SURVEY OF FUNGI, AFLATOXINS AND ZEARALENONE CONTAMINATION OF MAIZE IN NIGER STATE

By

TIJANI, ABIOLA SHADIAT
M. TECH/SSSE/2001/2002/771

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF TECHNOLOGY DEGREE (M. TECH) IN BIOCHEMISTRY.

CERTIFICATION

This research work was undertaken under my supervision and has been read, examined and found to meet the requirements for the award of the degree of Masters of Technology in Biochemistry of the Federal University of Technology, Minna, and is approved for its contribution to knowledge. The work reported is original and to the best of my knowledge has not been submitted either in part or in full for the award of any other degree or diploma of this or any other University.

Prof. T.A. Gbodi **Supervisor**	10th October 2005 **Date**
Dr. E. O. Ogbadoyi **Head of Department**	10/10/05 **Date**
Prof.(Mrs) H.O. Akanya **Dean, SSSE.**	**Date**
Prof. J.A. Abalaka **Dean, Postgraduate School**	8/12/05 **Date**
Prof. Z.S Okoye **External Examiner.**	29 June 2005 **Date**

DECLARATION

I hereby declare that this research project /Thesis is my original work and to the best of my knowledge has not been presented in any form for the award of a degree or any other certificate in any other Institution.

TIJANI ABIOLA S. _____ DATE

DEDICATION

This work is dedicated to Alh. and Mrs. Lawal, my beloved brothers and sisters, my mother and my friends.

ACKNOWLEDGMENTS

My unreserved gratitude and thanks goes to almighty Lord who has endowed and granted me the knowledge of how this project should be carried out and who guide me to the right path.

My profound appreciation to my brother and sister Alhaji M. M. Lawal and Mrs. F. O. Lawal for their immeasurable assistance and encouragement all through the programme and for triggering the light to acquire more knowledge.

My special and profound gratitude to my supervisor, Prof. T. A. Gbodi for fatherly support and experienced guidance throughout the period of this work, my role model, Prof. H. O. Akanya for her support and encouragement, Prof J. A. Abalaka who also contributed and cares for my progress, Dr. E O. Ogbadoyi for his concern, Mallam A.N. Saidu who always act as a brother in times of need, Alhaji A.A. Jigam for his pieces of advice and encouragement throughout the period of my work, Mr. H.A. Makun, a lecturer and a friend for his support, encouragement and pieces of advice towards achieving greater academic goal, Mallam A. Kabir for his pieces of advice and encouragement at various stages of the work.

My special thanks to Prof. U. J. Ijah, a friend and lecturer who is always attentive to every problems, the Chief Technologist of the Department, Alhaji S. A. Oyewo for his immeasurable assistance throughout my programme, Mallam Dauda Ibrahim, a true brother for his assistance, Mallam Shaibu, Mallam Hamidu and Mallam Kudu for their various assistances rendered towards the fulfillment of this task.. My sincere appreciation goes to Mallam Abdullahi, Mr. Yohanna, and Mallam Moh'd Dangana for their various services rendered throughout this work.

My special thanks to my big brother and friend, Dr. P.S. Akinyeye, for his unrelentless effort and assistance towards the success of this work. I cannot but appreciate the efforts of Musa, Emmanuel, Mustapha and Tony.

My profound gratitude also goes to Rev. K. Ladirin, Mrs. C. Olaleye, and Bro. S.K. Bolaji for their moral supports and prayers.

Finally my appreciation goes to my brothers, cousins, nephews, Aduke, Ronke, Victor and all others too.

ABSTRACT

Two hundred and eighty eight samples of maize were collected from field, markets and stores in the four agroecological zones of the twenty five local government areas of Niger state for three seasons and screened for their fungal and mycotoxins contaminations. Thirty one different species of fungi were identified with the major ones being *Aspergillus, Fusarium, Penicillium, Trichophyton and Microsporium spp.* The wet zones have the highest incidence of both fungal and mycotoxins contamination with the market being the highest place for contamination with a mean value range of 234 – 728ppb for aflatoxin and 356-721ppb for zearalenone. The presence of these toxigenic fungi in maize in Niger State, a major cereal producing state in the country indicates a potential health hazard to the nation.

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION

Fungi are eukaryotes with well-defined membrane bound nuclei. They are heterotrophic with a few of them having a well defined cell (Smith and Moss, 1985).Food spoilage fungi produce some harmful compounds that are toxic to man and animal; these harmful compounds are referred to as mycotoxins. Mycotoxins are poisonous compounds produced by certain food spoliage fungi; they are secondary metabolites that are produced by fungi on foods and feeds. Mycotoxins are haptens that elicit antibodies when bound to proteins, they have simple chemical structures with low molecular weight of below 500. There are about 300 mycotoxins but commonest one found in foods and feeds are aflatoxins, fumonisins, ochratoxins, zearalenone and deoxynivalenol (Bhat and Vasanthi, 2003).

The effect of poisoning by the mycotoxins is known as mycotoxicosis. Mycotoxins are capable of causing acute and chronic effects in man and animals ranging from disorder of central nervous system, cardiovascular system, pulmonary system and intestinal tract to death (WHO, 1999). They are also immunotoxic, teratogenic, nephrotoxic and mutagenic (WHO, 1999). Mycotoxins are of great public health importance because of their relevance in human hepatoma and esophageal cancer (Bhat and Vasanthi, 2003).

The existence of mycotoxins was not documented until 1960. However, the concept that mouldy food could lead to illness in people or domestic animals was long suspected before their existence was documented by science (Matossian, 1989). There have been outbreaks of mycotoxicoses; they were recurring during the ninth and tenth centuries in which there are vivid descriptions of limbs rotting and falling off (Mantle, 1977). The disease was

later known to be associated with the consumption of cereals, particularly, rye, contaminated with the sclerotic of the plant pathogenic fungus *Claviceps purpurea*.

Alimentary toxic aleukia (ATA) was a severe problem in parts of Russia (Bilay, 1960) and yellow rice disease caused grave concern in Japan, (Saito and Tatsuno, 1971) Amongst animals, sheep facial eczema has been the subject of considerable research in New Zealand while red clove disease, vulvovaginitis and mouldy corn toxicosis were all initially studied in the United States (Joffe, 1983). Though mycotoxins have had and impact mankind on since the beginning of organized crop cultivation, the scientific study of mycotoxins began in 1960 when a large number of turkey poults died in England due to consumption of contaminated groundnut meal imported from Brazil (Blout, 1961).

There is ample evidence that the inhabitants of sub-Sahara Africa are experiencing heavy dietary exposure to food borne mycotoxins. According to the World Development Report (1998), disease caused by mycotoxins lead to reduced life expectancy in developing countries. According to Miller (1996),40 % of the productivity lost to diseases in developing countries is due to diseases exacerbated by mycotoxins especially aflatoxins. Poor harvesting and storage facilities as well underdeveloped infrastructures such as processing facilities, transportations and skilled human resources. The West African countries have tropical climate with all year round high ambient temperature and relative humidity that provide optimal condition for the growth of toxigenic mould and mycotoxins production,

The incidence of mycotoxins, especially aflatoxin, in tropical diets is evident from the presence of aflatoxin M in human breast milk in Ghana, Nigeria, Sierra Leone and Sudan and in umbilical cord blood samples in Ghana, Kenya and Nigeria (CTA, 1997). Aflatoxins are the major food borne carcinogenic hepatotoxins of great public health importance in Africa. Their demonstrated presence in diets and human tissues in Nigeria and some West African states (Bankole and Adebanjo., 2003) may not only account for the relatively high incidence of hepatoma in tropical Africa but may cause exposed children to become stunted, underweight and more susceptible to infectious diseases later in life (Bhat and Vasanthi, 2003). Hospital records showed that Nigeria has liver cancer incidence of about 5 – 9 per 100,000 males, and it accounts for 72%, 19.2% and 10% of all cancer cases in Western rain forest, Northern and Eastern Nigeria, respectively (Gbodi, 1990).

Zearalenone, another mycotoxin found in Nigerian food (Gbodi *et al*; 1986), is a naturally occurring oestrogen produced by species of *Fusarium* causes infertility in sheep and pig (Bankole and Adebanjo., 2003). Although the association between zearalenone exposure and human diseases remain speculative at present, it was considered as a possible causative agent in the out breaks of precocious pubertal changes in thousands of young children in Puerto Rico (Saenz de Rodriguez *et al.*, 1985) and has been suggested to have a possible involvement in human cervical cancer.

Economic loses caused by fungi and mycotoxins contamination occurs at various levels; crops and livestock production and human health. The FAO has estimated that up to 25% of world's food crops are significantly contaminated with mycotoxin (WHO, 1999) and are valued at several billions of dollars that are lost annually. Nine African countries including Nigeria collectively lose 670 Million U S dollars in crop trade per year due to mycotoxin

weight, impaired resistance to infections and deteriorations in quality and loss of market for livestock products. The ill health and death of people due to mycotoxins adversely affects the nation's work force and consequent its economic statues.

Maize provides an excellent substrate for mould growth and mycotoxin contamination (FAO, 1983). It is the most economic source of starch for livestock and humans and therefore, highly cultivated and consumed in Nigeria. Susceptibility of maize to fungal growth and mycotoxins production has been shown around the world (Jelinck *et al*, 1989). Aflatoxin, zearalenone and ochratoxins A have been reported in maize in Plateau State of Nigeria (Gbodi, 1986) and various fungal species and mycotoxins has been shown to occur naturally in maize (Okoye, 1992). Niger State is one of the leading producer of maize in Nigeria. In 1999, about 823496 tonnes of maize were produced in the state (ADP, 1999).

Niger State is hot and humid for most part of the year, conditions that favour mycotoxin production on grains. In view of the foregoing, this study was done with the following research objectives:

i. To isolate and identify fungi contaminating maize in Niger state
ii. To identify and quantify aflatoxins and zearalenone in maize in Niger state.

The survey for aflatoxins and zearalenone and the mycotoxigenic fungi infesting maize (a highly cultivated and consumed staple food crop) in Niger state and in Nigeria would establish to what extent fungi and mycotoxins in maize will be indicative of the health risk of the consumption of maize in the State and indeed the nation with the adverse effect on food security, agricultural and economic growth of the nation with an increasing demand for food and founds that are scares.

CHAPTER TWO

2.0 LITERATURE REVIEW

The earliest record of mycotoxin problem was seen in China some 500 years ago but the catalyst awareness and knowledge of the effects of mycotoxins on animals and man was as a result of singular event when a feed related mycotoxicosis called " turkey X disease" caused death of thousands of turkey poults in 1960 in Britain which then stimulated the international interest that now exists in mycotoxin.

The first known mycotoxin caused epidemics of nervous derangement and gangrene of man and animals swept Europe from the ninth and tenth centuries. The disease became known as St. Anthony's fire during the Middle age (Bove, 1970).It was later discovered to be caused by the ingestion of ergot alkaloids produced by *Claviceps purpurea*. (Forsyth, 1991). In 1926, there was a report of an outbreak of another ergotism in Russia, Ireland in 1929, France in 1953 and Ethiopia in 1979 (King 1979).

Facial eczema in New Zealand, caused by consumption of dead grass contaminated by *Pithomyces chartarum* is another earliest mycotoxicosis; this caused massive hemorrhaging and large number of deaths of sheep (Uraguchi and Yamazaki, 1978). The outbreak of yellow rice disease in Japan in humans was caused by consumption of rice contaminated by several species of *Penicillium* and their metabolites (Ueno, 1984).

In the 1930s a disease of domestic animal called Stachybotrystoxicosis occurred in U.S.S R; this disease was caused by *Stachybotrys atra*. Alimentary toxic aleukia (ATA) outbreak in humans had been reported in the period 1942-1947 (Joffe, 1983). The disease was caused by consumption of grains infested by *Fusarium sporotrichioide* and *Fusarium poae*.

In 1934, in Midwestern states of United States, more than 5000 horses died because of "mouldy corn disease" (Uraguchi and Yamazaki, 1978).

Giberella ear rot also caused extensive feed-refusal problems in swine in the Corn Belt and in the Southeastern United States fescue toxicosis has been a common problem with fescue pastures for many years.

The international awareness of mycotoxins problems was however triggered in 1960, when approximately 100,000 turkey and other domestic birds died in England. The etiology of this major outbreak of what was initially referred to as turkey "X" disease took considerable part of the 1960 and it was discovered that the birds had been poisoned by a contaminant in the groundnut meal used as a protein supplement imported from Brazil (Spensely, 1963).

The implication of the Brazilian groundnut in turkey "X" disease gingered the Nigerian researchers into research of mycotoxins, aflatoxin in particular in groundnut, since Nigeria was then an exporter of groundnut, in order to save her export trade (Gbodi *et al.*, 1984). Since then Nigerian scientists have discovered different species of fungi and mycotoxins in the foods and foodstuffs including maize (Okoye, 1992).

2.1 MYCOTOXINS AND MYCOTOXICOSIS

Mycotoxins are toxic secondary metabolites of food spoliage fungal origin which when ingested, inhaled or absorbed through the skin caused lowered performance, sickness or death in human and animal (Bankole and Adebanjo, 2003). The consequence or effect (disease or pathological abnormalities) of ingesting toxin contaminated foods by men and animals is called mycotoxicosis. Mycotoxicosis may also result from consumption of animal products such as milk from livestock exposed to contaminated feed. (Bankole and Adebanjo, 2003). Mycotoxins may be produced directly by growth of moulds on animal feeds or human foods and illness arising from eating such food are referred to as primary mycotoxicosis. It is also possible that mycotoxins may pass through the food chain into animal products such

as meat which have not themselves been contaminated by mould growth and finally to man, illness arising from such sources are known as secondary mycotoxicosis.

Although mycotoxins are quite clearly defined in this manner, they are very diverse group of compounds produced by a taxonomically wide range of filamentous fungi and showing wide range of toxic effects.

Over 300 mycotoxins have been reported (Coker, 1979). However, based on extensive analytical studies (IARC, 1993) and detailed study of the distribution of fungi in nature, mycotoxins can cause acute or chronic intoxications depending on the animal, sex, breed and dosage (Coker, 1979).

Mycotoxins have attracted worldwide attention due to the significant losses associate with their impact on human and animal health, and consequently national economic implications (Bhat and Vashanti, 1999). The gravity of the mycotoxin contamination problem can be best illustrated by considering a few popularly known and studied mycotoxins

2.1.1 Aflatoxins and Aflatoxicosis.

Aflatoxins are a group of secondary metabolites produced by certain strains of *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nominus* (Bradburn *et al.* 1993). They are highly substituted coumarines fused to a dihydrofurofuran moiety; they are highly toxic and have been epidemiologically implicated as environmental carcinogens in man and animals (Wogan, 1976). The four major aflatoxins are B1, B2, G1 and G2. Aflatoxin M1 and M2 are hydroxylated metabolites of aflatoxins B1 and B2, respectively, in animal. In some animals especially diary cattle, aflatoxins B1 and B2 are partially metabolized to give hydroxylated derivatives named as M1 and M2 (Holzapfel *et a.l,* 1966) because they were first isolated from the milk of lactating animals fed with aflatoxins containing feeds. Aflatoxins are crystalline substances, freely soluble in moderately polar solvents such as

chloroform and methanol and dissolve in water to the extent of 10-20mg/litre; they fluoresce under ultraviolet radiation.

The incidences of aflatoxins in both humans and animals have been reported by many workers. In November 4, 1985, a three year old boy was admitted in the hospital in Northeast Thailand suffering from fever, vomiting, coma and convulsion, he died six hours later after eating rice contaminated with heavy dose of aflatoxins and autopsy revealed oedema in the brain with degradation of neurons as well as change in the fatty tissues associated with the liver, kidney and heart. These symptoms were discovered to be similar to these associated with childhood illness of unknown aetiology occurring in Australia and known as Rye's Syndrome (Smith and Moss, 1985).

Aflatoxins occupy the most important position among mycotoxins in view of their potent carcinogenic effect and high frequency of occurrence under natural conditions as well as their pharmacological properties (Stoloff, 1989). The presence of aflatoxins in a particular commodity is usually associated with poor post-harvest handling of crops such as drying and storage but contamination by aflatoxins has been shown to occur before harvest (Munkvold, 1994) of commodities like maize (Udòh, 1997), groundnut (McDonald and Harkness, 1967) and cottonseed (Marsh *et al.*, 1973).

Aflatoxin is a very powerful hepatocarcinogen, and naturally occurring mixtures of aflatoxin have been classified as class 1 human carcinogen (IARC, 1993). Aflatoxin contaminated diet has been linked with the high incidence of liver cancer in Africa (Bababunmi *et al.*, 1978). In a recent study in China, Li *et al*, (2001) found that the levels of aflatoxins were significantly higher in corn from the high incidence area for human hepatocellular carcinoma. Aflatoxin synergies other agents such as hepatitis B in the causation of liver cancer (Turner *et al.*, 2000).

Reports on the detection of aflatoxin B1 in the livers of children in Sudan who had the protein energy malnutrition syndrome, kwashiorkor have led to the speculations about the role of aflatoxin in the causation of the disease (Hendrickse, 1984). But Hendrickse, (1991) has shown that kwashiorkor and aflatoxin exposure appear to be seasonally linked in tropical regions because children with kwashiorkor have tested positive for aflatoxin in blood, urine and livers than similar age-matched children. Aflatoxin positive kwashiorkor children showed severity of oedema, increased number of infections, lower haemoglobin levels and longer duration of hospital stay than aflatoxin negative kwashiorkor children (Ramjee, 1996). Gong *et al.*, (2002) demonstrated that children in Togo and Benin who ate food contaminated with aflatoxins showed the kind of stunted growth and are under weight which are symptoms associated with malnutrition.

Aflatoxins have also been shown to be immunotoxic to both livestock and man. Turner *et al.*, (2003) detected aflatoxin albumin adducts in 93% of sampled children (6-9years) in Gambia and provided evidence that IgA in saliva may be reduced because of high dietary levels of aflatoxin exposure. Aflatoxin B1 is immunosuppressive and nephrotoxic in animals and human (IARC, 1993). In animal studies, aflatoxin B1 suppresses cell mediated immunity and some aspect of innate immunity.

In 2001, at least 12 people died in Meru North district of Kenya after eating contaminated maize, it was reported that, they had liver complications which was attributed to aflatoxin (Pro MED mail 2001).

In Northern states of Nigeria, aflatoxins have been detected in grains and foodstuffs (Gbodi *et al.*, 1984). In one hospital at Nsukka, Enugu State, aflatoxin B1 has been detected in the urine of liver disease patients (Obidoa Gugnani, 1992).

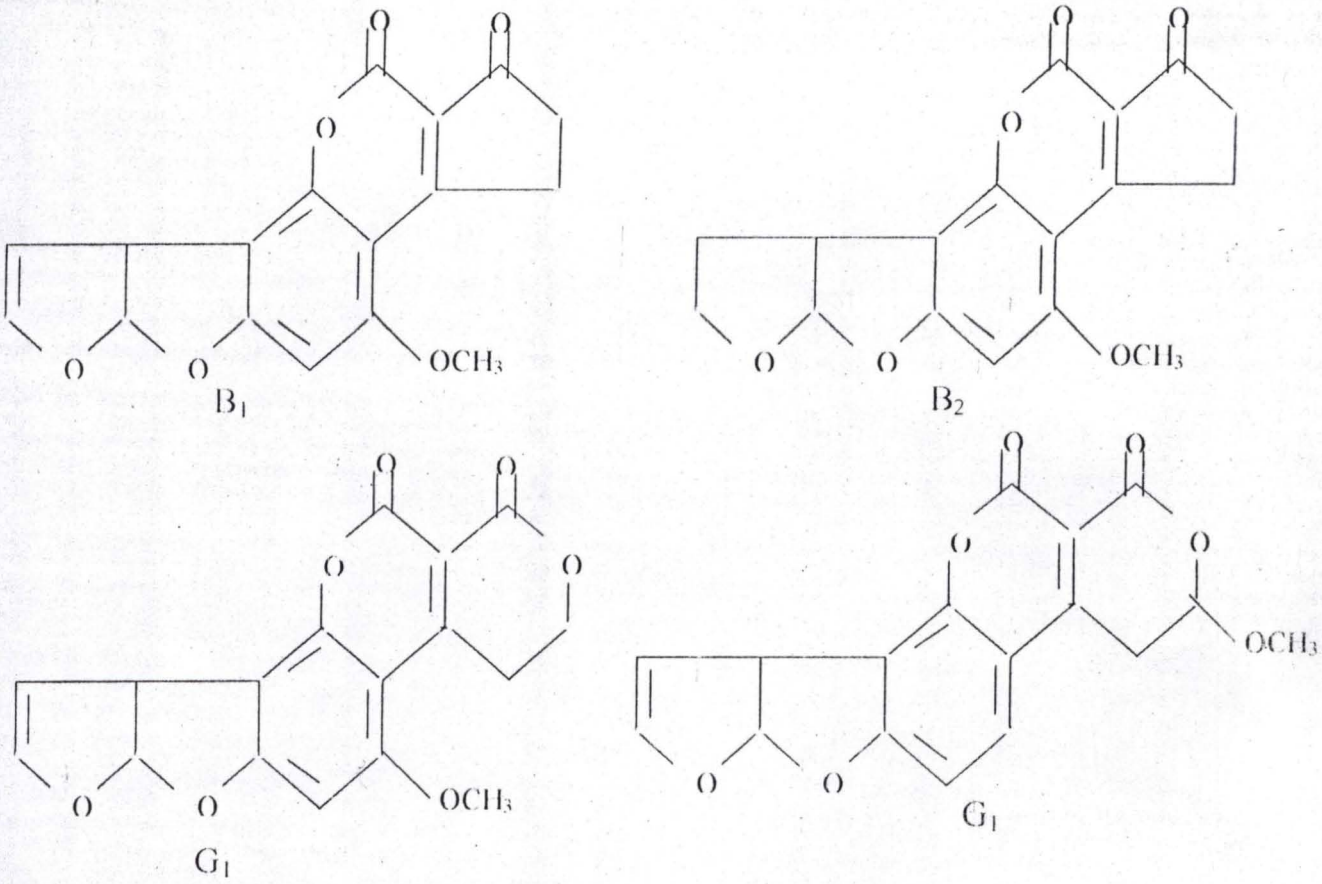


Fig1: Structures of the Major Aflatoxins

Acute aflatoxin toxicity has been demonstrated in a wide range of mammals, fish rabbits and dogs. Ducks, turkey and trout are all highly susceptible. Age, sex and nutritional states all affect the degree of toxicity. Aflatoxins have also been implicated in sub acute and chronic effects in human. Aflatoxin B1 is a potent mutagen (Hall *et al.*, 1994) causing chromosomal aberrations in a variety of plant, animal and human cells. The mutagenicity and carcinogenicity of aflatoxins are considered to arise as a result of the formation of reactive epoxide at the 8 and 9 positions of the terminal furan ring and its subsequent covalent binding to nucleic acid (IARC, 1993).

2.1.2 Zearalenone and Zearlenone Toxicosis

Zearalenone is describe chemically as a phenolic resorcyclic acid lactone and can be produced by a number of *Fusarium* species including

Fusarium graminearum, Fusarium culmorum, Fusarium crookwellense, Fusarium oxysporum, Fusarium moniliforme; Fusarium tricinctum and Fusarium roseum (Krska, 1999). It has been reported world wide in cereals crops like maize, oat, barley, rice, sorghum (Tanaka *et al.*, 1988). Zearalenone occurrence in food and feed has been demonstrated on corn in New Zealand (di Menna *et al.*, 1997), South East Asia (Yamashita *et al.*, 1995) and Africa (Doko *et al.*, 1996).

Zearalenone is a white crystalline stable compound that exhibits blue-green fluorescence when excited by long wave-length ultraviolet light (360nm) and more intense green fluorescence when excited with short wavelength ultraviolet light (260nm) (Aziz *et al.*, 1997).

Zearalenone is a non-steroidal estrogenic mycotoxin that has been implicated in numerous mycotoxicoses in farm animals, especially pigs (Chang and Mirocha,1979).Various oestrogenic effects like decreased fertility, increased embryo lethal resorptions, change in weight of adrenal, thyroid and pituitary glands and change in serum level of progesterone and estradiol have been observed in pig and sheeps (JECFA, 2000). Trial feeding of prepubertal female pigs, demonstrated that higher doses produced distinct redness and swelling of the vulva, swelling of the mammaries with numerous vesicular follicles and some cystic follicles on the ovaries.

Zearalenone has been suspected to be a causative agent in an epidemic of precocious pubertal changes in young children in Puerto Rico between 1978 and 1987 (Sáenz de Rodriguez *et al.*, 1985).Increased incidence of early telearche has been reported from south-east region of Hungary where zearalenone has been found in serum samples of patients (Szuetz *et al.*, 1997).

Zearalenone have low acute toxicity after either oral or intraperitoneal administration in mice, rats and guinea pig. In oral toxicity studies, the effects appeared to be dependent on interactions of zearalenone or its metabolites

with the endoplasmic reticulum. Chronic studies of zearalenone on mice shows an incidence of pituitary adenomas in both males and females and pituitary carcinomas were also found in both males and females mice (Forsell *et al.*, 1986).

Zearalenone has been tested for genotoxicity in a number of systems (Ghedira-Chekir *et al.*, 1998). It induced sister chromatid exchanges, chromosomal aberrations and polyploidy in Chinese hamster ovary *in vitro* and in cultured human lymphocytes, sister chromatid exchange was weakly induced (Kuiper-Goodman *et al.*, 1987). Zearalenone contaminated food consumed by cattle has also been reported to cause fertility disturbance and prolonged heat in a herds of cattle (Osweiler, 1990).

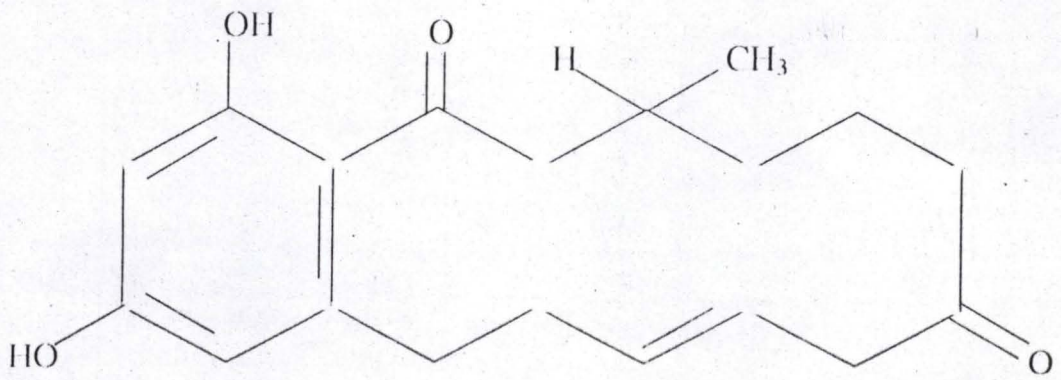


Fig 2. Structure of Zearalenone

2.2 CONDITIONS FAVOURING FUNGAL GROWTH AND MYCOTOXIN PRODUCTION

Niger State has favorable climatic conditions for the production of maize as well as other cereals. The state is generally warm and humid throughout the year (with an average annual rainfall of 1200mm and temperature of 28.5°C – 35.5°C), this is suitable for fungal growth and mycotoxins production(Moss, 1984).

Generally, production of mycotoxins by fungi is influenced by both the genotype of the organism and the physicochemical environment in which it is

growing and in view of the widespread occurrence of some species and the danger they pose to human health, it is important to appreciate that the production of any particular mycotoxins depends on the strain and not only on the species. For example, aflatoxins are known to be produced by *Aspergillus flavus* and *Aspergillus parasiticus*, but there are strains of both species that are non-aflatoxigenic.

Even if a strain of mould has the genetic potential to produce a particular mycotoxin, the level of production will be influenced by the nutrient available. It has been reported that toxin production is proportional to the concentration of the substrate (Pitt, 1995) and to aeration, although the substrates differ in their ability to support mycotoxin production (Madyastha *et al.*, 1990).

Physical parameters such as temperature and water activity are also required for fungal growth and mycotoxins production (Pitt, 1995). In case of *Aspergillus parasiticus,* which is essentially a subtropical species, the optimum temperature for growth is 35°C but maximum aflatoxin production occurs at 25°C – 30°C. But for *Fusaria* toxin, physiology of toxins formulation is radically different in that they are stimulated to produce toxins under cold temperature and water activity of 85 – 88% (Pitt, 1995). The laboratory behavior of these *Fusaria* accord well with known field behaviour since it is the ingestion of over wintered grains that caused ATA (Joffe, 1971) which was reported to occur in the cold months particularly in Missouri (Cliver, 1990).

In nature there are many factors interacting with the growth and metabolism of a mould other than those mentioned, for example antimicrobial agents produced by other microorganisms or by the plant hosting the mould or added biocides. The plant genome is also known to influence the amount of mycotoxins formed by a fungus which has successfully colonized the crop pre or post harvest (Zuber, 1977).

Storage atmosphere also affect the growth of fungi and mycotoxins production. Fungi are generally aerobic organisms, even though oxygen requirement may vary from species to species. Storage atmosphere deficient in oxygen will lead to reduced metabolism as well as toxin production, Detroy (1971) found that a reduction of oxygen content of the storage atmosphere from 5% to 1% dramatically reduced the growth of *Aspergillus flavus* and aflatoxin production. Modified atmosphere have shown promise in controlling the growth of *A. flavus* and aflatoxin production in groundnut (McDonald *et al.*, 1965) and in corn (Wilson *et al.*, 1975).

Insect attack is also a factor that can contribute to fungal growth and mycotoxins production in agricultural products. Insects damage crops they attack and thus rendering them more susceptible to fungal attack. *A. flavu, is a* fungus associated with rice weevil in stored rice and wheat (Moss, 1984). Insects carry spores of fungi into crops they infest and their metabolic activities may lead to increase in moisture and temperature of the crops, thus providing an excellent condition for fungal growth and production of toxin.

Storage system adopted also contributes to the growth of the fungi and their toxins production in some crops(Bhat and Vasanthi ,2003) Storage system will lead to moisture migration which will increase the danger of fungal attack and mycotoxins production in stored crops. The conventional metal silo is particularly vulnerable in this respect in tropics. Some crops like corn which are by nature hygroscopic need to be stored in such a way that will keep the moisture away from then to avoid fungal damage.

2.3 IMPLICATION OF MYCOTOXINS IN ANIMAL DISEASE

Mycotoxins can profoundly influence the health of most animal species but their effect are particularly noticeable in assembled groups of farm animals, such as, dairy and feedlot cattle, pigs and poultry, since their normal feeding practices involve a high intake of concentrated feeds (Hamilton,

1977). However, mycotoxicosis represent a diagnostically difficult problem to the veterinarian since generally the mycotoxin induced disease syndromes are slight and can be easily confused with other diseases caused by pathogenic micro organisms or by nutrient deficiencies. Individual mycotoxin may also affect more than one system of diseased animal species.

Although the poisonous nature of some fungi has been appreciated for many centuries, it was not until the nineteenth century that toxins produced by fungi were implicated in human illness. The appreciation of the significance of mycotoxins in human health continued to evolved and increase.

Animals can demonstrate variable susceptibilities to mycotoxins depending on physiological factors, genetic factors and environmental factors. The effect of mycotoxin on animals has been discussed under primary and secondary mycotoxin diseases (Pier *et al.*, 1980). Primary mycotoxicosis is further classified as acute primary and chronic primary mycotoxicosis.

Acute mycotoxicosis generally has been reported to cause marked signs of disease or death of affected animals with varying symptoms depending on the nature and concentration of the mycotoxins. Most of the effects are produced when high concentration of these toxins are consumed, causing a specific observable acute disease condition like haemorrhage, hepatitis, nephritis, necrosis of oral and enteric epithelia or death (Moss ,1984).

Pathological studies have shown that at acute levels of mycotoxins, virtually every system of an animal's body can be affected by one or combination of mycotoxins (Morehouse, 1979). For example it has been reported that acute aflatoxicosis manifest symptoms that include moderate to severe liver damage, appetite loss,diarrhea,immune suppression and premature motality(Hendrickse,1997)Normally, natural contamination levels

of mycotoxins are usually not high enough to cause overt mycotoxicoses. Reports of naturally occurring out breaks of acute mycotoxicoses are limited in both number and description. Field or feed contamination with mycotoxins will cause chronic mycotoxicosis symptoms or secondary mycotoxin diseases.

Chronic primary mycotoxicosis, on the other hand,is characterized by lack of visible changes in the infected animals and this prevent an easy diagnosis based on symptoms (Smith and Moss, 1985). In chronic aflatoxin mycotoxicoses in pigs, poultry and calves, the effects appear as reduced productivity, reduced food conversion efficiency resulting in reduced weight gains and a general lack of thrift in animals (Burditt *et al.*,1983).

Secondary mycotoxicosis disease is as a result of consumption of a low level of the mycotoxin which can lead to impairment of the native and acquired resistance to infectious diseases causing health related economic losses and most times vaccine failures (Pier *et al.*, 1978). The effects of secondary mycotoxicosis disease are to enhance the infectious processes to which the host animal is naturally predisposed. For example,it has been reported that low concentration of aflatoxin induced immunologic deficiency believed to be associated with the specific failure to function as cell mediated immune system, while at slightly higher levels, antibody production may also be impaired (Bababunmi and Bassir, 1982).

2.3.1 Biochemical and Molecular Basis of Mycotoxicosis.

The effect of mycotoxins on animals occur at the cell or molecular level.most mycotoxins have been examined for their influence on biochemical resctions within the cell, in particular with respect to energy metabolism, carbohydrate and lipid metabolism, protein synthesis and expression of DNA and RNA (Ueno, 1983).For example it has bee reported that the toxicological effect of Aflatoxins only occur after the metabolic activation of the molecules by a microsomal mixed-function oxidase system

(Miller, 1994). Several mycotoxins including aflatoxin B_1 inhibit oxygen uptake in whole tissue homogenates from several animal species. (Austwick, 1984). Aflatoxin B1 has also been reported to act on the electron transport system by inhibiting adenosine triphosphatase activity to varying degrees

There have been reported cases with several animal species, a reduction of hepatic glycogen levels after exposure to aflatiosxin B1, these chances has reported may arise from the effect of mycotoxin on the synthetic enzymes or by inhibition of glycogenesis, depression of glucose transport into hepatocytes (Hayes, 1980).Several mycotoxins including aflatoxin B1 have also been reported to cause accumulation of hepatic lipids.

Many mycotoxins including aflatoxin B_1, have pronounced effects on nucleic acid and protein synthesis in several animals, aflatoxin B_1, have been reported to affect protein synthesis at the level of DNA-dependent RNA polymerase (Yu *et al.*, 1994). In China it has been reported that aflatoxin B_1, decreased the assimilation of vitamin A in mice (Liu and Zou, 1989).

Mycotoxin exhibit a wide array of mycotoxicoses in animals and individual mycotoxins can be mutagenic, carcinogenic, teratogenic or oestrogenic. These effects are influenced by sex of the animal, nutritional statue, environmental factors, species of the animal and interaction with other chemicals.

Several mycotoxins have been shown to cause cancers in variety of animal species (Linsell, 1982). Aflatoxins are carcinogenic to mice, rat, ducks and monkeys. Aflatoxin B_1 primarily causes hepatocellular carcinoma and cholangio carcinoma in the liver. Several other mycotoxins such as zearalenone, sterigmatocystin, patulin, rugulosin, citrinin etc, have also been shown to induce tumor formation in animals.

Microsomal activation has been shown to be necessary for aflatoxin to exhibit mutasgenicity, which arises as a result of the formation of a reactive epoxide at the 8, 9 position of the terminal furan ring and its subsequent

covalent binding to nucleic acid. aflatoxin B_1, can also cause chromosomal aberrations and DNA breakage in animal cells. Zearalenone and other mycotoxins have also been reported to be mutagenetic. (IARC, 1993)

Prenatal effect has been documented with experimental animals which suggest that ochratoxin A, aflatoxin B1 are teratogenic (Hayes, 1980). Mycotoxins which are potent inhibitors of protein synthesis in eukaryotes must impair differentiation in sensitive primordia.

Zearalenone has been reported to be oestrogenic causing enlargement of vulva and uterus in animals especially pigs. Mammaries enlargement, reduced testes, and enlarged glands are all attributed to the oestrogenic effect of zearalenone in animals (Chang *et al*; 1979)

Mycotoxins like ochratoxin A, citrinin and aflatoxin B induced variable level of renal damage in animals (Hayes, 1980). Porcine mycotoxic nephropathy has been documented in Denmark and North America. Some mycotoxins are tremogens. They cause idiopathic disorders of sheep and cattles such as rye-grass staggers and marsh staggers in New Zealand (Gallagher *et al.*, 1981).

2.4 MYCOTOXINS AND HUMAN HEALTH

The evidence of human mycotoxicoses dated back to the nineteenth century. Then, a toxic compound ergot alkaloid produced by *Claviceps purpurea* was implicated in a disease condition called ergotis also known as St. Anthony's fire. Other human disease outbreaks in which mycotoxins have been implicated include alimentary toxic aleukia (Joffe, 1983) yellow rice disease, acute aflatoxicosis and Balkan nephropathy (Austwick, 1975).

Acute Aflatoxicosis has been reported and cases of lethal toxic hepatitis attributable to consumption of aflatoxins contaminated maize have occurred (Krishnamachari *et al.*, 1975). In India in 1975, 272 people were admitted to the hospital with clinical symptoms of aflatoxicosis and there was

27% mortality (Mehan *et a.l*, 1991). In Kenya and Mozambique in 1981 there was 20 hospital admissions and 60% mortality (Marasas, 1988).

Naturally occurring aflatoxins and other mycotoxins are carcinogenic to human (IARC, 1993), thus IARC assignation of aflatoxin as a Group 1 carcinogen. Aflatoxin contaminated diet has been linked with the high incidence of liver cancer in Africa (Bankole and Adebanjo, 2003). In a recent study in China, Li *et al*, (2001) found that the levels of aflatoxins B_1, B2 and G1 were high in corn from the high incidence area for human hepatocellular carcinoma. Fumonisins (metabolite of *Fusarium moliniforme)* have been reported as primary casual factors in esophageal cancer in Transkei, South Africa (Marasas, 1988), Ochratoxin A has also been reported as a renal carcinogen and there is evidence that it contributes to human renal and urinary tumors (Pestka and Bondy 1994).

Reports on the detection of aflatoxin B_1, in the livers of children in Southern Africa shows that there is an interaction of mycotoxins with protein and vitamin A assimilation in human. The children have protein energy malnutrition syndrome, kwashiorkor which led to the speculation about the role of mycotoxin in the causation of the disease (Hendrickse, 1984). Children with kwashiorkor tested positive for aflatoxins in blood and urine stayed longer in hospital and suffered more infections (Adhikari *et al* 1994). It was suggested that aflatoxins acted in conjunction with kwashiorkor by immune suppression to worsen the prognosis (Wild and Hall 1996). In China study showed that aflatoxin B decreased assimilation of Vitamin A, (Liu and Zou, 1989).

Recent studies carried out in West African countries like Benin, the Gambia and Togo indicate chronic exposure of population groups and fetuses to dietary aflatoxins and children exposed to aflatoxin have stunted growth, under weight and more susceptible to infectious diseases in childhood (FAO,

1997). In Kenya, the mean weight of the offspring of women exposed to aflatoxin prenatal was lower (Hendrickse, 1997).

Mycotoxins are immunosuppressive in human (FAO, 2001). Turner *et al, (*2003) detected aflatoxin albumin adducts in 93% of sampled children in Gambia. Trichothecenes and other *Fusarium* toxins are immunosuppressive causing body defence mechanism against infections to be weak (Pestka and Bondy, 1994).

Mycotoxins especially aflatoxin B1 has consistently genotoxic, producing adducts in human cell *in vivo*. In human cells, in culture, it produces DNA damage, gene mutation and chromosomal anomalies (IARC 1993).

Zearalenone has been reported to be a causative agent in an epidemic of precocious pubertal changes in young children in Puerto Rico (Sáenz de Rodriguez *et al.*, 1985). Increased incidence of early telearche in children has also been reported from south-east region of Hungary (Szuatz *et a.l*, 1997) and has been linked to zearalenone.

2.5 ECONOMIC IMPLICATION OF MYCOTOXIN CONTAMINATION OF FOOD

Several toxic effects of mycotoxins on livestock consuming contaminated feeds result in economic losses to the farmer such losses include;

Mortality- which is the most drastic effect, decline in productivity, infertility, feed refusal syndrome, carry over of mycotoxins into animal products, bruising syndrome in poultry birds, growth inhibition, impaired resistance to infections, decreased efficienty of feed digestion and utilization, reduced production of eggs by laying hens, underpigmentation of chicken meat and reduced adaptability to environment (Okoye, 1992).

Farmers are therefore obliged to spend money and other resources on Programmes to detect and ameliorate fungal metabolites (SHANE, 1994).

Mycotoxins losses and cost of mycotoxins management are overlapping areas of concern, costs of mycotoxins management include research production, testing and research necessary to prevent the toxins from appearing in food and feed products of affected commodities. Economic losses result from;

a) Lowered animal production and human toxicity attributed to the presence of the mycotoxins, for example reduction in milk production by diary cattle, infertility and abortion.

(b) The presence of the toxin in the affected commodity will lowers its market values as well as

(c) Secondary effects on agriculture production and agriculture communities..

In developed countries, mycotoxins in food are usually not of a public health risk because of their strict surveillance and toxins management procedures (Miller, 1994). For example, losses from mycotoxins in the United States of America are associated with regulatory losses, as opposed to lowered production, illness and /or death from the effects of the mycotoxins. This is particularly the case for human food but increasingly it has become the case for animal feeds, as strict feed quality control programs become norms for large-scale animal production units.

Between 1980-1990, the EEC reduced imports of some agricultural commodities by about 5%., Such action protect the health of humans and animals in Developed World but causes hardship in the developing world because of loss of market and local population consumes the rejected and possibly high contaminated products.

The negative trade impact of tightening the standards has been calculated by the World Bank for peanut from African exporting countries

(Otsuki *et al.*, 2001). A third United Nation Conference on the Least Developed Countries in Brussels on May 14, 2001, Secretary-general Kofi Annan said "a World Bank study has calculated that European Union regulation on aflatoxins cost Africa 750 million US dollars each year in export of cereals, dried fruits and nuts" (Annan, 2001).

The investment in research programme by government primarily to prevent mycotoxins in crops can be considered a major cost of mycotoxin management .The USDA's Agricultural Research Service(ARS) has a mycotoxin research programme of 17.7 million US Dollars in 2000 fiscal year, that focused on prevention of the fungi and toxins production in crops.

In developing countries like Nigeria, there is little doubt that high levels of exposure to food-borne mycotoxins are a serious threat to public health. It is a developmental issue, which embraces childhood survival, demographic, immune system function, the economic and human resource drain due to cancer as well as food security where livestock feeds are contaminated.

2.6 LEGISLATIONS ON MYCOTOXINS IN FOOD

Globalization of trade has complicated the way of dealing with mycotoxins in that regulatory standards often become a bargaining chip in the world trade negotiations (Cardwell *et al.*,2001) While developed countries have well-developed infrastructures for monitoring of internal food quality standards, people in developing countries are not protected by food quality monitoring and enforcement of safe standards within their countries. On the other hand, foods being exported are expected to comply with CODEX Alimentarius standards, thereby possibly inadvertently resulting in higher risk of exposure in developing countries because only the best quality foods leave the country. Although the FAO has assisted most countries in sub-Saharan

African to enter CODEX Alimentarius standards into law, monitoring of food quality for foods destined for local consumption is rare.

According to World Health Organization (WHO), the permitted level in food products of aflatoxin, for example, is zero part per billion (0ppb) for children, 20ppb for adults and 55ppb for animals(Cardwell,2001a).These norms are not respected in West Africa((Cardwell,2001b)But some countries have set regulatory standards for most of their food and feedstuff as shown in the table 1(as compiled from the FAO publication on worldwide regulations for Mycotoxins 1995 and from Ministry of Health resolution, published in the Diário official da União of October 16, 2002 in Brazil).

Table 1

COUNTRY	FOOD ITEMS	LEGISLATED AMOUNT ng\Kg
Brazil	peanut(shelled, roasted and paste)	Aflatoxins 20 µg/Kg
	corn (whole grains, ground and mashed)	Aflatoxins 20 µg/Kg
	fluid milk	Aflatoxins M1: 0.5 µg/L
Argentina	Baby foods	Aflatoxins 0 µg/Kg
	Peanut, corn and by-products	Aflatoxins 20 µg/Kg
Canada	Nut and products	Aflatoxins 15 µg/Kg
	Soft wheat	Deoxynivalenol 2000 µg/Kg
	Cattle and poultry feeds	Deoxynivalenol 5000 ppb HT-2 toxin 100µg/Kg
United States of America	Foods	Aflatoxins 20 µg/Kg
	Ready to eat wheat products	Deoxynivalenol 1000 µg/Kg
	Milk products	Aflatoxins 0.5 µg/Kg
European Union Countries	Peanuts, nuts, and dried fruits	Aflatoxins 4 µg/Kg
	Raw cereals	Ochratoxin A 5 µg/Kg
	Dried vine fruits	Ochratoxin A 10 µg/Kg
	Raw milk or milk products and heat treated milk	Aflatoxins Mi 0.05 µg/L
	Spices and alike	Aflatoxins 10 µg/Kg
	Raw materials for rations	Aflatoxins B1 50 µg/Kg
	Complete rations for swine and birds	Aflatoxins B1 20 µg/Kg
	Complete rations for fattening cattle, sheep, bovine except young animals	Aflatoxins B 50 µg/Kg
France	Cereals, vegetables oils	Zearalenone 200 µg/Kg
	Cereals	Ochratoxin 2 µg/Kg
South Africa	All foods	Aflatoxins 10 µg/Kg
Ivory coast	Rations ingredients	Aflatoxins 100µg/Kg
Nigeria	All foods	Aflatoxins B1 5µg/Kg
	Infant foods	Aflatoxins B1 0µg/Kg
	Fluid milk	Aflatoxins M1 1µg/Kg
	Rations	Aflatoxins B1 50 µg/Kg
Egypt	Peanut and products	Aflatoxins 10 µg/Kg
	Corn	Aflatoxins 20 ppb
	Milk and dairy products	Aflatoxins 0 µg/Kg
	Foods for animals and birds	Aflatoxins 20 ppb

Source: (FAO,1997)

2.7 CONTROL OF MYCOTOXINS

The main entry of mycotoxins into human food chains is from agricultural products such as cereal grains and oil seeds or from products derived from these sources (Stoloff, 1976). From practical standpoint, the best means of restricting mycotoxins contamination is by prevention, in particular, by excluding or reducing toxigenic mould growth in these raw and processed materials (Marth and Doyle, 1979a).

Prevention can be accomplished by reducing fungal infections in growing crops, by rapid drying and correct storage of the harvested crops, or by using effective anti mould preservatives (Burditt and Hamilton, 1983a). When mycotoxins contaminated materials is identified, it may in part be salvaged by the removal of contaminated material by mechanical separation techniques, by chemical extraction of the mycotoxins or by detoxification of the material by physical, chemical or biological techniques (Williams, 1983) However the use of each method will depend on the type of contaminated material and the purpose is to be used for as well as the nutritional valve of the material (Hall, 1970).For example corn to be used for compounding feed will not be treated the same way as those that will be used for human consumption .

2.7.1 Prevention

Toxigenic mould spore are almost universally present in agricultural crops and produce, they can be found growing on living plants, decaying plants materials and stored products and successful containment programmes will involve the development of methods to inhibit the germination and proliferation of these spores. To do this pre harvest and post harvest treatments are necessary (Goldblatt and Dolleal., 1977).

2.7.2 Pre-Harvest Treatments

Many environmental conditions have been identified which promote mycotoxin formation in growing crops, such conditions include insect infestation, drought conditions, varietal susceptibility or resistance, mechanical damage, nutritional deficiencies and unseasonal temperature and rainfall (Bullerman, 1979). Many mycotoxins especially the *Fusarium* mycotoxins zearalenone and trichothecenes, the tremogens, the ergot alkaloids and more recently observed aflatoxins can be formed during the growing stages of certain crops.

Pre-harvest treatment will evolved selection and breeding of crop variety that are genetically resistance to field infestation by mould, careful use of insecticides and fungicides, early harvest and drying and irrigation to prevent drought conditions (Marth and Dollear., 1979).

2.7.3 Post-Harvest Treatments

Post-harvest contamination with mycotoxins can be controlled only through the use of current technology and knowledge derived from a broad spectrum of scientific disciplines (Liberman, 1983). These treatments involve physical and chemical methods.

The physical methods of controlling mycotoxins production include heating (Hell *et al.*, 2000a) pasteuirizing, sterilization, cooling, vacuum packaging, canning, drying (Awuah and Ellis 2002) and irradiation to reduced water content of the susceptible crops (Lieberman, 1983). Alternative physical method of controlling fungal growth and mycotoxins productions is reduction of storage time (Christensen, 1974).

Chemical methods involve the use of chemical inhibitors of mould growth. International regulations vary somewhat in the type and level of these chemicals/preservatives that are acceptable. The table 2 shows some of the preservatives and their acceptable daily intakes (ADI) recommended by the

Joint FAO/WHO Expert Committee on Food Additive. A successful preservative must have very low mammalian toxicity but possess wide and long lasting microbial inhibitory properties (William, 1983).

Table 2: Acceptable daily intake (ADI) of preservatives.

Preservatives	ADI ($mgkg^{-1}$ body weight per day)
Propionic acid	no limit
Sorbic acid	0-25
P-Hydroxybenzoic acid esters	0-10
Nitrate	0-5
Benzoic acid	0-5
Formic acid	0-3
Sulphur dioxide, Sulphites	0-0.7
Natamycin	0-0.3
Nitrite	0-0.2
Hexamethylene tetramine	0-0.15
Gentian violet	not specified.

Source: Smith and Moss, 1985.

2.7.4 Elimination Of Mycotoxins From Products

Once a product is contaminated with mycotoxins there are only two options if it is to be used for human or animal consumption (Lord and Lacey, 1978).These are ;

1.the toxin can be removed

i. the toxin can be degraded into less toxic or non-toxic compounds.

The stability of mycotoxins under normal biological conditions affects the elimination of mycotoxins in food and its products. The most studied mycotoxin is aflatoxin which appears to be quite stable under normal biological conditions. Elimination involves physical separation (Udoh, 1997)

chemical separation (Giga and Biscoe, 1989) and detoxification (Philips, 1997) of mycotoxins.

Physical Separation:-

Prior sorting out by hand or mechanical means of damaged or mouldy lots will eliminate mycotoxins (Udoh, 1997). When aflatoxin contamination occurs in peanut, Brazilian nut or almond, the contaminated kernels are normally confined in any batch to a small number of the seeds or kernels; when these seeds are discarded the remaining kernels are relatively free of aflatoxins (Hirano *et al.*, 2001).Off coloured kernels or seeds normally imply mould contamination and such kernels can be separated either by hand picking or by passing through colour sorters (Martin *et al.*, 1999)

Chemical Separation:-

Numerous processes have been developed to remove mycotoxin from contaminated materials by various chemicals extraction techniques. A research programme initiated at Texas A and M University in collaboration with the USDA – ARS Research Laboratory at College Station, Texas, demonstrated the capacity of zeolites to selectively bind mycotoxins in feed. Hydrated sodium calcium aluminosilicate (HSCAS) available commercially as Movasil, can selectively combine with aflatoxin B1 forming a bond resistant to the action of various solvents with a range of PH values and ambient temperatures (Philips., 1997).

Simultaneous solvent extraction of oil and aflatoxin and selective extraction of aflatoxins from peanut oil with sodium hydroxide and bleaching earth have also been demonstrated (Maerck *et al.*, 1980).

Detoxification:-

Many physical, chemical and biological methods have been developed to degrade mycotoxins present in raw materials.

Physical methods include irradiation by ultraviolet light. Heating of the product will also detoxify some of the mycotoxins present in food and feedstuff especially aflatoxins. With dry heat, such as, roasting, temperatures approaching the melting point (250°C) of aflatoxins must be used to effect degradation of the toxins but in presence of moisture the time of heat is always increased (Castegnaro *et al.*, 1980).

Chemical methods of detoxification involve the use of wide range of chemicals such as acids, alkalis, aldehydes, oxidizing agent and several gases. Ammonia used as an anhydrous gas at elevated temperatures and pressure can cause 95-98 percent reduction in total aflatoxin concentration in peanut meal (Maerck et al., 1980). Oxidizing agents like hydrogen peroxide also has been used in aflatoxin decontamination, also Sodium bisulphate has been used to degrade aflatoxin B1 in naturally contaminated maize (Maerck *et al.*, 1980).

Biological method of degrading mycotoxins lies on the natural ability of microorganisms to degrade them. Many microorganism like bacteria, yeasts, moulds, algae and actinomycetes show varying abilities to degrade mycotoxins. *Flavobacterium aurantiacum* in aqueous solution would take up and metabolize aflatoxins B1, G1 and M1 (Castegnaro *et al.*, 1980).

The prevention of mycotoxin formation in agricultural produce and other foodstuffs represents both a pre and post-harvest problem of regulating the environmental factors influencing fungal growth. The grower must practise:-

1. Good farm management by controlling insects and fungal pests infestation; harvest with minimal damage to seed coats and use of

adequate drying techniques that will reduce seed moistures to safe levels.

2. Food processing should aim to exclude mould at all stages of production and distribution and should analyse raw materials and products for the presence of mycotoxins.
3. Strict adherence to the multidisciplinary principles of mycotoxin control would reduce the exposure of human and animal populations to the effects of mycotoxins.

2.8 THE NATURE OF MYCOTOXINS PROBLEM

The currently intractable global debate on the nature of the mycotoxin contamination revolves around concern for human health. In this regard, the mycotoxins contamination problem has some important characteristics which may constitute the cornerstone and primary point of departure in the quest for solutions to them.

Forgacs and Carllen (1962) enumerated these characteristics as follows:-

a. Mycotoxin problem frequently arise as a problem whose true cause is not immediately identifiable.
b. The disorders (mycotoxicoses) are not transmissible from one person to another, and is neither infectious nor contagious,
c. Treatment with drugs or antibiotics usually has little effect on the course of the disease,
d. Field outbreak of the trouble is often seasonal, as particular climatic sequences favour toxin production by the mould,
e. Careful studies indicate association with specific foodstuffs e.g. peanut, corn, rice etc.
f. Examination of the suspected foodstuffs reveals signs of fungal activity.

In the light of all these and in order to closely identify the mycotoxin problem with a view of diagnosing and finding lasting solution to it, it is necessary, therefore, to look into the aspect of toxicity of mycotoxins.

2.9 MAIZE AND ITS USES

Maize (*Zea mays*), also refered to as corn, India corn, *mais* (French), *milho* (Portuguese) and *maiz* (Spanish) belongs to the family Gramineae and was discovered in the primitive wild state by the Indians of Mesoamerica and developed extensively by them and it became a principal foodstuff of the great Indian civilization of Mexico, Central America and Andean countries (Grolier, 1994). Although maize is of tropical origin, man has selected grains that are adapted to temperate climate with warm summer season. Its cultivation is now extended to all the inhabited continents (Macrae and Sadler, 1994).

Maize is a tall, coarse grass that bears individual spikes or ears and is the leading grain crop cultivated in the United States (Grolier, 1994). It is one of the most widely distributed of all food plants and rank second only to wheat in the total numbers of acres planted throughout the world. Maize is generally the most economical source of starch (Grolier, 1994). It is a staple food crop and plays an important role in the diet of billions of people because of its capacity to produce a large amount of dry matter per hectare of land, its ease of cultivation, versatile food uses and storage characteristics. There are various types of corn among which are dent corn, flunt corn, flour corn, popcorn, sweat corn, pod corn, waxy corn etc. (Grolier, 1994). Maize is a primary source of food, for man, it is the most important cereal in the western hemisphere

Maize is one of the predominant cereal crops in Nigeria. In most parts of the country, fresh grains are eaten roasted or boiled on the cob. The ripe grains are cooked in combination with millet and boiled as porridge "ogi" or

"eko" in Yoruba. In the North maize is milled with rice and used for a type of stiff porridge called "tuwo" in Hausa. Milled maize are also rolled into flat cakes and baked to produce the traditional tortilla chips or maize chips or the milled corn is boiled to make corn starch or kenkey (Gbodi *et al.*, 2000).

The maize'stalk constitutes a raw material for the manufacture of many products ranging from upholstery filler to explosives. Maize is an important raw material in fermentation industries.

Both the kernel and the other parts of the corn plant are used to manufacture a wide range of products, The husk are usually boiled in sugar, pressed and dried to make cigarette papers; yarn are made from the spongy tissues of the corn stalks. Corn cobs are a source of furfurals, a raw material for making nylons, plastics, synthetic rubbers and explosives (Grolier, 1994).

The protein zein which is found in the corn kernel is made into artificial fibre similar to wool and industrial grades of corn oil are used in the manufacture of rubber substitute soaps and inexpensive paint. Corn starch is used in laundering and as fabric finisher in the textile industries or as industrial alcohol. It has been documented that maize has the following nutrient composition (table 3)

Table 3: Proximate composition of maize

Parameter Analyzed	Values (in %)
Lipid	4.06
Protein	9.56
Ash	3.31
Calcium	0.0066
Sodium	0.009
Potassium	0.02
Crude fibre	1.13

Source; (Gbodi *et al.*, 2000)

Maize grain on average contains 75% of starch, with high calorific value, 9% of protein that has low levels of essential amino acid, lysine and tryptophan and 4% of fat of which about 80% is in the germ. Zein is the predominant form of protein found in maize.

Because maize contains high carbohydrate and protein content that can supply both the energy and the amino acids requirement needed for the growth of the fungi as well as fat for additional energy, maize is one of the ideal substrate foi moulds growths (FAO, 1983).

2.10 FUNGI AND MYCOTOXINS IN MAIZE.

The mycoflora and mycotoxins found in maize have been documented (Uraguchi and Yamazaki, 1978). Research results support the contention that generally soft endosperm maize are more susceptible to seed pest than harder endosperm types and that the biggest problem with the opaque genotype is endosperm softness (Grolier, 1994). It appears that the genetic modifications resolve the drawbacks of the original opaque corns through the improvement of endosperm hardness. Despite insecticide application to reduce corn earworm, these insects caused significant cob damage in the predisposition of plants to seed mould damage by providing entry point and vector moulds. If a substrate is spoiled by the growth of mould, this is caused above all by the production of mycotoxins (Hepperly *et a.l,* 1989). This makes maize one of the most susceptible cereal to fungal and mycotoxins contamination.

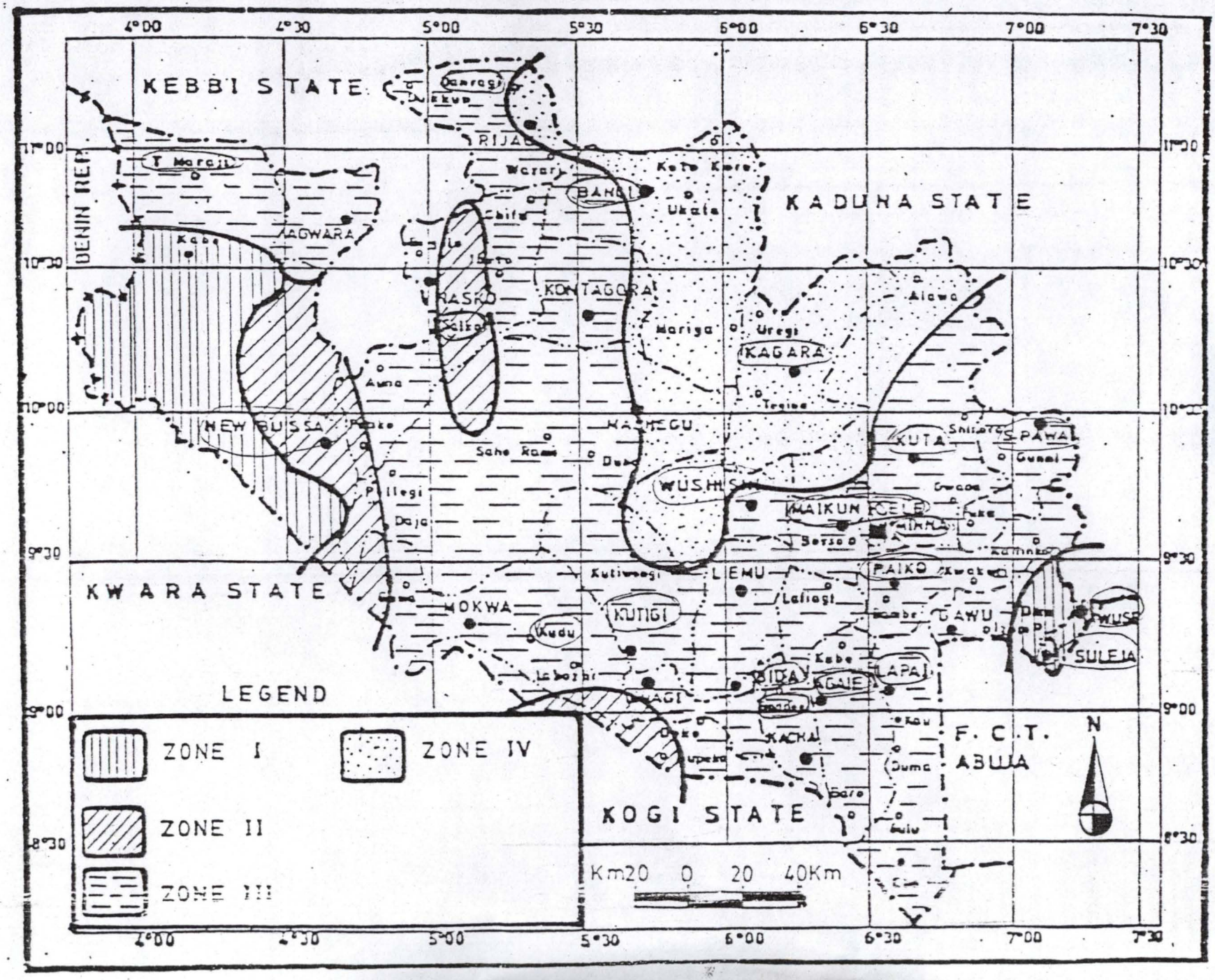
Sydenham *et al,* (1990) studied the high incidence of oesophageal cancer in the Transkei section of South Africa and reported an earlier study done in China and found out that this was the result of consumption of maize contaminated with *Fusarium moniliforme* and its mycotoxins.

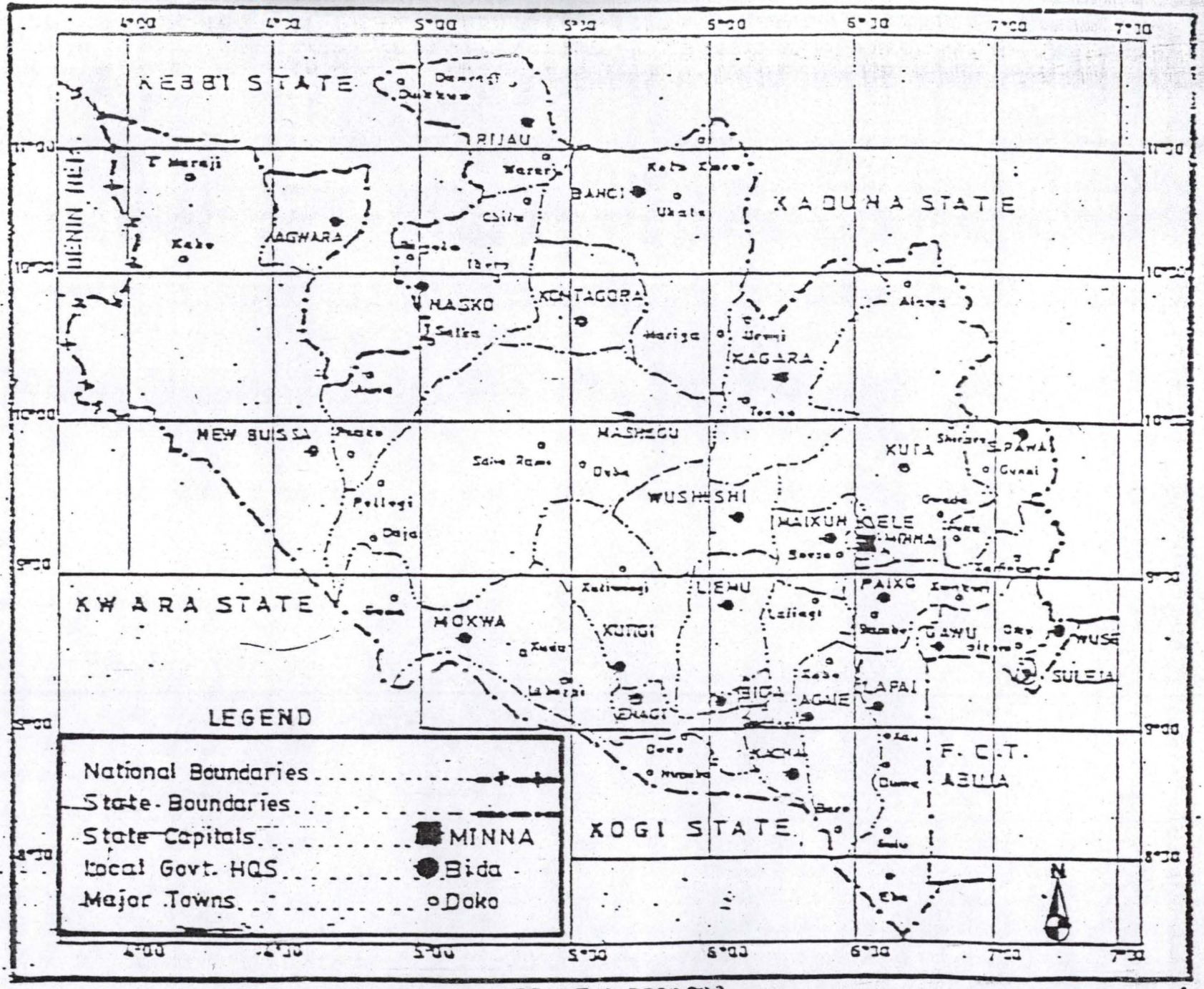
Mycotoxins occur in maize either before or after storage and in products manufactured from maize. Yamashita *et al,* (1995) investigated the level of aflatoxin B1and zearalenone in maize and found that the

concentration of aflatoxin B1 were slightly higher in maize consumed in Argentina than in maize to be exported. While zearalenone levels were in excess of 90 ppb in the sample of maize.

Gbodi, (1986) investigated the mycoflora and mycotoxins produced on maize in Plateau state of Nigeria and found that *Fusarium* species were the commonest fungi found in maize during the dry harmattan and dry hot humid periods and that the common mycotoxins contaminants of maize was zearalenone. Other mycotoxins detected in mouldy maize of Plateau state include T-2 toxin and moniliformin (Okoye, 1992) and deoxynivalenol, nivalenol, fusarenon-X and HT-2 toxin (Okoye 1993). However, there appears to have been no similar systematic investigation on mycoflora and mycotoxins of maize in Niger state, Nigeria, hence the need for the present study.

THE AGRO-CLIMATIC ZONATION MAP OF NIGER STATE BASED ON PRECIPITATION EFFECTIVENESS





SCALE-1: 2000,000

Fig·2.2:NIGER STATE MAP SHOWING THE LOCAL GOVERNMENT AREAS AND

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials.

3.1.1 Collection of Samples

Samples of maize grains were collected randomly from the four microclimatic zones of Niger State. The samples were collected during the harmattan (November – February), the rainy season (June - October) and the hot dry season (March - May) seasons, respectively. The samples were collected between November, 2003 - October, 2004 from the four microclimatic zones under which all the local government areas in Niger state were grouped. Zone I was classified as the wettest zone with mean annual rainfall of above 1400mm and the zone is composed of two local government areas; Suleja, Tafa and part of Gurara. Zone II was known as the wet zone with annual mean rainfall ranges between 1,200 – 1,400mm, it covers two Local Government Areas namely; Borgu and Magama. Zone III is the largest area and covers the following local government areas; Munya, Shiroro, Bosso, Lapai, Paiko, Agaie, Katcha, Bida, Gbako, Edati, Mokwa, Kontagora, Rijau, Mashegu, Agwara, Lavun, Minna and Gurara. This zone is known as the dry zone with annual mean rainfall of 1000 – 1200mm. Zone IV is the driest with annual mean rainfall of less than 1000mm and comprises of Wushishi, Mariga, part of Mashegu and Rafi.

Two hundred and eighty eight samples were collected. Three samples collections were made from the fields, stores and markets (all in duplicates from chosen villages and towns for the three seasons. For every season, twenty four samples were collected from each zone.

All the samples were collected directly from each unit, most stored samples were from sack and rumbu (local mud silo) in dried forms while

samples from field between June and October were wet and fresh others collected from the field for other seasons were dried. Samples from market between June and October were mixture of wet and dried samples while for other periods of collection, samples were purchased as dried samples.

Each sample was collected from source using polythene bags and stored in deep freezer after taking portions for the microbial analysis on arrival from the collections. All the samples were collected randomly from various villages and towns within each microclimatic zone.

The field, market and stored samples were screened and analyzed for mycotoxins, aflatoxins and Zearalenone. Aftatoxins standard was obtained form Makor Chemical Ltd. Jerusalem, Isreal and zearalenone standard was obtained from USDA Southern Regional Research Center, New Orleans, USA (courtesy of Prof. T.A. Gbodi).

3.1.2 Chemicals

Sobouraud dextrose agar, obtained from Biotech Laboratories Ltd, Ipswich, UK. Sodium hypochlorite solution, obtained from Reckitt Benckiser ™, Reckit Benckiser (Nig) Ltd, Agbara Industrial Estate, Ogun State Nigeria, methylene chloride from Maxheal Pharmaceuticals (India) Muribi, 4000102; n-hexane, sodium hydroxide pellets, citric acid, sulphuric acid, methanol and potassium dichromate were all obtained from May and Baker Limited, Dagenham, England. Diethyl ether, ethanol, phosphoric acid, benzene, anhydrous sodium sulphate, sodium hydrogen carbonate, acetone and silica gel 60 – 120mesh were obtained from BDH Chemical Ltd, Poole, England. Acetic acid (Hopkins and Williams, Chad wellhealth, Essex, England). Silica gel GF – 254 (Fluka Chemical AGCH 9470 Buchs, Switzerland). Chloramphenicol sodium succinate for injection (Medrel GMBH – D – 25421, Pinneberg, Germany).

3.13 Equipments

Autoclave AUX-261, BFJ-510-P electronic weighing balance, BFE-610 analytical weighing balance, BJE-750 multiple place rectangular water bath, glass chromatography tanks, columns chromatography, LCF-610 UV lamp, Olympus MKU-350 microscope, OUB-305 oven, PMP-720 micropipette, SGL-700 shaker, SPR-610 spectrophotometer, all made by Gallenkamp and company Ltd, London.

3.2 CULTURE MEDIUM PREPARATION

The culture medium used was Sabouraud dextrose agar (Biotech Laboratories Ltd, Ipswich, United Kingdom) and was prepared by weighing 20 grammes in 1 litre distilled water and sterilized by autoclaving at 121°C for 15minutes pressure after 0.5gramme of chloramphenicol (antibiotic) was added to inhibit bacteria growth (Fawole and Oso, 1995). The sterilized medium was then poured into Petri dishes for solidification. Some of the medium was put into slants and were later used in the sub-culturing of the fungi isolates from the cultured maize.

3.3 ISOLATION OF FUNGI

About ten grains were taken from each sample and washed with ten successive 10ml portions of sterile distilled water and surface sterilized using 5.25% sodium hypochlorite solution, Five grains were placed into each petri dish containing Sabouraud dextrose agar used as medium. The dishes were incubated at 27°C and were examined daily for 7 – 10 days and fungi from plated grains were sub cultured to get pure culture and for identification.

3.4 IDENTIFICATION OF FUNGI.

Pure cultures of fungal isolate were characterized based on colour and structure of hyphae, shape and kind of asexual spore appearance and characteristic of spore head. For the microscopic examination, the isolates were placed in a drop of lactophenol blue on a clean slide, covered with slip and examined under microscope. The fungi were identified by comparing their characteristic features with those of known taxa using the scheme of Rhode and Hartmann (1980).

3.5 MYCOTOXINS DETERMINATION

sA multi mycotoxin assay method developed and used in U.S. Department of Agriculture, Southern Regional Research Center New Orleans

was used (Ehrlich and Lee, 1984). The method utilizes methylene chloride and phosphoric acid for the simultaneous extraction of aflatoxins and zearalenone. Portions of the initial methylene chloride and phosphoric acid were subjected to a specific clean up procedure for mycotoxins.

3.5.1 Extraction And Identification Of Mycotoxins

Glasswares used for extractions and identification were decontaminated by sterilizing them in 10% sodium hypochlorite solution for one hour and dried at 110°C in an oven until they were completely dried.

Two hundred grammes portion of each maize sample was grinded, in a Hammermill. 25g portions of the resultant powder were weighed accurately into a conical flask, 12.5ml, of 0.1M phosphoric acid and 125ml methylene chloride were added.

The flasks were corked and agitated on a wrist shaker (Griffin and George Ltd. England) for 30minutes and the contents were filtered under reduced pressure on a Buchner funnel fitted with 18cm circle rapid filter paper. At least 100ml of the filtrate were collected from each sample.

A glass column was mounted on a retort stand with a plug of glass wool placed tightly at the lower end of the column. 150ml of methylene chloride was then poured into the column and drained half way followed by addition of 5 spatulafulls of anhydrous sodium sulphate; the remaining half of the methylene chloride was then drained. Four spatulafulls of silica gel G-60 was then made into slurry with methylene chloride and poured into the column. This was allowed to settled and another four spatulafulls of anhydrous sodium sulphate were then added to the column and the remaining methylene chloride drained off after the column have settled.

Twenty-five milliliter aliquot of the sample filtrate was measured accurately and poured into the column and drained to the top of the column followed by addition of 65ml of hexane which was similarly drained off. 65ml of diethyl ether was later added and drained off the column. A new beaker was then placed under the column and 65ml of diethyl ether – methanol-water (96:3:1) were added into the column which was collected with the extract and evaporated to dryness on a steam bath (Griffin and George Ltd England). The residue was quantitatively transferred into a vial with 10mls of methylene chloride and dried; this was stored for the thin layer chromatography for identification and quantitation. This same procedure was used for all the samples for aflatoxin extraction.

For zearalenone extraction, the procedure used was that of Thorpe and Ware (1978). And the procedure is as follows, 25ml of the sample filtrate was poured into a separating funnel mounted on a retort stand and 25ml of

4% sodium hydroxide solution was added and shook manually for about 2 minutes, this was then allowed to settled and separate for about 15minutes, the lower layer was then discarded and 25ml of 7% citric acid solution was added with 25ml of benzene, shaken manually for 3minutes and allowed to settle and separate The lower layer was then transferred into another separatory funnel and 25ml of benzene added to it and was shaken manually for about 2minutes after leaving it for separation. The lower layer in the second separatory funnel was discarded and the upper layer was combined with the content in the first separating funnel and 25ml of distilled water was added, shaken and then allowed to settle and separate. The lower aqueous layer was discarded and the upper benzene layer was passed through an anhydrous sodium sulphate column and collected in a conical flask, and evaporated on a steam bath. 10ml portion of methylene chloride was used to transfer the extract into a vial and evaporated to dryness. The extract was stored in refrigerator pending thin layer chromatography. This procedure was used for all the samples.

3.5.2 Thin Layer Chromatographic Plate (Tlc Plates)

The dried extract for aflatoxins is the vials were dissolved with 200ul of benzene-acetonitrile (98:2).Aliquots of the sample were then spotted on the chromatographic plate using disposable micropipette. The pipette tips were changed after each sample applications and were done in such a way that the resultant spots were very compact. Then different volumes 5µl, 10µl, 15µl and 20µlof aflatoxin standard were spotted on the plate as shown in the figure 4.

Fig4. Schematic representation of spots on chromatographic plates

The dried extracts for zearalenone in the vials were dissolved with 50ul of benzene – acetonitrile (98:2). Different volumes of the samples were then spotted on the plate using disposable micropipette. The tips of the pipette were changed after each sample application, then different volumes of zearalenone standard were spotted on the same plate as shown in Figure 4 and these plates were developed in different developing solvents.

The plate for aflatoxin samples were developed using diethyl ether – methanol – water (96:3:1) as developing solvent. The solvent was poured into chromatographic tank and allowed to stand for about 30minutes for equilibration. The plate was then placed in the tank and developed to a solvent front of 10cm. The plate was then removed and dried in air for some minutes.

Plates for zearalenone extract were developed first in benzene – hexane (75:25) followed by development the in methylene-ethanol (97:3). The first solvent was poured into a tank and allowed to equilibrate before placing the spotted plate into it. When the solvent front was about 10cm, the plate was removed and dried before placing in the second tank containing the second developing solvent (methylene-ethanol). This also was allowed to reach about 10cm solvent front, then the plate was removed from the tank and dried in air for some minutes.

3.5.3 Confirmatory Tests And Quantitation

The presence of aflatoxin presence was confirmed by spraying the thin layer chromatographic plate with aqueous sulphuric acid (50/50 v/v), dried and viewed under 365nm uvlight when the spots fluoresced yellow. The presence of zearalenone was confirmed by spraying the thin layer chromatographic plate with alcoholic aluminium chloride (20g/100ml), dried and viewed under 365nm uvlight where the fluorescence intensity increased.

The quantitation of both mycotoxins was done by using "Comparison of standards" techniques. This involved the comparison of the fluorescence intensities of the spots of various R_f of the mycotoxins in the samples with those of their corresponding standard spots to determine which of the sample spots matches any of the standard. The corresponding aliquot volumes were then recorded and the concentration of the mycotoxin in the sample in $\mu g / kg$ was then calculated using the formula:-

For aflatoxin:-

$$\text{Aflatoxin content } (\mu g / kg) = \frac{S \times Y \times V}{W \times Z}$$

Where

S = Volumes of standard with same colour intensity as Sample (in µl)

Y = Concentration of aflatoxin standard used in µg/ml

V = Volume of solvent required to dilute sample contained in final extract.

W = Effective weight (g) of original sample contained in final extracts

Z = Volume of spotted sample equivalent to standard

3.6 DETERMINATION OF THE CONCENTRATION OF MYCOTOXIN STANDARDS

The concentrations of mycotoxin standard solutions were determined using U.V absorption spectrophotometers (toloff, 1980). The spectrophotometer (Spectronic 601, Gallenkamp was calibrated as described below.

3.6.1 Calibration of the Spectrophotometer

1ml of concentrated Tetraoxosulphate (VI) acid (98%H_2SO_4,Sp.gr 1.84), was diluted and made up to 2 litres with distilled water to give a solution of H_2SO_4 (approx. 0.018N). 125mg of potassium dichromate was weighed out, dissolved and made up to 1 litre with the 0.018N solution of the suphuric acid. The molarity of this was then calculated thus:

$$\text{Molarity in mM} = \frac{\text{weight of dichromate in mg}}{\text{Molecular weight of dichromate}}$$

$$= \frac{125}{312.5} = 0.4\text{mm}.$$

Two accurate successive serial dilutions of the acidified dichromate solution were made to prepare solution of approximately 0.2mM and 0.1mM, respectively, by making the acidified solution up with 0.018N solution of tetraoxosulphate (VI) acid. The absorbances of the three solutions (0.4, 0.2, and 0.1mM) were determined at 350nm wavelength using sulphuric acid as the solvent blank.

The absorptivity or molar extinction coefficient (E) for each of the solutions was calculated using the equation:

$$E = \frac{\text{absorbance} \times 1000}{}$$

Concentration (mM)

The average of the three values obtained then gave $\bar{E}$ and the correction factor (CF) for the instrument and the cell used was determined by applying the equation;

$$CF = \frac{3160}{E}$$

3.6.2 Determination of Concentration of Mycotoxin Standard Solutions

Mycotoxin standards received as dry films or crystals were dissolved in appropriate solvent by means of a syringe through the rubber septum of the vial. A known volume of this solution was then withdrawn from the concentrated solution and transferred to a volumetric flask and diluted to the required concentration. The concentration of each mycotoxin was then determined by measuring absorbance (A) at wavelength of maximum absorption and using the following equation.

$$\text{Concentration } (\mu g/m/) = \frac{1000 \times A \times Mwt \times CF}{\bar{E}}$$

Mwt = Molecular weight of the mycotoxin

CF = Correction factor

$\bar{E}$ = Molar extinction coefficient of the mycotoxin.

3.7 STATISTICAL ANALYSIS

Data generated were assessed using analysis of variance (ANOVA). This was used to evaluate variation due to factors (occurrence and concentration) of the toxins from the sampling sites (fsield, market and store). Probability level was maintained at 0.05 (confidence limit) and was used for the significant test of the variation.

CHAPTER FOUR

RESULT

Tables 3, 4 and 5 show the different species of fungi isolated from maize samples collected in Niger State and their incidence. In all, nine hundred and seventy one (971) isolates were cultured and identified. Ninety six maize samples were collected during the dry cold harmattan period (November – February); *Aspergillus spp* were the most common fungi isolated from the samples followed by *Penicillium spp*; *Fusarium spp*, *Trichophyton spp*, *Cephalosporium spp Microsporium spp*, *Mucor spp*, *Rhizopus spp* and *Trichothecium spp*, in that order. During the hot dry season (March-May), *Fusarium spp* were the commonest maize spoilage fungal moulds followed by *Aspergillus spp*, Penicillium *spp*, *Trichophyton spp*, *Microsporium* species, *Rhizopus spp*, *Mucor spp*, *Cephalosporium spp*, *Cladosporium spp*, *Trichoderma spp* and *Trichothecium spp* in that order. 96 samples were collected during the period. The 96 samples collected during the warm humid season of the year (June – October) were mostly infested by *Fusarium spp*, *Aspergillus spp*, *Penicillium spp*, *Microsporium spp*, *Trichophyton spp*, *Mucor spp*, *Rhizopus spp*, *Cephalosporium spp*, *Cladosporium spp*, *Trichothecium spp*, and *Trichoderma spp* .

Table 6 shows the incidence of contamination by aflatoxins and zearalenone in maize samples collected during the cold dry period, 15 local government areas samples were contaminated with aflatoxin B_1 (454 –728 ppb), 5 local government areas were contaminated with aflatoxin B_2 (234 ppb). On the other hand, maize samples from 19 local government areas were contaminated with zearalenone (356 – 721ppb). Aflatoxins $G_1$and G_2 were not detected in any samples.

Table 6-9 show the levels of contamination by Aflatoxins and Zearalenone in maize samples collected from different local government areas in different climatic periods. During the hot dry period, samples from 8 local government areas were contaminated with aflatoxin B1 (454 -908ppb) while one local government area samples was contaminated with aflatoxin B_2 (234ppb). On the other hand, maize samples from 16 local government areas were contaminated with zearalenone (356 -1424ppb). Aflatoxin G_1 and G_2 were not detected in any samples.

Of all the 96 sample collected during the warm humid period, samples from 18 local government areas were contaminated. AflatoxinB_1 (454 – 728ppb) while those from 4 local government areas were contaminated with zearalenone (356 –712 ppb)..Aflatoxins B_2, G_1 and G_2 were not collected in any of the sample.

Statistical analysis carried out on the mycotoxins (aflatoxins and zearalenone) contamination from spoilage by moulds from the four microclimatic zones at different times of the year in three units samples (field, store and market) indicated that there was a significant difference at 5% probability level of mycotoxins contamination in the samples from the market while there were no significant differences in the level of the mycotoxins contamination in samples from the field and the store.

Maize samples collected from seventeen out of the 25 local government areas of the state were contaminated with both aflatoxins and zearalenone while those from all the 25 local government areas were contaminated by either of the two mycotoxins.

Table 3: Fungi Isolated from Maize and their occurrence in the four microclimatic zones in Niger State during dry cold harmattan period (November - February).

Fungi Isolated	Zone I	Zone II	Zone III	Zone IV	Incidence	%
Aspergillus Flavus	7/24	8/24	8/24	6/24	29	9.9
A. niger	3/24	6/24	7/24	4/24	20	6.8
A. nidulans	4/24	2/24	1/24	-	7	2.4
A. fumigalus	5/24	7/24	4/24	7/24	24	8.2
A. parasiticus	4/24	7/24	5/24	6/24	22	7.5
A. versicolar	2/24	3/24	1/24	3/24	9	3.1
A. glaucus	1/24	2/24	3/24	1/24	7	2.4
Fusarium oxysporium	2/24	3/24	1/24	1/24	7	2.4
Cephalosporium spp.	1/24	2/24	1/24	1/24	5	1.7
Cladosporium spp	2/24	1/24	1/24	2/24	6	2.1
Fusarium nivale	1/24	1/24	3/24	2/24	7	2.4
F. poae	2/24	3/24	2/24	3/24	10	3.4
F. tricinctum	3/24	1/24	1/24	2/24	7	2.4
F. moniliforme	6/24	2/24	3/24	2/24	13	4.5
Microsporium scanis	1/24	1/24	2/24	3/24	7	2.4
Microsporium gypsium	1/24	1/24	-	2/24	4	1.4
Mucor spp	3/24	2/24	4/24	3/24	12	4.1
Penicillium expansum	1/24	3/24	2/24	3/24	9	3.1
P. notatum	1/24	1/24	2/24	1/24	5	1.7
P. rubrum	3/24	2/24	1/24	3/24	9	3.1
P. ruqulosum	1/24	3/24	2/24	2/24	8	2.7
P. viridicatum	2/24	5/24	1/24	3/24	11	3.8
Rhizopus spp.	1/24	2/24	3/24	1/24	7	2.4
Trichothecium	-	1/24	2/24	1/24	4	1.4
Trichophyton gallinae	1/24	2/24	1/24	2/24	6	2.1
T. mentagrophyte	1/24	2/24	3/24	3/24	9	3.1
T. rubrum	3/24	4/24	1/24	1/24	9	3.1
T. violaceum	1/24	3/24	1/24	2/24	7	2.4
Penicillium cyclopium	1/24	1/24	1/24	1/24	4	1.4
Penicillium citreoviride	1/24	2/24	2/24	3/24	8	2.7
Total	**65**	**83**	**69**	**75**	**292**	
Percentage %	**22.3**	**28.4**	**23.6**	**25.7**		

Table 4: Fungi isolated from maize and their occurrence in the four microclimatic zones of Niger state during hot and dry period (march – may)

Fungi Isolates	Zone I	Zone II	Zone III	Zone IV	Incidence	%
Aspergillus flavus	3/24	3/24	4/24	3/24	13	3.2
A. niger	3/24	4/24	5/24	3/24	15	3.6
A. fumigatus	1/24	3/24	4/24	6/24	14	3.4
A. nidulans	1/24	1/24	3/24	2/24	7	1.7
A. parasiticus	4/24	3/24	1/24	3/24	11	2.7
A. versicolor	1/24	2/24	-	2/24	4	1.0
A. glaucus	2/24	1/24	1/24	2/24	6	1.5
Fusarium oxysporium	5/24	4/24	6/24	8/24	23	5.6
Cephalosporium spp	2/24	1/24	1/24	2/24	6	1.5
Cladosporium spp	1/24	1/24	3/24	1/24	6	1.5
Fusarium nivale	13/24	12/24	12/24	16/24	53	12.9
F. poae	11/24	10/24	11/24	12/24	44	10.7
F. tricinctum	12/24	11/24	12/24	11/24	46	11.2
F. moniliforme	11/24	14/24	15/24	14/24	54	13.1
Microsporium canis	1/24	2/24	1/24	1/24	5	1.2
Microsporium gypseum	2/24	1/24	3/24	2/24	8	1.9
Mucor species	1/24	2/24	3/24	1/24	7	1.7
Penicillium expansum	2/24	2/24	1/24	3/24	8	1.9
P. notatum	1/24	2/24	3/24	1/24	7	1.7
P. rubrum	2/24	1/24	3/24	1/24	7	1.7
P. rugulosum	1/24	3/24	1/24	2/24	7	1.7
P. cyclopium	2/;24	1/24	2/24	1/24	6	1.5
P. citreoviride	2/24	1/24	2/24	2/24	7	1.7
Rhizopus species	3/24	4/24	2/24	1/24	10	2.4
Trichothecium species	1/24	2/24	1/24	1/24	5	1.2
Trichoderma species	2/24	1/24	-	2/24	5	1.2
Trichophyton gallinae	2/24	1/24	1/24	2/24	6	1.5
T. mentagrophyte	2/24	1/24	3/24	2/24	8	1.9
T. rubrum	1/24	2/24	1/24	2/24	6	1.5
T. violaceaum	3/24	1/24	1/24	2/24	7	1.7
TOTAL	**98**	**96**	**106**	**111**	**411**	
PERCENTAGE %	**23.8**	**23.4**	**25.8**	**27.0**		

Table 5: Fungi isolated from maize and their occurrence in the four microclimatic zones of Niger State during the humid period (June – October)

Fungi isolates	Zone I	Zone II	Zone III	Zone IV	Incidence	%
Aspergillus flavus	5/24	7/24	6/24	4/24	22	8.2
A. niger	3/24	4/24	3/24	2/24	12	4.5
A. nidulans	2/24	1/24	-	1/24	4	1.5
A. fumigatus	1/24	1/24	4/24	2/24	8	3.0
A. parasiticus	-	2/24	4/24	1/24	7	2.6
A. versicolor	1/24	1/24	3/24	-	5	1.9
A. glaucus	2/24	1/24	2/24	1/24	6	2.2
Cephalosporium spp	3/24	124	2/24	3/24	9	3.4
Cladosporium spp	2/24	3/24	1/24	3/24	9	3.4
Fusarium nivale	6/24	4/24	5/24	4/24	19	7.1
F. poae	4/2	6/24	3/24	4/24	17	6.3
F. tricinctum	5/24	3/24	2/24	5/24	15	5.6
F. moniliforme	3/24	2/24	4/24	3/24	12	4.3
Microsporium canis	2/24	3/24	5/24	3/24	13	4.8
Microsporium gypseum	1/24	2/24	3/24	1/24	7	2.6
Mucor spp	3/24	2/24	4/24	3/24	12	4.5
P expansum	1/24	2/24	1/24	1/24	5	1.9
P. notatum	2/24	3/24	1/24	3/24	9	3.4
P. rubrum	1/24	1/24	3/24	2/24	7	2.6
P. ruqulosum	2/24	3/24	1/24	3/24	9	3.4
P. cyclopium	1/24	1/24	2/24	4/24	6	2.2
P. citreoviride	2/24	3/24	2/24	1/24	8	3.0
Rhizopus spp	1/24	2/24	4/24	3/24	10	3.7
Trichothecium spp	1/24	2/24	2/24	1/24	6	2.2
Trichoderma spp	1/24	1/24	1/24	-	3	1.1
Trichophyton gallinae	-	1/24	1/24	-	2	0.7
Trichophyton mentagrophite	1/24	2/24	2/24	1/24	6	2.2
T. rubrum	1/24	2/24	1/24	2/24	6	2.2
T. violaceaum	1/24	1/24	1/24	1/24	4	1.5
Fusarium oxysporium	3/24	4/24	2/24	1/24	10	3.7
TOTAL	**61**	**71**	**75**	**61**	**268**	
PERCENTAGE %	**22.8**	**26.5**	**28.0**	**22.8**		

Table 6a: Incidence of aflatoxins in samples of maize collected at different times of the year in the four microclimatic zones of Niger State of Nigeria.

SAMPLING PERIODS	CONDITIONS	Incidence Of Aflatoxins											
		Zone I			Zone II			Zone III			Zone IV		
		Field	Market	Store	Field	Market	Store	Field	Market	Store	Field	Market	Store
Nov – Feb	Dry , cold harmattan	5/8	1/8	2/8	4/8	3/8	5/8	4/8	3/8	4/8	3/8	2/8	3/8
% Incidence		62.5	12.5	25	50	37.5	62.5	50	37.5	50	37.5	25	37.5
Mar – May	Hot, dry	2/8	2/8	5/8	4/8	3/8	4/8	5/8	4/8	3/8	3/8	4/8	5/8
% Incidence		25	25	62.5	50	37.5	50	62.5	50	37.5	37.5	50	62.5
Jun – Oct	Hot, humid	1/8	2/8	3/8	2/8	3/8	1/8	2/8	4/8	1/8	2/8	2/8	1/8
% Incidence		12.5	25	37.5	25	37.5	12.5	25	50	12.5	25	25	12.5

Table 6b: Incidence of zearalenone in samples of maize collected at different times of the year in the four microclimatic zones of Niger State of Nigeria.

SAMPLING PERIODS	CONDITIONS	Incidence Of Zearalenone											
		Zone I			Zone II			Zone III			Zone IV		
		Field	Market	Store	Field	Market	Store	Field	Market	Store	Field	Market	Store
Nov – Feb	Dry , cold harmattan	4/8	5/8	4/8	3/8	4/8	5/8	28	4/8	4/8	2/8	5/8	4/8
% Incidence		50	62.5	50	37.5	50	62.5	25	50	50	25	62.5	50
Mar – May	Hot, dry	4/8	4/8	5/8	4/8	3/8	4/8	3/8	3/8	4/8	4/8	4/8	5/8
% Incidence		50	50	62.5	50	37.5	50	37.5	37.5	50	50	50	62.5
Jun – Oct	Hot, humid	2/8	4/8	3/8	2/8	3/8	3/8	2/8	4/8	3/8	2/8	4/8	2/8
% Incidence		25	50	37.5	25	37.5	37.5	25	50	37.5	25	50	25

TABLE 7: Levels of contamination of maize (*Zea mays*) sample by aflatoxin and zearalenone during dry-cold harmattan period (Nov-Feb) in Niger state (Concentration in µg/kg or ppb)

LGA/TOWN	B_1(ppb)	B_2	G_1	G_2	Zearalenone (ppb)
Agaie/Agaie	454	234	ND	ND	721
Agwara/T. Maraji	454	ND	ND	ND	721
Bida/Bida	454	ND	ND	ND	721
Borgu/New Bussa	ND	ND	ND	ND	356
Bosso/Mekunkele	454	ND	ND	ND	ND
Edati/Sakpe	ND	234	ND	ND	356
Gbako/Lemu	ND	ND	ND	ND	356
Gurara/Izom	ND	ND	ND	ND	721
Katcha/Badegi	454	ND	ND	ND	721
Kotangora/Salta	ND	234	ND	ND	721
Lapai/Lapai	454	ND	ND	ND	356
Lavun/Kutigi	454	ND	ND	ND	721
Magama	ND	ND	ND	ND	356
Mariga/Bangi	454	ND	ND	ND	ND
Mashegu/Daffan	454	ND	ND	ND	ND
Mokwa/Kudu	454	ND	ND	ND	721
Minna/Chanchaga	454	ND	ND	ND	721
Munya/Seriki pawa	454	ND	ND	ND	356
Paikoro/Paiko	ND	ND	ND	ND	356
Rafi/Kagara	ND	ND	ND	ND	ND
Rijau/Danragi	454	ND	ND	ND	356
Suleja/Madala	728	234	ND	ND	721
Shiroro/Kuta	454	ND	ND	ND	ND
Tafa/Sabo Wuse	728	234	ND	ND	721
Wushishi/Zungeru	ND	ND	ND	ND	ND

Total Number of Samples: 288

LGA = Local Government Area

ND = Not Detected

Table 8: Levels of contamination of maize samples by aflatoxins and zearalenone during hot dry period (Mar-May) in Niger State (Concentration in µg/Kg or ppb)

LGA/TOWN	B_1(ppb)	B_2	G_1	G_2	Zearalenone (ppb)
Agaie/Agaie	908	ND	ND	ND	1,424
Agwara/T. Maraji	908	ND	ND	ND	1,424
Bida/Bida	ND	ND	ND	ND	1,424
Borgu/New Bussa	454	ND	ND	ND	712
Bosso/Mekunkele	ND	ND	ND	ND	ND
Edati/Sakpe	ND	ND	ND	ND	1,424
Gbako/Lemu	454	ND	ND	ND	ND
Gurara/Izom	ND	ND	ND	ND	ND
Katcha/Badegi	ND	ND	ND	ND	1,424
Kotangora/Salka	454	ND	ND	ND	712
Lapai/Lapai	ND	ND	ND	ND	ND
Lavun/Kutigi	ND	ND	ND	ND	712
Magama	ND	ND	ND	ND	356
Mariga/Bangi	454	ND	ND	ND	ND
Mashegu/Daffan	ND	ND	ND	ND	ND
Minna/Chanchaga	ND	ND	ND	ND	712
Mokwa/Kudu	ND	ND	ND	ND	356
Munya/Seriki pawa	454	ND	ND	ND	ND
Paikoro/Paiko	ND	ND	ND	ND	712
Rafi/Kagara	ND	ND	15	ND	ND
Rijau/Danragi	ND	ND	ND	ND	ND
Suleja/Madala	454	234	ND	ND	356
Shiroro/Kuta	ND	ND	ND	ND	356
Tafa/Sabo Wuse	ND	ND	ND	ND	712
Wushishi/Zungeru	ND	ND	ND	ND	712

Total Number of Samples: 288

LGA = Local Government Area

ND = Not Detected

Table 9: levels of contamination of maize samples by aflatoxins and zearalenone during hot Humid period (Jun-Oct)) in Niger State (Concentration in µg/Kg or ppb)

LGA/TOWN	B1(ppb)	B2	G1	G2	Zearalenone (ppb)
Agaie/Agaie	ND	ND	ND	ND	356
Agwara/T. Maraji	454	ND	ND	ND	ND
Bida/Bida	454	ND	ND	ND	ND
Borgu/New Bussa	454	ND	ND	ND	ND
Bosso/Mekunkele	454	ND	ND	ND	712
Edati/Sakpe	454	ND	ND	ND	ND
Gbako/Lemu	ND	ND	ND	ND	ND
Gurara/Izom	454	ND	ND	ND	356
Katcha/Badegi	454	ND	ND	ND	ND
Kotangora/Salka	454	ND	ND	ND	ND
Lapai/Lapai	ND	ND	ND	ND	ND
Lavun/Kutigi	454	ND	ND	ND	ND
Magama	728	ND	ND	ND	ND
Mariga/Bangi	ND	ND	ND	ND	ND
Mashegu/Daffan	ND	ND	ND	ND	ND
Minna/Chanchaga	454	ND	ND	ND	ND
Mokwa/Kudu	728	ND	ND	ND	ND
Munya/Seriki pawa	908	ND	ND	ND	ND
Paikoro/Paiko	728	ND	ND	ND	ND
Rafi/Kagara	ND	ND	ND	ND	ND
Rijau/Danragi	454	ND	ND	ND	ND
Suleja/Madala	454	ND	ND	ND	356
Shiroro/Kuta	454	ND	ND	ND	ND
Tafa/Sabo Wuse	ND	ND	ND	ND	ND
Wushishi/Zungeru	454	ND	ND	ND	ND

Total Number of Samples: 288

LGA = Local Government Area

ND = Not Detected

CHAPTER FIVE

DISCUSSION

Mycotoxigenic fungi are capable of growing on various substrates and substrates differ in their ability to support mycotoxins production (Madyastha *et al.*, 1990). Even if a strain of mould has the potential to produce a particular mycotoxin, the level of production would be influenced by the nutrient available (Madyastha *et al.*, 1990). Typically, fungi require a source of energy in form of carbohydrate or vegetable oil and a source of nitrogen and available water for growth and toxin production (Heseltine, 1976).

Niger state which is generally warm and humid throughout the year (average annual rainfall of 1400mm, 85% of relative humidity and temperature of between 28.5-35.5°C) has suitable climate conditions to support fungal growth and mycotoxins production on cultivated cereals like maize.

Fungi found infesting cereals have been classified into field, storage and advanced decay fungi (Christensen, 1965) but Pelhate (1968) subdivided the mycoflora of grains into three groups based on their ability to persist as ephemeral, mesobiotic and persistent fungi. The first group consists of the field mycoflora while the second and the third groups comprise of the storage mycoflora. However, Christensen (1971) discovered that in some cases a sharp distinction is not possible because the prevalence of storage fungi was noticed in harvested maize that had been damaged.

Field fungi identified from this study are *Cladosporium*, *Fusarium*, *Microsporium*, *Cephalosporium* and some *Trichophyton species*. These moulds were also observed as field fungi by Bankole (1994). The storage

fungi observed in this are *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus species*. They are also in accord with the classification of Christensen (1970).

The stored grains collected from all the four microclimatic zones have higher incidence of fungal growth than the field and market samples. There appears to be a relationship between the fungal growth and the moisture content of the stored grains because at the onset of rainy season the moisture content of the stored maize rose to a level that facilitate the inversion and proliferation by the storage fungi. This result agrees with the report of other workers (Martins and Gilman, 1976).

Insect infestation was also a contributing factor to the high incidence of fungal growth in stored maize, because insects generate moisture and distribute fungi spores in commodities especially stored cereals and most stored grains especially in the Tropics are infested with insects as in the case of those grains collected for this research, this is also in accordance with Setamou *et al.*, (1998).

The incidence, (36.5%, 30.2% and 41.3%) of fungi infestation of maize samples collected from the market samples for the three seasons were higher than that of the field samples (7.2%, 6.8% and 24.3%). This may also probably be as a result of mechanical damage to the grains which then paved way for human and environmental factors that are suitable for mycoflora infestation. The high incidence of fungal growth in the market samples also may be due to the fact that the maize apart from being infected from the field by the fungi has also been subjected to storage fungal invasion before been taken to the market.

Maize samples from zones II (wet zone) and zone III (dry zone) of the four microclimate zone have high incidence of fungal growth throughout the

study period and in all the units (field, market and store) of collections. This observation could be attributed to favourable climate conditions as well as poor storage facilities used in the zones and the various farming practices including methods of harvesting the crops. This discovery is also in accordance with the work of Udoh *et al*, (2000). However some of the fungi isolated and identified belong to the genera known to produce mycotoxins.

The result shows that there is a high incidence of aflatoxin and zearalenone in maize samples found in different parts of Niger state. Both have been presumed to occur mainly in grains in temperate countries with some reports from Zambia where up to 4mg of zearalenone per litre was found in maize grain (Scott, 1978) and Setamou *et al.*, (1997) reported aflatoxins contamination in maize samples from Benin.

The presence of aflatoxins and zearalenone in high concentration from market samples show that the level of mycotoxin production by the identified fungi was very high as it is suspected that most of these fungi are toxigenic in nature.

Some areas (the wet zones) have high concentrations of aflatoxins and zearalenone, has observed during the course of this work, out of all the four microclimate zones in Niger state. This is in agreement with the work of Udoh *et al*, (2000). This observation correlated with the incidence of fungal growth in these zones and from the statistical analysis it was discovered that the market maize samples have high concentration of aflatoxin which is also in accordance with the work of Setamou *et al*, (1998). Aflatoxin contamination in these zones could be attributed to insect infestation, poor storage facilities and favorable environmental conditions as stressed by Payne (1992) and Hell *et al*, (2000a).

High dietary aflatoxin through consumption of contaminated maize can also be immunotoxic to both livestock and human as maize serves as a popular food base and ration in livestock feeds. The incidence of high concentration of zearalenone in maize samples collected from various zones in Niger State on the other hand, also poses a very serious health hazard to the populace as it has the mycotoxin been implicated as a potential human mutagen and carcinogen and speculated to be a causative agent in precocious pubertal changes in young children in Puerto Rico (Saenz de Rodriguez *et al.*, 1985).

Apart from the nutritional loss and heath hazard of fungal contamination and mycotoxins production in maize, the fungal growth and mycotoxin production in Nigerian maize may also had to economic losses as some of the countries importing this cereal grains have enacted laws stipulating permissible levels of these mycotoxins for their countries.

CONCLUSION

This study has shown that stored and marketed maize samples have highest fungal contaminations than the field samples. This indicates that the methods of storage and handling of the crop were inadequate.

The presence of Aflatoxins and zearalenone in maize grain has serious nutritional and health implications for human and livestock as well as economic implications. What then should be done to prevent fungal invasion and mycotoxins contamination of maize in Niger State?

RECOMMENDATION AND SUGGESTION FOR FURTHER WORK

Prevention of fungal invasion of commodity is by far the most effective method of avoiding mycotoxin problems. Attention must be paid to drying of the commodity, early harvest of the crop, adoption of good agronomic practices, sanitation of the storage bin, sorting out of damaged crops before storing, use of improved and resistant varieties of the crop, fumigation, enlightenment programmes in form of workshop and seminars, education and extension programmes and minimum exposure to conditions that may aid fungal growth.

As much has been reported on mycotoxins by various workers, mycotoxins considerations should be a component of an integrated commodity management programme focusing on the maintenance of commodity quality from the field to the consumer. Much more work needs to be done in the Nigerian scene to elucidate the possible role of mycotoxins in diseases like cancer, precocious pubertal changes in young children, protein energy malnutrition syndrome in relation to growth rate of our children such studies should include an examination of: the geographical distribution of the diseases and its aetiology and pathogenesis in the region.

Recommendation is also made that some of the fungi isolated should be investigated for their mycotoxin production and chemotaxonomy in maize substrate.

REFERENCES

Adhikari, M., Ramjee, G. and Berjak, P. (1994). Aflatoxin, Kwashiorkor and Morbidity. Natural Toxin, 2:1-3

Agricultural Development Project (1999). Niger State Branch Quarterly Report.

Annan, K. (2001). Secretary General tells special event on poverty eradication, "best hope" for least developed countries would be new round of global trade negotiation. *Press Release* G/SM/7802 Dev/2311, 14, May, 2001.

Austwich, P.K.C.(1975). Balkan nephrophty. Proceedinqs of the Royal Society of Medicine, 68:219-221.

Austwick, P.K.C. (1984). Human mycotoxicosis – Past, present and future. *Chemistry and Industry*, 547-551.

Awuah, R.T. and Ellis, W.O (2002). Effects of some groundnut packaging methods and protection with *Ocium* and *Syzygium* powders on the kernel infection by fungi, *Mycopathologia,* 154:29-36.

Aziz, N.H., Attia, E.S. and Faraq, S.A. (1997). Effect of gamma-irradiation on the natural occurrence of *Fusarium* mycotoxins in wheat, flour and bread, *Nahrung,* 41:34-37.

Bababunmi, E. A., Uwaifo, A. O. and Bassir, O. (1978). Hepatocarcinogens in Nigerian foodstuffs. *World Reviews on Nutrition and Dietetics,* 28: 188-209.

Bababunmi, E. A. and Bassir, O. (1982). A delay in blood clotting of chickens and ducks induced by aflatoxin treatment. *Poultry Science,* 61:166-168.

Bankole, S.A. (1994). Changes in moisture content, fungal infection and kernel germinability of maize in storage. *International Journal for Tropical Plant Diseases,* 12;213-218.

Bankole, S. A. and Adebanjo, A. (2003). Mycotoxins in foods in West Africa. Current situation and possibilities of controlling it. *African Journal of Biotechnology,* 2 (a): 254 – 263.

Bhatt, R. V. and Vashanti, S. (1999). Occurrence of aflatoxins and its economic impact on human nutrition and animal feed. The new regulation. Agricultural Development, 23:50-57.

Bhatt, R. V. and Vashanti, S. (2003) Food Safety Risk in Developing Countries. IFPRI. Brief 3. September 2003.

Blout, W. P. (1961) Turkey 'X' disease. *Journal of British Turkey Federation,* 9:5-61.

Bove, F.J. (1970). The story of ERGOT. New York Book of Journals. paqination

Bradburn, N., Blunden, G. Coker, R.D. and Jewers, K. (1993) Aflatoxin contamination of maize. *Tropical Science,* 33:418-428.

Bullerman, L. B. (1979) Significance of mycotoxins to food safety and human health. *Journal of Food Protection,* 11:65-86.

Burditt, S. J. and Hamilton, P.B. (1983a). Mould inhibitory activity of an N-oxide ether. *Poultry Science,* 62:2183-2186.

Burditt, S. J. Hamilton, P. B. (1983b). Mould inhibitory activity of an N-oxide either. *Poultry Science*, 62:2183-2186.

Burroughs, R. and Sauer, D. B. (1971). Growth of fungi in sorghum grain stored at high moisture content. *Phytopathologia,* 61:767-772.

Cardwell, K.F. (2001a). Mycotoxin contamination of food in Africa and Anti-nutritional factors. *Food and Nutrition Bulletin*, 21:488-492.

Cardwell, K.F. (2001b). Mycotoxin contamination of food in Africa and Anti-nutritional factors. *Food and Nutrition Bulletin,* 21:488-492.

Cardwell, K.F. Desjardins, A., Munkvold, G. and Robens, J. (2001). The cost of mycotoxins management to the USA: Management of Aflatoxins in United State, Agricultural Research, Service, United State Department of Agriculture, Beltsville, MD./-34.

Castegnaro, M. G., Telling, G. M., Van Egmond, H.P. and Walker, E. A. (1980) *Laboratory Decontamination and Destruction of Aflatoxins B1, B2, G1*

and G2 in Laboratory Wastes. International Agency for Research on Cancer, IARC Scientific Publication, Lyon.

Chang, W. M. Kuttz, J. H. and Mirocha, C. J. (1979). Effects of the mycotoxin zearalenone on swine production *American Journal of Veterinary Research*, 40:1260-1267.

Christensen, C.M. (1965). Fungi in cereal grains and their products. In: Mycotoxins in Foodstuffs. G. N. Wogan (ed). M.I.T. Press, Cambridge, Massachusetts, 9-14.

Christensen, C. M. (1971). Moisture content, moisture transfer and invasion of stored grain seeds by fungi. *Phytopathologia,* 60:280-283.

Christensen, C.M. (1974). Storage of Cereal Grains and Their Products. The American Association of Cereal Chemists, St. Paul, Minnesota.

Cliver, D. O. (1990). Food Borne Diseases. New York. Academic Press.

CODEX Alimentarius Commission (2000). Proposed Draft Code of Practice for the Prevention of Contamination by Ochratoxin A in Cereals. CX/FAC00/17, Rome.

Coker, R. D. (1979). Aflatoxin. Past, present and future, *Tropical Science,* 21:143-161.

CTA (1997). Technical Centre for Agricultural and rural Cooperation. Technical leaflet No. 3.

Detroy, R. W., Lillehoj, E. B. and Ciegler, A. (1971). Aflatoxins and Related compounds. *Microbial Toxins,* VI:3-178.

diMenna, M. E., Lauren, D. R. and Hardacre, A. (1997). Fusaria and Fusarium Toxins in New Zealand Maize Plants. *Mycopathologia,* 139:165-173.

Doko, M. B., Canet, C. Brown, N. Sydenham, E.W., Mpunchane, S. and Siame, B.A. (1996). Natural occurrence of fumonisins and zearalenone in cereal and cereal based food from Eastern and Southern Africa. *Journal of Agriculture and Food Chemistry,* 44:3240-3243.

Ehrlich, K. C. and Lee, L. S. (1984). Mycotoxin grain dust: method of analysis of aflatoxin, Ochratoxin A, Zearalenone, Vomitoxin and Secalonic acid. *Journal of the Association of* Official Analytical Chemists, 67:963.

Emato, P. and Madueno, I. (1974).In: Mycotoxins (ed. Purchase), Elsevier, Amsterdam, 303.

Fawole, M.O. and Oso, B. A. (1995). Laboratory Manual of Microbiology. Spectrum Books Limited, Ibadan, p14-35.

FAO (1983a). Post harvest losses in quality of food grains. FAO Food and Nutrition paper No. 29:103.

FAO (1983b). Post harvest losses in quality of food grains. FAO Food and Nutrition paper No. 29:103.

FAO, (1997). Food and Agricultural Organization. Worldwide regulations for mycotoxins, FAO Food and Nutrition Paper 55. FAO, UN, Rome.

FAO (1997). Agricultural Food and Nutrition for Africa. A resource book for teachers of Agriculture. Healthy Environment and Sustainable Development. Congo Brazzaville. Fact Sheet 5°.

FAO (2001). Manual on the Application of the HACCP System in Mycotoxin Prevention and Control. Healthy Environment and Sustainable Development. Congo Brazzaville. Fact Sheet 5°.

Forgacs, J. and Carllen, W. T. (1962). Mycotoxicosis. *Advances in Veterinary Sciences,* 7:273-382.

Forsell, J. H., Witt, M. F., Tai, J. H., Jensen, R. and Pestka, J. J. (1986). Effect of 8weeks exposure of the B6C3F1-mouse to dietary deoxynivalenol, vomitoxin and zearalenone. *Food and chemical Toxicology,* 24:213-219.

Forsyth, A. (1991). Fungal foes, fungal friends. *Equinox,* 10 (57):29-31.

Gallagher, R. T., White, E. P. and Mortimer, P.H. (1981). Ryegrass staggers: isolation of potent neurotoxins loliterm A and loliterm B from stagger-producing pastures. *New Zealand Veterinary Journal,* 29:188-190.

Gbodi, T. A., Nwude, N., Aliu, Y. O. and Ikediobi, C. O. (1984). The mycoflora and some mycotoxins found in maize. (Zea mays) in plateau state of Nigeria. *Mycopathologia.* In press.

Gbodi, T. A. (1986). The mycoflora and mycotoxins found in acha, maize and cottonseed in Plateau state Nigeria. Ph. D. thesis, Ahmadu Bello University, Zaria.

Gbodi, T. A. (1992). Occurrence of non-Aspergillus mycoflora and mycotoxins in food and feedstuff of the savannah zone of Nigeria. In: Okoye, Z. S. C (ed). Mycotoxin contaminants of Nigerian Food Crops. ISBN 978-166-069-4. Jos University Press Ltd, 61-72.

Gbodi, T. A., Makun, H. A., Kabiru, Y.A., Ogbadoyi, E., Tijani, A. S., Lawal, S. A. and Bayero, R. A. (2000). Effect of local processing methods on aflatoxin contents of some common Nigeria foods prepared from artificially contaminated maize, rice, and sorghum. *Journal of Agricultural Science*. Mansoura, University, 26(6):3759 -3770.

Ghedira-Cheki, L., Maaroufi, K. Zakhama, A., Elouz, F., Dhoub, S., Creppy, E.E. and Bache, H. (1998) induction of a SOS repair system in lysogenic bacteria by zearalenone and its prevention by vitamin E. *Chemical and Biological Interactions,* 113:15-25.

Giga, O. B. and Biscoe, J. (1989). Treating maize grain for storage with registered protectants in Zimbabwe. Technical, practical and economic considerations. *Zimbabwean Science News,* 23:101-103.

Goldblatt, L. A. and Dollear, F. G. (1977). Review of prevention, elimination and detoxification of aflatoxins. *Pure and Applied Chemistry*, 49:1739-1764.

Gong, Y. Y., Cardwell, K. F., Hounsa, A., Eggal, S. Turner, P.C., Hall, A.J. and Wild, C.P. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: a cross sectional study: - *British Medical Journal*, 325: 20-21.

Grolier, Incorporated (1994). Copyright. Encyclopedia Americana, 798 – 807.

Hall, D.W. (1970). Handling and storage of food grains in tropical and subtropical areas. FAO. Agricultural Development Paper No. 90, Rome, Italy.

Hall, A.J. and Wild, C.P. (1994). Epidemiology of aflatoxin related disease. In: The Toxicology of Alflatoxins: Human Health, Veterinary and Agricultural significance. D.A. Eaton and J.D.Goopman (Eds). Academic Press, 233-285.

Hamilton, P. B. (1977). Interrelationships of mycotoxins with nutrition. *Federation Proceedings*. 36:1899-1902.

Hayes, A.W. (1980). Mycotoxins, biological effects and their role in human disease. *Clinical Toxicology*, 17:45-83

Hell, K., Cardwell, K.F, Setamou, M. and Pochling, H.M. (2000a). The influence of storage practices on aflatoxin contamination in maize in four agroecological regions of Benin. *African Entomology*, 6:169-177.

Hell, K., Cardwell, K.F., Setamou, M. and Schulltess, F.(2000b). The influence of insect infestation on aflatoxin contamination of stored maize in four agroecological regions, in Benin. *African Entomology*, 6:169-177.

Hendrickse, R.G. (1981). Aflatoxin and kwashiorkor: epidemiology and clinical studies in Sudanese children and findings in autopsy liver sample from Nigeria and South Africa. *Bulletin of Society of Pathology Exotique*, 76:559-566.

Hendrickse, R.G. (1984). The influence of aflatoxin on child health in the tropics with particular reference to kwashiorkor. *Trans. Royal Society. Tropical Medicine and Hygiene, 78:427-435*

Hendrickse, R.G. (1997). Of sick turkey, kwashiorkor, malaria, perinatal mortality, heroin addicts and food poisoning: research on the influence of aflatoxins on child health in tropics. *Analytical Tropical Medicine and Parasitology, 19:787-793.*

Hepperly, P.R., Wessel-Beaver, L. and Cardona-Castro, C. (1989). Mycoflora and germinat on of maize seed.

Hesseltine, C.W. (976). Conditions leading to mycotoxin contamination of foods and s eds. In: J.D Roderick (ed). *American Chemical Society.* Washington DC. 1-22

Hirano, S., Shima, T. and Shimade, T. (2001), Proportion of aflatoxin B, contaminated kernels and its concentration in imported peanut samples. *Journal of Food Hygiene Society.* Japan, *42:237-242*

Holzapfel, C.W. (1966). Tetrahedron Letters, 25: 2799.

IARC (1993) Some natural occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins. IARC Monographs on Evaluation of C0arcinogenic Risk of Chemical to Human, Lyon, France. International Agency for Research on Cancer, Vol. 56:397-444, Journal Agriculture Univ P.R.73 (2).

JECFA (2000). Joint FAO\WHO Expert Committee on Food Additives, 532nd . .Safety Evaluation of Certain Food Additives. Series 44 (In print).

Jelinck, C.F., Pohland, A.E. and Wood, G.E. (1989). Review of mycotoxin in food contamination Worldwide occurrence of mycotoxins in food and feeds. An update: *Journal of the Association of Official Analytical Chemists, 72 (2):223-229.*

Joffe, A.Z. (1971). Microbial Toxins VII (Cigler, Kadis and Ajl -eds). Academic Press, New York.

Joffe, A.Z. (1983). Food-borne disease: Alimentary toxic aleukia. In: Handbook of Food-borne Disease of Biological Origin, M.Rechcigle (ed). CRC Press, Florida, 193.

Kellerhals, M.D. (2000). E.U food safety may cost Africa $700 million in trade. World Bank says rule does little to protect human health. [Available on line] http://usinfo.state/govKing, B. (1979). Outbreak of Ergotism in Wollo, Ethiopia *Lancet* ,1, 1831:1411

Krishnamachari, K. A. V. R., Bhat, R. V., Nagarajan, V. And Tilak, T. B. G. (1975). Hepatis due to aflatoxicosis-An outbreak in Western India. Lancte, I:1061-1063.

Krogh, P. (1977). Ochratoxins. In: Mycotoxins on Human and Animal Health, J. V. Rodericks, C. W. Hesseltine and M. A Mehlmen (eds), Pathotox Illinos, 489-498.

Krska, R. (1999). Mycotoxins of growing interest. Zearalenone. (MYC-CONF/99/5D). Third Joint FAO/WITO/UNEP International Conference on Mycotoxins. Tunis, 3-6 March.

Kubena, L.F., Philips, T.D, Creger, C.O. (1983). Toxicity of ochratoxin A, and tannic acid to growing chicks. *Poultry science* 62:1786-1792.

Kuiper-Goodman, T., Scott, P.M. and Watanabe, H. (1987). Risk assessment of the mycotoxin zearalenone. *Regulatory Toxicology and Pharmacology*, 7:253-306

Li, F., Yoshizawa, T., Kawamura, O., Luo, X. and Li Y. (2001). Aflatoxins and fumonisins in corn from the high incidence area for human hepatocelilular carcinoma in Guangxi, China: *Journal of Agricultural Food Chemistry*, 49:4122-4126.

Liberman, M. (ed). (1983). *Post Harvest physiology and crop preservation*, NATO Advanced Study Institutes Series, A. (1982), Carcinogenicity of mycotoxins. In: *Environmental carcinogens selected methods of Analysis.* Some mycotoxins, H. Egan, L. Stolloff, M. Castegnaro, P. Scott, I.K.

Linsell, A.(1982). Carcinogenicity of mycotoxins. In: *Environmental Carcinogens Selected Methods of Analysis.* International Agency for Research on Cancer, IARC Scientific Publications, Lyon, 3-14.

O'Neil and H.Bartsch (Eds), International Agency for Research on Cancer, IARC Scientific Publications, Lyon, 3-14.

Liu, Z.L. and Zou, (1989). The effect of aflatoxin B_1, on vitamin A status and on microsomal mixed function oxidase in male mouse: Chung Hua Yu Fang I Hsueh Tsa Chih, 23:218 - 223.

Lord, K.A. and Lacey, J. (1978). Chemical to prevent the mouldy of hay and other crops, *Journal of the Science of Food and Agriculture,* 29:574-575.

Madhyastha, S.M., Marguardt, R.R., Frohlich, A.A, and Abrason, D. (1990). Effects of different cereals and oilseed substrates on growth and production of toxin by *Aspergillus ochraceous* and *Penicillium verrucosum. Journal Agricultural Food Chemistry,* 38:1506-1510.

Macrae, R., Sadler, M.J. (1994). Food Technology and Nutrition. Hull University of Reading Consultancy Nutritionist, Ashford, U.K. *Food Science,* 2825-2831

Maerck, K.E., McElfrsh, p., Wholman, a. and Hillton, B.W. (1980). Aflatoxion destruction in corn using sodium bisulfite, sodium hydroxide and aqueous ammonia. *Journal of Food Protection, 43:571-574.*

Mantle, P. G. (1977). Chemistry of *Claviceps* mycotoxins. In : Mycotoxigenic fungi, mycotoxins, mycotoxicosis : an Encyclopaedic handbook, T. D.Wyllie and L.G. Morehouse, (eds). Marcel Dekker, New York, 421-426.

cMarasas, W.F.O. (1988). Medical relevance of mycotoxin in Southern Africa. *Microbilogie – Aliments – Nutrition,* 6:1-5

Marsh, P.B., Simpson, M.E., Craig, G.O., Donoso, J. and Ramey, H. H. (1973). Occurrence of aflatoxins in cottonseeds at harvest in relation to location of growth and field temperatures. *Journal Environmental Quality,* 2:276-281

Marth, E. H. and Doyle, M.P. (1979a). Update on Moulds: degradation of aflatoxin. *Food Technology,* 81-86.

Marth, E. H. and Doyle, M.P. (1979b). Update on Moulds: degradation of aflatoxin. *Food Technology,* 81-86

Martin, P.M. and Gilman, G.A. (1976). A consideration of the mycotoxins hypothesis with special reference to the mycoflora of maize, sorghum,, wheat and groundnut. Tropical Products Institute, London.

Martin, J., Ba, A., Dimanche, P. and Schilhing, R. (1999). How groundnut contamination can be controlled? Work in Senegal. *Agriculture et Development,* 23:58-67

Matossian, M.K. (1989). Poisons of the Past: Moulds, Epidemics and History. Yale University Press, new Havens.

McDonalds, D. and Harkness, C.(1965). Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts. *Tropical Science*, 5: 143-154

McDonald, D. and Harkness, C. (1967). Aflatoxin in groundnut crop at harvest in Northern Nigeria. *Tropical Science,* 9:148 – 161

Mehan, V. K., McDonald, D., Haravu, L.K, and Jayanthi:s (1991). The groundnut aflatoxin problem, review and literature database. Patancheru, A.P. 502324, India. International Crop Research Institute for Semi-Arid Tropics.

Miller, J.D and Willson, D.M. (1994). In: the Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance D, Edaton, D and J. Groopman (eds). Academic Press, Chicago.

Miller, J. D. (1996). Mycotoxins. "In: Cardwell, K.F. (ed). Proceedings of the Workshop on Mycotoxins in Food in Africa. November 6-10, 1995 Cotonou, Benin. International Institute of Tropical Agriculture, Benin, 18-22

Morehouse, L.G.(1979). Mycotoxicoses of the bovine with reference to fungi and toxins associated with disease. *The Bovine Practitioner,* 14:175-180

Moss, M. O. (1984). Conditions and factors influencing mycotoxins formation in the field and during storage of foods. *Chemistry and Industry,* 533-536.

Munkvold, G. P. and McKean, J. (1994). Field survey for cornear rots and mycotoxins in 1993: Iowa St. *University Veterinary Medicine Extension Newsletter, 402*-751.

Obidoa, O. and Gugnani, H.C. (1992). Mycotoxins in Nigerian foods: Causes, consequences and remedial measures. In: Okoye, Z.S.C (ed) Mycotoxin Contaminants of Nigerian Food Crops ISBN 978-166-069-4. Jos University Press Ltd. 95-114.

Okoye, Z. S. C. (1992). An Overview of Mycotoxins likely to contaminate staple food stuffs. In: Okoye,Z.S.C (ed) Mycotoxin Contaminants of Nigerian Food Crops ISBN 978-166-069-4. Jos University Press Ltd. 9-26.

Okoye, Z.S.C. (1993). *Fusarium* Mycotoxins nivalenol and 4 – acetyl-nivalenol (Fusarenon-x) in mouldy maize harvested from farms in Jos district, Nigeria. *Food Additives and Contaminants,* 10:375-379.

Onyemelukwu, G.C; Ogbadu, G. H. and Salifu, A. (1982). Aflatoxin B1, B2 and G2 in primary liver cell cancirioma. *Toxicologyl Letters,* 10:309-312

Osweiler, G. D. (1990). Mycotoxins and livestock. What role do fungal toxins play in illness and production losses? *Journal of Veterinary Medicine,* 85:89-94.

Otsuki, T, Wilson, J.S and Sewadeh, M. (2001). What price precaution? European Harmonization of Aflatoxin Regulations and African Food Expert Development Research Group (DECRG). The World Bank, Washington D. C.

Payne, G. A. (1992). Aflatoxin in maize. *Criteria Revolution of Plant Science,* 10:432-440.

Pelhate, J. (1968). Evolution de la mycoflora des bles en cours de conservation. *Industrial Alime Agriculture*, 85:769-773.

Pestka, J.J and Bondy, G.S. (1994). Immunotoxic effects of mycotoxins. In: Mycotoxins in Grain, Compounds Other Than Aflatoxin. J. D. Miller (ed). Egan Press, St. Paul, MN. 339-358.

Philips, T.D. (1997). Detection and decontamination of aflatoxin contaminated food products. In: J. H. Williams. D. C., Cummins, G. Hutto and A. King (eds). Impacts and Scientifics Advances through Collaborative Research on Peanut CRSP, Griffin, GA, 117-130.

Pier, A. C. Richard, J. L. and Thurston, J. R. (1978). The influence of mycotoxins on resistance and immunity. In: Interaction of Mycotoxins in Animal Production, National Academy of Sciences. Washington DC, 56-66.

Pier, A. C, Richard, J. L. and Cyzewski, S. J. (1980). Implication of mycotoxins in animal disease. *Journal of the American Veterinary Medicine Association,* 176:719-724.

Pitt, J. I. (1995a) Under what conditions are mycotoxins produced?. Field fungi. *Australian Mycotoxin Newsletter*, 6(2):1-2.

Pitt, J. I. (1995b). *Aspergillus spp. Australian Mycotoxins Newsletter* 6(3):1-2.

ProMED-mail:Promed@Promed.isid.harvard.edu (Available on line) http://www.oneworld.o.ips2/aug00/07 39 002. html

Ramjee, G. (1996). Aflatoxin, kwashiorkor and morbidity. In: Cardwell, K. F. (ed). Proceeding of the Workshop on Mycotoxins in Food in Africa. November 6-10, 1995 at Cotonou, Benin. International Institute of Tropical Agriculture, Benin 25.

Rhode, B. and Hartmann,G(1980). Introducing mycology by examples,1:45-123

Roland, J. O. and Beuchat, L. R. (1984). Influence of temperature and water activity on growth and patulin production by *Byssochlamys nivea* in apple juice. *Applied and Environmental Microbiology,* 47:205-207.

Sáenz de Rodriguez., C. A., Bonqiuoantz, A. M. and conde de Borreq. (1985a). An epidemic of precocious development in Puerto Rican children. *Journal of Pediatrics,* 107:393-396.

Sáenz de Rodriguez., C. A., Bonqiuoantz, A. M. and conde de Borreq. (1985b). An epidemic of precocious development in Puerto Rican children. *Journal of Pediatrics,* 107:393-396.

Saito, M. and Tatsuno, T. (1971). "Toxin of *Fusarium nivale,*" *"Microbial Toxins"* Vol. 7, S. Kadis, A. Ciegler, S. J. Ajl, (eds). Academic, New York.

Sampson, R. A., Hoekstra, E. S. and Van Oorschot, (1981). *Introduction to Food -Borne Fungi,* Central Bureau for Schimmelcultures, Delft.

Scott, P. M. (1978). Mycotoxins in food and ingredients and their origin. *Journal of Food Protection,* 41:385.

Setamou, M., Cardwell, K.F., Schulthess, F. and Hell, K. (1997). *Aspergillus flavus* infection and aflatoxin contamination of preharvest maize in Benin. *Plant Dis.* 81:1323-1327.

Setamou, M., Cardwell, K. F., Schulthess, F. and Hell, K. (1998). Effect of insect damage to maize ears, with special reference to *Mussidia nigrivenella* (Lepidoptera; Pyralidae), on *Aspergillus flavus* infection and aflatoxin production in maize before harvest in the Republic of Benin. *Journal Economical Entomology,* 91(2):433-438.

Shane, S. M. (1994) Economic issues associated with aflatoxicosis. In:. *The Toxicology of Aflatoxins,* DL. Eaton and J. D. Groopmen (eds) Academic Press, Inc. San Diego, California, 513-527.

Smith, J.E. and Moss, M. O. (1985). Mycotoxins, formation, analysis and significance, 4:50-54.

Spensely, P.C. (1963). Aflatoxin, the active principle in turkey 'X' disease. *Endeavour,* 22:75-79.

Stoloff, L. (1976). Occurrence of mycotoxins in foods and feeds. In: Mycotoxins and other Fungal Related Food Problems, J.V. Rodricks (ed.), American Chemical Society, Washington , 23-50

Stoloff, L. (1980). Mycotoxins methodology. *Journal of the Association of Official Analytical Chemists.* Washington DC.

Stoloff, L. (1989). Aflatoxin is not a probable carcinogen. The published evidence is sufficient. *Regulatory Toxicology and Pharmacology*, 10:272-283.

Sydenham, E. W., Thiel, P. G., Marasas, W.F.O., Shephard, G. S., Van Schalkwyk, D. J. and Koch, K. R. (1990). Natural occurrence of some *Fusarium* mycotoxins in corn from low and high oesophageal cancer prevalence area of the Transkei, South Africa. *Journal of Agriculture and Food Chemistry.*

Szuetz, P., Mesterhazy, A., Falkay, G. Y. amd Bartok, T. (1997). Early telearche symptoms in children and their relations to zearalenone contamination in foodstuff. *Cereals Research Communication*, 25:429-436.

Tanaka, T., Hasagawa, A., Yamamoto, S., Lee, U.S., Sugiura, Y. and Ueno, Y. (1983). Worldwide contamination of cereals by the *Fusarium* mycotoxins, nivalenol, deoxynivalenol and zearalenone. 1. Survey of 19 countries. *Journal of Agriculture and Food Chemistry,* 36:979-983.

Turner, W., Brand Aldridge, D.C. (1983). *Fungal Metabolites* II, Academic Press, London.

Turner, P.C., Mendy, M., White H., Fortuin, M., Hall, A.J. and Wild, C.P. (2000). Hepatitis B infection and aflatoxin biomarker level in Gambian children. *Tropical Medicine of Internal Health,* 5:837-841.

Turner, P.C., Moore, S. E., Hall A. J., Preantice, A. M. and Wild, C. P. (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives,* 111:217-220.

Udoh, J. M. (1997). Aflatoxin content of maize as affected by agricultural practices in five agroecological zones of Nigeria. PhD thesis, University of Ibadan, Nigeria.

Udoh, J. M., Ikotun, T. and Cardwell K.F. (1999). Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. *Journal Stored Product Research,* MSE 255 (in Press).

Udoh, J. M., Cardwell, K. F. and Ikotun, T.(2000). Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. Journal Stored Products Research, 36:187-201

Ueno, Y. (Ed.) (1983). Trichothecenes, Chemical, Biological and Toxicological Aspects, Elsevier, Amsterdam, New York

Ueno, Y. (1984). Hydroxyanthraquinones (skyrines). In: Mycotoxin Production, Isolation, Separation and Purification. V. Betina (ed). Amsterdam, Elsevier/North Holland Biomedical Press, 329-350.

Uraguchi, K. and Yamazaki, M. (1978). Toxicity, Biochemistry and Pathology of Mycotoxins. Helsted Press. Japan, 1-278.

Wogan, G. N. (1976). Aflatoxin and their relation to hepatocellular carcinoma. In: Hepatocellular carcinoma. K. Okuda and R.L. Peters (eds). New York, Wiley Publishers, Inc., 25-41

World Health Organization Food Safety Unit (FOS). (1998) Mycotoxins fact sheets {Available on line} www.afro.who.int.des.

World Health Organization (1999). Basic Food Safety for Health Workers.

Wild, C. P. and Hall, A. J. (1996). Epidemiology of mycotoxins-related disease. The mycota VI. Human and Animal Relationships. Howard/Miller (eds). Springer Verlag, Berlink, 213-225.

Wilson, D. M. and Jay, E. (1975). Influence of aflatoxin production in high moisture corn. *Applied Microbiology*, 29:224-228.

Williams, P. C. (1983a). Maintaining nutritional and processing quality in grain crop during handling, storage and transportation. In: Post-Harvest

Physiology and Crop Preservation, M. Liberman (Ed), NATO Advanced Study Institutes Series, series A, 46:425-444

Williams, P. C. (1983b). Maintaining nutritional and processing quality in grain crop during handling, storage and transportation. In: Post-Harvest Physiology and Crop Preservation, M. Liberman (Ed), NATO Advanced Study Institutes Series, series A, 46:425-444

Yamashita, A., Yashizawa, T. Aiura, Y., Sanchez, P. C., Dizon, E. I., Arim, R. H. and Sardjono. (1995). Fusarium mycotoxins (fumonisins, nivalenol and zearalenone) and aflatoxins in corn from Southeast Asia. *Bioscience, Biotechnology and Biochemistry,* 59:1204-1807.

Yu M. W., Zhang, Y. J., Blaner, W.S. and Santella, R. M. (1994). Influence of vitamins A, C and E and Beta-carotene on aflatoxinB1 binding to DNA in woodchuk hepatocytes. *Cancer,* 73:596-604.

Zuber, M. S. (1977). Influence of plant generics on toxin production in corn. In: Mycotoxins in Human and Animal Health J. V. Rodricks, C. W. Hesseltine and M. A. Mehlman (Eds), Pathotox, Illinois.

Appendix 1: Incidence of mould fungi in maize samples collected in three units of samples collection from each zone during Dry and Cold Harmattan season (November – February)

Fungi Isolates	Zone I			Zone II			Zone III			Zone IV			Incidence
	field	store	market	field	store	market	field	store	market	field	store	market	
Aspergillus flavus	1	4	2	-	6	2	1	4	3	-	3	3	29
A. niger	-	2	1	-	3	3	-	3	4	1	2	1	20
A. nidulans	-	2	2	-	2	-	-	1	-	-	-	-	7
A. fumigatus	-	2	3	1	3	3	1	2	1	1	3	4	24
A. glaucus	-	1	-	-	1	1	-	2	1	-	1	-	7
A. parasiticus	1	2	1	1	5	1	1	3	1	1	3	2	22
A. versicolor	-	1	1	-	1	2	-	1	-	-	1	2	9
Cephalosporium spp	-	1	-	-	1	1	-	1	-	-	1	-	5
Cladosporium spp	-	1	1	-	-	1	-	1	-	-	1	1	6
Fusarium nivale	-	1	-	-	1	-	-	2	1	-	1	1	7
F poae	-	1	1	-	2	1	1	1	-	-	2	1	10
F. tricintum	-	1	2	-	1	-	-	-	1	1	-	1	7
F. moniliforme	1	2	3	-	1	1	-	1	2	-	2	-	13
F. oxysporium	-	2	-	1	2	-	-	1	-	-	1	-	7

Appendix 1: Continued

Fungi Isolates	Zone I			Zone II			Zone III			Zone IV			Incidence
Microsporium canis	-	1	-	-	1	-	-	1	1	-	2	1	7
Microsporium gypseum	-	-	1	-	-	1	-	-	-	-	1	1	4
Mucor species	-	2	1	-	1	-	-	-	-	-	1	1	12
Penicillium expansum	-	1	-		1	2	-	1	1	-	2	1	9
P. notatum	-	1	-	-	1	-	-	1	-	-	1	-	5
P. rubrum	-	1	2	-	1	1	-	1	-	1	1	1	9
P. ruqulosum	-	-	1	-	2	1	-	1	1	-	1	1	8
P. cyclopium	-	1	-	-	1	-	-	1	-	-	1	-	4
P. citreoviride	-	1	-	-	1	1	-	1	1	-	1	2	8
P. viridicantum	-	1	1	1	3	1	-	1	-	-	2	1	11
Rhizopus species	-	-	1	-	2	-	-	2	1	-	-	1	7
Trichothecium species	-	-	-	-	-	1	-	1	1	-	1	-	4
Trichophyton gallinae	-	1	-	-	1	1	-	-	1	-	2	-	6
T. mentagrophite	-	1	-	-	-	2	1	2	-	1	2	-	9
T. rubrum	-	1	2	1	2	1	-	1	-	-	-	1	9
T. violaceum	-	-	1	-	2	1	-	1	-	-	1	1	7
TOTAL	**3**	**35**	**27**	**5**	**49**	**29**	**7**	**40**	**22**	**6**	**40**	**29**	**292**
PERCENTAGE	**1.0**	**12.0**	**9.2**	**1.7**	**16.8**	**9.9**	**2.4**	**13.7**	**7.5**	**2.1**	**13.7**	**9.9**	

Appendix 2: Incidence of mould fungi in maize samples collected in three units of samples collection from each zone during Hot and Dry season (March – May)

Fungi Isolates	Zone I			Zone II			Zone III			Zone IV			Incidence
	field	Store	market	field	store	market	field	store	market	field	store	market	
Aspergillus flavus	-	2	1	-	1	2	-	3	1	-	2	1	13
A. niger	-	1	2	-	3	1	-	3	2	-	3	-	15
A. nidulans	-	1	-	-	1	1	-	2	1	-	2	-	7
A. fumigatus	-	1	-	-	2	1	1	2	1	1	3	2	14
A. glaucus	-	1	1	-	1	-	-	1	-	-	2	-	6
A. parasiticus	-	3	1	-	3	-	-	1	-	-	1	2	11
A. versicolor	-	1	-	-	1	-	-	-	-	-	2	-	4
Cephalosporium spp	-	1	1	-	1	-	-	1	-	-	2	-	6
Cladosporium spp	-	1	-	-	2	-	-	3	2	-	2	-	6
Fusarium nivale	2	7	4	1	7	4	-	7	5	2	8	6	53
F poae	1	7	3	1	6	3	1	8	2	2	8	2	44
F. tricintum	2	8	2	2	5	4	3	3	6	2	6	3	46
F. moniliforme	1	6	4	1	7	6	1	8	7	2	5	6	54
F. oxysporium	-	4	1	-	3	1	1	4	1	1	6	1	23
Microsporium canis	-	1	-	-	2	-	-	1	-	-	1	-	5
Microsporium gypseum	-	1	1	-	1	-	-	2	1	-	2	-	8

Appendix 2 Continued

Fungi Isolates	Zone I			Zone II			Zone III			Zone IV			Incidence
Mucor species	-	1	-	-	2	-	-	1	2	-	1	-	7
Penicillium expansum	-	1	1	-	2	-	-	1	-	-	2	1	8
P. notatum	-	1	-	-	1	1	-	2	1	-	1	-	7
P. rubrum	-	2	-	-	1	-	-	2	1	-	1	-	7
P. ruqulosum	-	1	-	-	1	2	-	1	-	-	1	1	7
P. cyclopium	-	1	1	-	1	-	-	2	-	-	1	-	6
P. citreoviride	-	2	-	-	1	-	-	1	1	-	1	1	7
Rhizopus species	-	1	2	--	2	2	-	--	2	--	-	1	10
Trichothecium spp	-	1		-		2		1		-	1	-	5
Trichoderma spp	-	2	-	-	1	-	-	-	-	-	2	-	5
Trichophyton gallinae	-	1	1	-	1	-	-	1	-	-		2	6
T. mentagrophite	-	1	1	-	-	1	-	2	-	-	1	1	8
T. rubrum	-	1		-	2	-		1		-	1	1	6
T. violaceum	-	3		-		-		1		-	2	-	7
TOTAL	**6**	**65**	**27**	**5**	**61**	**30**	**7**	**64**	**36**	**10**	**69**	**31**	**411**
PERCENTAGE	**1.5**	**15.8**	**6.8**	**1.2**	**14.8**	**7.3**	**1.7**	**15.6**	**8.8**	**2.4**	**16.8**	**7.5**	

Appendix 3: Incidence of mould fungi in maize samples collected in three units of samples collection from each zone during humid period (June - October)

Fungi Isolates	Zone I			Zone II			Zone III			Zone IV			Incidence
	field	Store	market	field	store	market	field	store	market	field	store	market	
Aspergillus flavus	2	1	2	3	1	3	2	-	4	1	1	2	22
A. niger	1	-	2	1	1	2	1	-	2	-	-	2	22
A. nidulans	1	-	1	-	-	1	-	-	-	-	-	1	4
A. fumigatus	-	-	1	-	-	1	2	-	2	1	-	1	8
A. glaucus	1	-	1	-	-	1	1	-	1	1	-	-	6
A. parasiticus	-	-	-	-	1	1	1	1	2	1	-	-	7
A. versicolor	1	-	-	-	-	1	1	1	1	-	-	-	5
Cephalosporium spp	1	1	1	1	-	-	1	-	1	1	1	1	9
Cladosporium spp	1	1	-	1	2	-	-	1	-	1	-	2	9
Fusarium nivale	1	3	2	1	1	2	1	2	2	1	1	2	19
F poae	1	1	2	2	1	3	-	2	1	1	2	1	17
F. tricintum	-	2	3	-	1	2	-	2	-	1	3	1	15
F. moniliforme	-	1	2	-	1	1	1	2	1	-	1	2	12
F. oxysporium	1	1	1	1	2	1	1	-	1	1	-	-	10

Appendix 3: Continued

Fungi Isolates	Zone I			Zone II			Zone III			Zone IV			Incidence
Microsporium canis	1	-	1	1	-	2	2	1	2	1	1	1	13
Microsporium gypseum	1	-	-	-	1	1	1	1	1	1	-	-	7
Mucor species	-	2	1	1	1	-	1	1	2	-	1	2	12
Penicillium expansum	-	-	1	1	1	-	1	-	-	-	1	-	5
P. notatum	-	1	1	-	2	1	-	-	1	1	-	2	9
P. rubrum	1	-	-	-	1	-	1	1	1	1	-	1	7
P. ruqulosum	1	-	1	-	1	2	-	1	-	-	1	2	9
P. cyclopium	-	1	-	-	-	1	-	1	1	-	2	-	6
P. citreoviride	-	1	1	1	1	1	-	1	1	-	1	-	8
Rhizopus species	-	1	-	1	1	-	1	1	2	-	2	1	10
Trichothecium spp	-	1	-	1	1	-	-	1	1	-	1	-	6
Trichoderma spp	-	-	1	1	1	-	-	-	1	-	-	-	3
Trichophyton gallinae	-	-	-	-	1	-	-	1	-	-	-	-	2
T. mentagrophite	-	1	-	-	2	-	-	1	1	-	-	1	6
T. rubrum	-	1	-	-	1	1	-	1	-	1	1	-	6
T. violaceum	-	1	-	-	-	1	-	1	-	-	1	-	4
TOTAL	**15**	**21**	**25**	**16**	**26**	**29**	**19**	**24**	**32**	**15**	**21**	**25**	**268**
PERCENTAGE	**5.6**	**7.8**	**9.3**	**6.0**	**9.7**	**10.8**	**7.1**	**9.0**	**11.9**	**5.6**	**7.8**	**9.3**	

APENDIX 4: Incidence of mould fungi from maize sample and their occurrence in three units of sample collection at different time of the year.

	Fungi isolates	Dry cold hammarttan Nov-Feb	Dry hot season Mar-May	Warm Humid period Jun-Oct
FIELD	*Aspergillus spp*	10	2	22
	Cephalosporium spp	NIL	NIL	1
	Cladosporium species	NIL	NIL	4
	Fusarium spp	3	2.6	14
	Microsporium spp	NIL	NIL	8
	Mucor spp	1	NIL	2
	Penicillium spp	1	NIL	8
	Rhizopus spp	NIL	NIL	2
	Trichothecium spp	NIL	NIL	1
	Trichophyton spp	1	NIL	1
MARKET	*Aspergillus spp*	44	19	35
	Cephalosporium spp	1	1	3
	Cladosporium spp	3	1	2
	Fusarium spp	15	66	30
	Microsporium spp	5	2	5
	Mucor spp	4	2	5
	Penicillium spp	19	11	18
	Rhizopus spp	3	7	3
	Trichoderma spp	NIL	NIL	2
	Trichothecium spp	2	2	1
	Trichophyton species	11	12	4
STORE	*Aspergillus species*	64	49	7
	Cephalosporium species	4	5	2
	Cladosporium species	3	5	4
	Fusarium species	25	123	29
	Microsporium species	6	11	4
	Mucor species	7	5	5
	Penicillium species	32	24	18
	Rhizopus species	4	3	5
	Trichoderma species	NIL	5	1
	Trichothecium species	2	3	4
	Trichophyton species	17	19	13

APENDIX 5: Percentage of fungi isolated from maize in three units of samples collected from the four microclimate zones at different times of the year in Niger State.

SEASON	Zone I	Zone II	Zone III	Zone IV	Total (%)
		FIELD			
Dry cold Harmattan (Nov-Feb)	1.0	1.7	2.4	2.1	7.2
Hot Dry season (Mar-May)	1.5	1.2	1.7	2.4	6.8
Hot Humid Season (Jun-Oct)	5.6	6.0	7.1	5.6	24.3
		MARKET			
Dry cold Harmattan (Nov-Feb)	9.2	9.9	7.5	9.7	36.5
Hot Dry season (Mar-May)	6.6	7.3	8.8	7.5	30.2
Hot Humid Season (Jun-Oct)	9.3	10.8	11.9	9.3	41.3
		STORE			
Dry cold Harmattan (Nov-Feb)	12.0	16.8	13.7	13.7	56.2
Hot Dry season (Mar-May)	15.8	14.8	15.6	16.8	63.0
Hot Humid Season (Jun-Oct)	7.8	9.7	9.0	7.8	34.3

APPENDIX 6: Statistical Analysis of Variance (ANOVA) carried out on Aflatoxins and Zearalenone concentration in (at 5% probability level) field, market and stored samples at different times of the year in the four microclimatic zones of N.S.

Source of Variation	Degree of Freedom	Sum of Square	Mean sum of Square	F. Cal	HO
Btw R	r-1	$\frac{1}{c}\sum R^2 \quad Cf$	$\frac{SSR}{r-1}$	$\frac{MSSR}{MSE}$	
Btw C	C-1	$\frac{1}{r}\sum C^2 \quad Cf$	$\frac{SSC}{C-1}$	$\frac{MSSC}{MSE}$	
Residual (Error)	rc-c-r+1	$S-\frac{1}{r}\sum C^2-\frac{1}{c}\sum R^2+CF$			
Total	rc-1	S-CF			

Btw R=Between Row (Occurrence of mycotoxins)

Btw C=Between Column (Concentration of Mycotoxins)

SS= Sum of Square of Variables

MSS = Mean sum of Square of Variables

F. cal = Fischer test Calculated

F. tab = Fischer table

Ho = Null Hypothesis

CF = Correction factor $(1/rc \times T^2)$

T2 = Grand Total

Residual = Error due to variation

1. Field samples Analysis for Aflatoxins

	Zone I	Zone II	Zone III	Zone IV	Total
A	62.5	50.0	50.0	37.5	200.0
B	25.0	50.0	62.5	37.5	175.0
C	12.5	25.0	25.0	25.0	87.5
Total	100.0	125.0	137.5	100.0	462.5

A = November – February Collections

B = March – May Collections

C = June – October Collections

S.V	DF	SS	MSS	Fcal	HO
Btw R	2	1744.7925	872.3963	6.091	5.99
Btw C	3	351.563	117.1877	0.820	4.76
Residual (error)	6	857.37	143.2291	1	
Total	11	2955.73			

Aflatoxin concentrations from field samples were not significant at 5% probability level.

2. Markets samples Analysis for Aflatoxins

	Zone I	Zone II	Zone III	Zone IV	Total
A	12.5	37.5	37.5	25.0	112.5
B	25.0	37.5	50.0	50.0	162.5
C	25.0	37.5	50.0	25.0	137.5
Total	62.5	112.5	137.5	100.0	412.5

A, B and C as in 1

S.V	DF	SS	MSS	Fcal	HO
Btw R	2	312.500	156.250	3.00	5.99
Btw C	3	976.5625	325.521	6.25	4.76
Residual (error)	6	312.500	52.083	1	
Total	11	1601.5625			

Aflatoxins concentrations from the market samples were significant at 5% probability level

3. Store samples Analysis for Aflatoxins

	Zone I	Zone II	Zone III	Zone IV	Total
A	25.0	62.5	50.0	37.5	175.0
B	62.5	50.0	37.5	62.5	212.5
C	37.5	12.5	12.5	12.5	75.0
Total	125	125	100	112.5	462.5

A, B and C as in 1.

S.V	DF	SS	MSS	Fcal	HO
Btw R	2	1119.792	559.896	9.923	5.99
Btw C	3	52.0837	17.361	0.308	4.76
Residual (error)	6	338.5413	56.424	1	
Total	11	1510.4167			

Aflatoxins concentrations from the store samples were not significant at 5% probability level.

4. Field samples Analysis for Zearalenone.

	Zone I	Zone II	Zone III	Zone IV	Total
A	50.0	37.5	25.0	25.0	137.5
B	50.0	50.0	37.5	50.0	187.5
C	25.0	25.0	25.0	25.0	100
Total	125.0	112.5	87.5	100.0	425.0

A, B and C as in 1

S.V	DF	SS	MSS	Fcal	HO
Btw R	2	963.5417	481.7708	10.091	5.99
Btw C	3	260.4167	86.8056	1.8181	4.76
Residual (error)	6	286.4583	47.7431	1	
Total	11	1510.4167			

Zearalenone concentrations from the field samples were not significant at 5% probability level.

5. Market Samples Analysis for Zearalenone.

	Zone I	Zone II	Zone III	Zone IV	Total
A	62.5	50.0	50.0	62.5	225.0
B	50.0	37.5	37.5	50.0	175.0
C	50.0	37.5	50.0	50.0	187.5
Total	162.5	125.0	137.5	162.5	587.5

A, B and C as in 1

S.V	DF	SS	MSS	Fcal	HO
Btw R	2	338.5417	169.2709	13.0000	5.99
Btw C	3	351.5626	117.1875	9.90000	4.76
Residual (error)	6	78.1250	13.0208	1	
Total	11	768.2292			

Zearalenone concentrations from the market samples were significant at 5% probability level.

6. Stores samples Analysis for Zearalenone.

	Zone I	Zone II	Zone III	Zone IV	Total
A	50.0	62.5	50.0	50.0	212.5
B	62.5	50.0	50.0	62.5	225.0
C	37.5	37.5	37.5	25.0	137.5
Total	150.0	150.0	137.5	137.5	575.0

A, B and C= as in 1

S.V	DF	SS	MSS	Fcal	HO
Btw R	2	1119.7917	559.8959	10.6802	5.99
Btw C	3	52.0834	17.3611	0.330	4.76
Residual (error)	6	338.5146	52.42.36	1	
Total	11	1510.4167			

Zearalenone concentrations from the store samples were not significant at 5% probability level.