Kadmi, Y., Khan, M. U., & Shariati, M. A. (2017). Assessment of Ochratoxin A in Commercial Corn and Wheat Products. *Current Nutrition & Food Science*, 13 (0), 1-5.

Makun, H. A., Adeniran, A. L., Mailafiya, S. C., Ayanda, I. S., Mudashiru, A. T., Ojukwu, U.J., Jagaba, A. S., Usman, Z., & Salihu, D. A. (2013). Natural occurrence of ochratoxin A in some marketed Nigerian foods. *Food Control*, 31, 566 – 571.

Makun, H. A., Dutton M. F., Njobeh P. B., Phoku J. P., & Yah C. S. (2011). Incidence, phylogeny and mycotoxigenic potentials of fungi isolated from rice in Niger State, Nigeria. *Journal of Food Safety*, 31, 334–349.

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. *Mycotoxin Research*.

Makun, H. A., Anjorin, S.T., Moronfoye, B., Adejo, F.O., Afolabi, O.A., Fagbayibo, G., Balogun, B. O., & Surajudeen, A. A. (2010). Fungal and aflatoxin contamination of some human food commodities in Nigeria. *African Journal of Food Science,* 4(4), 127 – 135.

Makun, H.A., Gbodi, T. A., Akanya, O. H., Ezekiel, A., Salako, E. A., Godwin, H., & Ogbadu, G. H. (2009). Fungi and Some Mycotoxins Found in Mouldy Sorghum in Niger State, Nigeria. *World Journal of Agricultural Sciences*, 5 (1), 05-17.

Makun, H.A., Gbodi, T. A., Akanya, O. H., Ezekiel, A. Salako, E. A., Godwin, H., & Ogbadu, G. H. (2007). Fungi and some mycotoxins contaminating rice (Oryza Sativa) in Niger State, Nigeria. *African Journal Biotechnology*, 6 (2), 099-108.

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.

Mannaa, M., & Kim, K. D. (2016). Microbe-Mediated control of mycotoxigenic grain fungi in stored rice with focus on aflatoxin biodegradation and biosynthesis inhibition. *Mycobiology*, 44(2), 67.

Maragos, C. M. (2018). Complexation of the Mycotoxin Cyclopiazonic Acid with Lanthanides Yields Luminescent Products. *Toxins*, 10 (285), 1-15.

Martinez-Blanco, H., Reglero, A., Fernhdez-Valverde, M., Ferrero, M. A., Moreno, M. A., Peiialva, M. A. & Luengo, J. M. (1992). Isolation and Characterization of the Acetyl-coA Synthetase from *Penicillium* chrysogenum. *The Journal of Biological Chemistry*, 267(8), 5474-5481.

Mejia, D., & Lewis, B. (1999). Sorghum post-harvests operations. Food of Agriculture Organization of united Nation page 1 – 32.

Milani, J. M. (2013). Ecological conditions affecting mycotoxin production in cereals: a review. *Veterinarni Medicina*, 58 (8), 405–411.

Mohammad, K. H., & Olurunmowaju, Y. B. (2014). Natural occurrence of aflatoxins and ochratoxin A in raw and roasted groundnut from Niger State, Nigeria. *Mycotoxicology Society of Nigeria,* 1, 35-45.

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

Mundia, C. W., Secchi, S., Akamani, K., & Wang, G. (2019). A Regional comparison of factors affecting global sorghum production: The case of North America, Asia and Africa's Sahel. *Sustainability*, 11 (2135), 1-18.

Nafuka, S. N., Misihairabgwi, J. M., Bock, R., Ishola, A., Sulyok, M., & Krska, R. (2019). Variation of fungal metabolites in sorghum malts used to prepare Namibian traditional fermented beverages Omalodu and Otombo. *Toxins*, 11(3), 165.

Nalle, C. L., Angi, M. A. H., Supit, M. A. J. & Ambarti, S. (2019). Aflatoxin and Ochrtoxin A contamination in Corn Grains and Sago (Putak meal) from different sources for poultry in West Timor, Indonesia. *International Journal of Poultry Science*, 18 (8), 353-360.

Njobeh, P. B. (2009). The Prevalence and health effects of fungi and mycotoxins in food commodities from Cameroon. A dissertation submitted to the faculty of health Science, University of Johannesburg, South Africa, in fulfillment of the requirements of an award of a Doctorate Degree in Technology: Biomedical Technology.

Odjugo, P. A. O. (2011). Perception of climate change in the Niger Delta region of Nigeria. Centre for Population and Environmental Development (CPED), Policy Paper Series. Benin City, Nigeria: Ambik Press Ltd.

Odusanya, R. (2018). Maize production in Nigeria: is it profitable in 2018. Legit.

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.

Onyeke, C. C. (2020). Review of mycotoxins in foods in Nigeria. *Food Control*, 107376.

Organization for Econmoic Co-operative and Development (OECD) and food and agricultureOrganization (FAO) (2019). OECD-FAO Agricultural outlook 2019-2028, oecd publishing, paris/Food and Agriculture organization of the United Nations, Rome.

Oliveira, C.A.F., Sebastiao, L. S., Fagundes, H., Rosin, R. E., & Fernandes, A. M. (2008). Aflatoxins and cyclopiazonic acid in feed and milk from dairy farms in São Paulo, Brazil. *Food additives and Contaminants*: Part B, 1 (2), 147-152.

Ostry, V., Toman, J., Grosse, Y., & Malir, F. (2018). Cyclopiazonic acid: 50th anniversary of its discovery. *World Mycotoxin Journal*, 11 (1), 135-148.

Ostry, V., Malir, F., Toman, J., & Grosse, Y. (2016). *Mycotoxins as human carcinogens—the IARC Monographs classification.* Society for Mycotoxin Research and Springer-Verlag, Berlin Heidelberg.

Ostry, V., Skarkova, J., Prochazkova, I., & Ruprich, J. (2005). The production of Cyclopiazonic acid in strains *Penicillium* candidum isolated from the Czech camembert type cheeses. Conference paper, the 27th Mycotoxin Workshop in Dortmund.

Oyedele, O. A., Ezekiel, C. N., Sulyok, M., Adetunji, M. C., Warth, B., Atanda, O. O., & Krska, R. (2017). Mycotoxin risk assessment for consumers of groundnut in domestic markets in Nigeria. *International Journal of Food Microbiology*.

Palavecino, P. M., Penci, M. C., Calderón-Domínguez, G., & Ribotta, P. D. (2016). Chemical composition and physical properties of sorghum flour prepared from different sorghum hybrids grown in Argentina. *Research Article*.

Palencia, E.R., Mitchell, T. R., Snook, M. E., Glenn, A. E., Gold, S., Hinton, D. M., Riley, R. T., & Bacon, C. W. (2014). Analyses of black *Aspergillus Species* of peanut and maize for chratoxins and fumonisins. *Journal of Food Protection*, 77 (5), 805–813.

Park, D. L. & Stoloff, L (1989). Aflotoxin control – how a regulatory agency managed risk from an unavoidable natural toxicant in food and feed. *Regulatory toxicoology and pharmacology*, 9, 10-15.

Park, J., Kim, D., Moon, J., An J., Kim, Y., Chung, S., & Lee, C. (2018). Distribution Analysis of Twelve Mycotoxins in Corn and Corn-Derived Products by LC-MS/MS to Evaluate the Carry-Over Ratio during Wet-Milling. *Toxins*, 10(8), 319-321.

Park, M. S., Chung, D., Baek, K., & Lima, Y. W. (2019). Three unrecorded species belonging to *Penicillium* section Sclerotiora from marine environments in Korea. *Microbiology*, 7 (5), 805–813.

Patricia C. and Association of Official Analytical Chemists (AOCA) (1995). Official methods of analysis of AOAC International 16th Ed. Washington, DC: AOAC

Perng-Kuang, C., Kenneth, C. E., & Isao, F. (2009). Cyclopiazonic Acid Biosynthesis of *Aspergillus flavus* and *Aspergillus oryzae*. *Toxins*, 2009 (1), 74-99.

Perrone, G., Gallo, A., & Logrieco, A. F. (2014). Biodiversity of Aspergillus section Flavi in Europe in relation to the management of aflatoxin risk. *Frontiers in Microbiology*, 5 (377), 1 – 5.

Pitt, J.I. (2000). Toxigenic fungi: which are important? *Medical Mycology*, 38 (1), 17-22.

Pitt, J. I. (1987). *Penicillium* viridicatum, *Penicillium* verrucosum, and Production of Ochratoxin A. *Applied and Environmental Microbiology*, 53 (2), 266-269.

Puntaric, D., Bošnir, J., Šmit, Z., Škes, I., & Baklaiæ, Z. (2001). Ochratoxin A in Corn and Wheat: Geographical Association with Endemic Nephropathy. *Croatian Medical journal*, 42(2), 175-180.

Qamar, S., Aslam, M., Huyop, F., & Javed, M. A. (2017). Comparative study for the determination of nutritional composition in commercial and noncommercial maize flours. *Pakistan Journal of Botany*, 49 (2), 519-523.

Reddy B., & Raghavender C. (2008). Outbreaks of fusarial-toxicoses in India. *Food and safety Toxicology*, 36, 321 – 325.

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

Refai, M., El-Yazid H. A., & Tawakkol (2015). Monogram on the genus *Penicillium* – a guide for historical, classicafication and identification of penicilli, hthier industrial applications and detrimental effects. Department of Microbiology, Faculty of Veterinary Medicine, Cairo University.

Resnik, S.L., Gonzalez, H.H.L., Pacin, A.M., Viora, M., Caballero, G.M., & Gros, E.G. (1996). Cyclopiazonic acid and Aflatoxins production by Apergillius favus isolated from Argentinian corn. *Mycotoxin Research*, 12, 61-66.

Reza, K. D., & Saied, R. T. (2017). Occurrence of T-2 Toxin and Afltoxin B1 in Cereals and Cereal Based products: A Short Review. *Journal of Chemical Health Risks*, 7(4), 331-341

Sallawu, H., Tanko, L., Coker, A. A. A., and Sadiq, M. S. (2016). Livelihood and income diversification strategies among rural farm households in Niger state. *Asian Journal of Agriculture Extension, Economics and Sociology*. 11, (4) 1 - 11

Sani, R.M., Haruna, R. & Sirajo, S. (2013). Economics of sorghum (*Sorghum bicolor(L) Moench*) production in Bauchi Local Government Area of Bauchi state, Nigeria. Invited paper presented at the 4th International Conference of the African Association of Agricultural Economists, Hammamet, Tunisia.

Sautour, M., Mansur, C. S., Divies, C., Bensoussan, M., & Dantigny, P. (2002). Comparison of the effects of temperature and water activity on growth rate of food spoilage moulds. *Journal of Industrial Microbiology & Biotechnology*, 28, 311 – 315.

Scudamore, K. A. (2005) Prevention of ochratoxin A in commodities and likely effects of processing fractionation and animal feeds. *Food Additives and Contaminants*, 22 (1), 17-25.

Sekiyama, B. L., Ribeiro A. B., Machinski P. A., & Junior M. M. (2005). Aflatoxins, ochratoxin A and zearalenone in maize-based food products. *Brazilian Journal Microbiology*, 36 (3), 12 -16.

Shah, H. U., Simpson, T. S., Alam, S., Khattak, K. F., & Perveen, S. (2010). Mould incidence and mycotoxin contamination in maize kernels from Swat Valley, North West Frontier Province of Pakistan. *Food and Chemical Toxicology*, 48, 1111–1116

Simon, R. F., Duntoye, D. B,. & Oyewole, K. (2018). The Effects of Urbanization on Micro-climate of Minna Urban Area. *Journal of Geography, Environment and Earth Science International*, 15(1), 1-13.

Soares, C., Calado, T., & Venâncio, A. (2013). Mycotoxin production by *Aspergillus niger* aggregate strains isolated from harvested maize in three Portuguese regions. *Revista Iberoamericana de Micología*, 30 (1), 9-13.

Tugba, K. K., Ahmet, A., Burhan, S., & Suzan, O. (2019). Biodiversity of *Penicillium* species isolated from Edirne Söğütlük Forest soil (Turkey). *The Journal of Fungus*, 10(1), 26-39.

Viegas, S., Viegas, C., Ramos, C., Silva, M., Sabino, R., Veríssimo, C., & Rosado, L. (2011). Risk Assessment of exposure to multiple mycotoxins in food. *Food and Environment*, 152, 81 – 87.

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.

Wielogorska, E., Mooney, M., Eskola, M., Ezekiel, C. N., Stranska, M., Krska, R., & Elliott, C. (2019). Occurrence and human-health impacts of mycotoxins in Somalia. *Journal of agricultural and food chemistry*, 67(7), 2052-2060.

Wolf, K., & Schweigert, F. J. (2018). Mycotoxin Analysis: A Focus on Rapid Methods. Partnership for Aflatoxin Control in Africa, African Union Commission, Addis Ababa, Ethiopia. http://www.aflatoxinpartnership.org/

Xiong, Y., Zhang, P., Warner, R. D., & Fang, Z. (2019). Sorghum Grain: From Genotype, Nutrition, and Phenolic Profile to Its Health Benefits and Food Applications. Comprehensive Reviews in Food Sciences and Food safety, 00, 1 – 22.

Yin, G., Zhang, Y., Pennerman, K. K., Wu, G., Hua, S. S. T., Yu, J., Jurick II, W. M., Guo, A., & Bennett, J. W. (2017) Characterization of blue mold *Penicillium* Species isolated from stored fruits using multiple highly conserved loci. *Journal Fungi*, 3(12), 2-10.

Zaied, C., Abid, S., Zorgui, L., Bouaziz, C., Chouchane, S., Jomaa, M., & Bacha, H. (2009). Natural occurrence of ochratoxin A in Tunisian cereals. *Food Control*, 20, 218–222.

APPENDIX A

Table: The Co-occurrence of CPA and OTA in each zone

Food Samples	Wettest Zone				Wet Zone				Dry Zone				Driest Zone				Total Occurrence per sample	% Co-occurence per sample
	Suleja Stores		Suleja Markets		Magama Stores		Magama Markets		Bosso Stores		Bosso Markets		Mariga Stores		Mariga Markets			
	OTA	CPA	OTA	CPA	OTA	CPA	OTA	CPA	OTA	CPA	OTA	CPA	OTA	CPA	OTA	CPA		
RS1	√	X	√	√	x	X	√	√	√	√	√	√	√	√	√	x	05	62.0 %
RS2	√	X	x	x	√	√	√	x	X	X	√	x	√	√	√	√	03	25.0 %
WS1	√	X	√	√	√	√	√	√	√	X	√	x	√	√	√	x	04	50.0 %
WS2	√	X	√	x	√	√	√	x	√	X	x	x	√	x	√	x	01	12.5 %
WM1	√	√	√	x	√	√	√	√	√	√	√	x	√	x	√	√	05	62.5 %
WM2	√	√	√	x	√	X	√	√	√	√	√	x	x	x	√	√	04	50.0 %
YM1	√	√	√	x	√	√	√	x	√	√	√	√	√	x	x	x	04	50.0 %
YM2	√	X	√	√	√	√	√	√	X	X	√	x	√	x	√	x	03	37.5 %
Total Co-occurrence	03		03		06		05		04		02		03		03		29	45.5 %
% Co-occurence	37.5 %		37.5 %		75.0 %		62.5 %		50.0 %		25.0 %		37.5 %		37.5 %		45.5 %	

KEY: RS=Red Sorghum, WS=White Sorghum, WM=White Maize, YM=Yellow Maize, √=Detected, x=Non-detect

APPENDIX B

RESEARCH QUESTIONAIRE

Department of Biochemistry, School of Life Sciences

Federal University of Technology, Minna Niger State

Title: The occurrence of ochratoxin A and cyclopiazonic acid in maize and sorghum grown in microclimatic zones of Niger State.

Research Questionnaires

In this questionnaire, there is no wrong or correct answer. What is required is just your opinion on maize consumption rate in Niger state. The outcome will assist in estimating the exposure rate to mycotoxin in sorghum grown in the state. This is a requirement for partial fulfillment of the award of postgraduate research project stemming from the Department of 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 ☐

Body 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:_____

No. of Men: _____ No. of Women: _____ No. of Children: _____

Section B:

Tick which ever applicable in the questions below:

1. Do you consume any maize grain? Yes_____ No_____
2. Do you consume boiled maize: Yes_____ No_____

   If yes, how many boiled maize do you consume per day (household)? _____
3. Do consume roasted maize? Yes_____ No_____
   a. If yes, how many roasted maize do you consume per day (household) _____
   b. If yes, how many roasted maize do you consume per day (individual) _____
4. Do consume Tuwo made from maize? Yes_____ No_____
   a. If yes, how often do you consume it?

      Daily_____ Weekly_____ Monthly_____ Occasionally_____
   b. If weekly or monthly, how many times / weekly (or monthly)? _____
   c. How many cups of maize make a meal size (individual)? _____
5. Do you take Cornflakes/Golden morn? Yes_____ No_____

   If yes, how often do you consume it?

   Daily_____ Weekly_____ Monthly_____ Occasionally_____

   If weekly or monthly, how many times / weekly (or monthly)? _____
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? _____