FUNGI AND SOME MYCOTOXINS FOUND IN GROUNDNUTS (*Arachis hypogea*) FROM NIGER STATE, NIGERIA

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M.TECH/SSSE/2008/1890

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MAY, 2012.

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTERS OF TECHNOLOGY (M.TECH) IN BIOCHEMISTRY.

MAY, 2012

DECLARATION

I hereby declare that this thesis title: "Fungi and Some Mycotoxins found in Groundnuts (*Arachis hypogea*) from Niger State, Nigeria" is a collection of my original research work and it has not been presented for any qualification anywhere. Information from other sources (published or unpublished) has been duly acknowledged.

Ifeji 15/06/2012

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CERTIFICATION

The thesis titled: "Fungi and Some Mycotoxins found in Groundnuts (*Arachis hypogea*) from Niger State, Nigeria" by IFEJI EBERE IFEYINWA (M.Tech/SSSE/2008/1890) meets the regulations governing the award of the degree of Master of Technology of Federal University of Technology, Minna and it is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to my parents Lay-Reader and Mrs. W. I Ifeji, they are the brain behind my academic success and to my siblings, they are to me "all things at all times".

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ABSTRACT

Groundnut samples were collected during rainy season May-October from different Local Government areas of Niger State and screened for fungal, Aflatoxins B_1, B_2, G_1, G_2 and Ochratoxin A. Ten (10) fungi genera were isolated from eighty two (82) groundnut samples collected. The major fungal contaminants of groundnut in Niger state were *A.niger*, *A. ochraeus*, *A.flavus*, *Rhizopus* and *Mucor*. AFB_1, AFB_2, AFG_1 and AFG_2 were found in 72, 61, 55 and 15 samples respectively out of 82 samples analyzed while OTA was detected in 73 out of 82 samples examined. The results showed samples from dry zone with highest AFB_1 range (5.6-188µg/kg) while wet zone has the lowest (4.0-13.6µg/kg). Wet zone had the highest concentration of OTA, AFB_2 and AFG_1 ranges (4.0-45.6µg/kg), (0.4-38.4µg/kg) and (20.0-516.0µg/kg) while dry zone has least OTA (1.2-30.4µg/kg) and wettest zone has least AFG_1 (8.0-340.0µg/kg). AFB_1 range was high in roasted samples (27.0-188µg/kg) while AFB_2 and AFG_1 was more in field samples (0.4-38.4µg/kg) and (8.0-516.0µg/kg). OTA was found to be highest in field samples (1.6-45.6µg/kg) and least in roasted (5.2-32.8µg/kg). While a significant difference ($p<0.05$) existed in AFB_1 between samples from dry zone (5.6-188.0µg/kg) and wet zone (4.0-13.6µg/kg), there was no significant difference in concentration of AFB_1, AFB_2, AFG_1 and OTA present in between the other zones and also there is no significant difference ($p>0.05$) in roasted, stored and farm samples. The detection of AFB_1, AFB_2, AFG_1 and OTA in many samples necessitates regular surveillance and some caution in the rampant consumption of groundnut.

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ABBREVIATIONS

AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M 2
DON	Deoxynivalenol
FAO	Food and Agricultural Organisation
OTA	Ochatoxin A
UNEP	United Nations Extended Programme
UNICEF	United Nations International Children Emergency Fund
WHO	World Health Organisation
ZEN	Zearalenone

CHAPTER ONE

1.0 INTRODUCTION

Mycotoxins are natural toxic chemical substances produced by fungi. They are the secondary metabolites of toxigenic fungi which commonly occur in various agricultural products including food and feed stuffs and are a potential threat to the health of humans and animals (CAST, 1989). The fungi are a vast assemblage of living organisms, called the moulds. Various genera of toxigenic fungi are capable of producing diverse mycotoxins such as the ergots of alkaloids, aflatoxins, rubratoxins, ochratoxins, fumonisins, zearalenone, patulin and trichothecenes. Mycotoxins, by-products of fungal metabolism, have been implicated as causative agents of adverse health effects in humans and animals that have consumed fungus-infected agricultural products (Kishore et al., 2002). Consequently, fungi that produce mycotoxins, as well as the mycotoxins themselves, are potential problems from both public health and economic perspectives. Due to the diversity of their toxic effects and their synergetic properties, mycotoxins are considered as risk to the consumers of contaminated foods and feeds (Yiannikouris and Jonany, 2002).

These toxins can contaminate an array of crops including maize, sorghum, barley, wheat, rice meal, cotton seed meal, groundnuts, tree nuts and other legumes. Health consequences related to consumption of mycotoxin-contaminated foods include impaired growth in children, liver carcinogenesis, teratogenesis, mutagenesis, tremor genesis, hemorrhage (ATA), hepatotoxicity, nephrotoxicity and neurotoxicity, immuno-suppression and synergism with hepatitis B and C viruses. Economic losses from mycotoxicoses in agriculture are due to effects on livestock productivity, losses in crops, growth inhibition, impaired resistance to infection, infertility, mortality, the

costs and effects of regulatory programs directed toward mycotoxins. It is estimated that annual economic losses in Asia and Africa as a result of grain mould are in excess of US$ 130 million (CAST 2003).

Groundnut (*Arachis hypogaea* L.) is one of the major sources for protein, livelihood for the rural poor and foreign exchange earnings for many West African countries. Apart from its use as food and oil, groundnut is also an important source of cash and cattle feed for farmers in Nigeria. It generates 60%, 42% and 21% of rural cash earnings among groundnut producers in Senegal, Niger and Nigeria respectively, and accounts for about 70% of rural employment in Senegal (Ntare et al. 2005). However, during the last four decades, West Africa lost its position in world groundnut production and export shares. Groundnut production share declined from 23% to 15% whereas export share declined from 55% to 20% (ITC 2001). However, since 1984, groundnut production in West Africa has been increasing by about 6% annually, mainly due to expansion of groundnut production area (CGIAR 2004-2005). Senegal and Nigeria are among the world's largest groundnut producers (Ntare et al. 2005). Groundnut is produced mainly in the Northern and Middle-belt of Nigeria and Mokwa Local Government of Niger State (Adoga and Obatomi, 1992). It is obvious that hot, humid conditions enhance the development of fungi and production of mycotoxins on foods and feedstuffs and Niger state has an average annual temperature of 31.7°C and average humidity of 51.6% (Umoh, 1997). It is hot and humid for most part of the year especially between May and October (29.5°C and 73.1%) and these climatic conditions are favourable for fungal growth and mycotoxin production in foods and feedstuffs.

Aflatoxins, a group of mycotoxins produced by *Aspergillus flavus* and zearalenone produced by *fusarium spp.* have been reported in the past literature as the major

mycotoxin that contaminates groundnut and affect its quality (Kishore *et al.,* 2002; Mehan *et al.,* 1985).

Low productivity, mycotoxin regulations, stricter grades and standards have limited the competitiveness of West African groundnut in domestic, regional and international markets.

Due to deleterious health hazards, mycotoxin contamination significantly restricts the volume of groundnut exports from sub-Saharan Africa (Freeman et al., 1999). International trade restriction is particularly serious, because of the European Union's (EU) imposition of a new mycotoxin regulation (Ntare et al., 2005).

To regain its competitiveness, groundnut productivity and production need to be increased significantly, technologies to reduce mycotoxin contamination must be promoted, and grades and standards met. Implementing programs to reduce the levels of mycotoxin contamination will generate social benefits. Technologies based on agronomic and cultural practices that can minimize the risk of mycotoxin contamination in groundnut and its products can be developed (Waliyar et al. 2005 and 2006).

1.1 Justification

Niger state is a major producer of groundnut in the country, but limited monitoring and surveillance activities seems to have been done on the fungi and their toxins in groundnut in the State. Since fungi and their toxins cause obvious reduction in crop and animal livestock production and diseases in human, the survey for mycotoxigenic fungi and mycotoxin contaminating groundnut in Niger state would produce data that would be used to place preventive measures towards food safety and so this study

would be of great importance with respect to public health, agricultural and economic growth of Nigeria. Based on these, this study was undertaken to determine the fungi and some mycotoxins contaminating groundnut in Niger State, Nigeria with a view to rationally speculate the types of mycotoxicoses expected following consumption of groundnut in Niger State and by implication in Nigeria.

1.2 Aim and Objectives

The aim of this work is to isolate and extract the fungi and aflatoxins (B_1, B_2 and G_1) and ochratoxin A contaminating groundnuts in the Niger State.

The study has the following specific objectives:

1. To isolate and identify fungi infecting groundnuts in Niger state during the wet season.
2. To determine the incidence and levels of aflatoxins and ochratoxin A contamination in field, stored and market groundnuts in Niger state.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fungi

A fungus is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and moulds, as well as the more familiar mushrooms. Fungi are classified as a kingdom that is separate from plants, animals and bacteria. Some common mycotoxicoses caused by common and widespread fungi such as *Aspergillus, Penicillium, Fusarium* and *Stachybotrys* result in severe illness and death. *Aspergillus* and *Penicillium* produce their toxins mostly in stored seeds, hay or commercially processed foods and feeds although infection of seeds usually takes place in the field. Javis (1971) has reported that storage fungi especially *Aspergillus, Penicillium, Rhizopus* and *Mucor* species infect grains after harvest and can grow on them during storage. The genera of mycotoxigenic fungi are mainly represented by *Aspergillus, Penicillium* and *Fusarium* but *Alternaria* among others are also important as food contaminants or pathogens of plants. *Alternaria* produces mycotoxins in grains such as rice and maize (Ominski *et al.*, 1994). Many *Aspergillus, Penicillium* and *Cladosporium* species are known to produce mycotoxins. According to Javis (1971), other toxigenic fungi frequently found on grains are *Alternaria, Trichoderma, Fusarium, Paecilomyces, Chaetomium* and *Acremonium.*

Fungi that contaminate food and feed can be classified broadly into field, storage and advanced decay. *Fusarium, Alternaria, Gibberella, Cladosporium and Helminthesporium spp* are fungi found before harvest and are commonly called field fungi. *Aspergillus, Penicillium, Rhizopus and Mucor* produce their toxin mostly in

stored seed so are commonly called storage fungi (Javis., 1971). Some fungi grow after considerable damage from other microorganism and are referred to as advanced decay fungi which consist of the genera of *Fusarium* and *Chaetomium*.

2.2 Fungal Genera

2.2.1 *Aspergillus spp*

Aspergillus is the most common genus of fungi in our environment with more than 180 different species of moulds (CAST 1989). Sixteen of these species have been documented as causing human disease. Aspergillosis is now the second most common fungal infection requiring hospitalization in the United States. Exposure to *Aspergillus* can often cause skin rashes and hair loss. Many vitamins and supplements are made with the *aspergillus* fermentation process or other types of fungi. *Aspergillus fumigates* is the most encountered species causing infection. It is seen abundantly in decomposing organic material, such as self-heating compost piles, since it readily grows at temperatures up to 55°C. People who handle contaminated material often develop hypersensitivity to the spores of *Aspergillus* and may suffer severe allergices.

Aspergillus flavus, the second most encountered fungi in cases of *Aspergillus* infection is also known to produce the mycotoxin aflatoxin, one of the most potent carcinogens known to man. In the 1960s, 100,000 turkey poultry in Great Britain died from ingesting contaminated feed (CAST 1989). Most countries have established levels for aflatoxin in food. However, the risks associated with airborne exposure are not adequately studied and no exposure standards exist.

Aspergillus niger, the third most common *Aspergillus* fungi associated with disease and the most common of any *Aspergillus* species in nature due to its ability to grow on a wide variety of substrates. This species may cause a "fungal ball", which is a condition where the fungus actively proliferates in the human lung, forming a ball. It does so without invading the lung tissue. It has also been linked to hearing problems including tinnitus and hearing loss. Other fungi from *Aspergillius spp.* include *Aspergillius glaucus, A.nidulans, A.ochraceus, A.parasiticus, A.versicolor, A.clavatus, A.terrues, A.niveus, A.oryzea, A.pseudotamarii, A.carbonarius, A.awamori, A.ostianus*. Common toxins produced by *aspergillus spp* are aflatoxins, ochratoxin A, gliotoxin, cyclopiazonic acid, sterigmatocystin, methyoxy sterigmatocystin, penicillic acid, restrictoxin.

2.2.2 *Fusarium spp.*

Cool, wet conditions favour the growth of *Fusarium* species moulds, which can produce several mycotoxins detrimental to livestock. *Fusarium* mould species can produce fumonisins, vomitoxin, and zearalenone (Mehan et al., 1985). This fungus is often found in humidifiers and has been isolated from water-damaged carpets and a variety of other building materials. Human exposure may occur through ingestion of contaminated grains and possibly through the inhalation of spores. *Fusarium* is high in agricultural field soil and it includes saprophytes that breakdown plant residues in the soil as well as pathogens that can cause rots, wilts,and other diseases (CAST, 2003). A limited number of *Fusarium spp* are found to be responsible for mycotoxin production in food and feed (Marasaa et al., 1984) and they mostly contaminate corn, rice, barley, oats, and other cereal grains (Campbell et al., 2000). *Fusarium spp* includes *F.graminearum, F.verticillioides, F.fujikuroi, F.poae, F.proliferatum. Fusarium spp.* is frequently involved with eye, skin, and nail infections. More

severely it can produce hemorrhagic syndrome (alimentary toxic aleukia) in humans which is characterized by nausea, vomiting, diarrhoea, dermatitis, and extensive internal bleeding.

Common toxins produced by the *fusarium spp* include fumonisin, zearalenone, trichothecenes (deoxynivalenol and T-2 toxin), diacetpxyscirpenol (DAS), 3-acetyl DON, T-2 tetraol (verrucarol) (Glenn 2007). Some of other major types of *Fusarium* toxins include beauvercin and ennitins, butenolide, equisetin and fusarins (Desjardins and Proctor 2007).

2.2.3 *Penicillium spp.*

These fungi are commonly found in soil, food, cellulose, grains, paint, carpet, wallpaper, interior fiberglass duct insulation, and decaying vegetation. *Penicillium* may cause hypersensitivity pneumonitis, asthma, and allergic alveolitis in susceptible individuals.

The genus *Penicillium* has several species. The most common ones include *Penicillium chrysogenum, Penicillium citrinum, Penicillium janthinellum, Penicillium marneffei*, and *Penicillium purpurogenum*.

The common occurrence of *Penicillium* species in food is a particular problem. Some species produce toxins and may render food inedible or even dangerous. On the other hand some species of *Penicillium* are beneficial to humans. Cheeses such as Roquefort, Brie, Camembert, Stilton, etc. are ripened with species of *Penicillium* and are quite safe to eat. The drug penicillin is produced by *Penicillium chrysogenum*, a commonly occurring mould in most homes. *Penicillium spp* are occasionally found in

grains, and infection is favoured by prolong wet weather or lodging of grain (Lindenfelser and Ciegler 1977).

Penicillium has been isolated from patients with keratitis, ear infections, pneumonia, endocarditis, peritonitis, and urinary tract infections. *Penicillium* infections are most commonly exhibited in immunosuppressed individuals. For example, *P. marneffei* is a fungus abundant in Southeast Asia that typically infects patients with AIDS in this area. Infection with *P.marneffei* is acquired via inhalation and initially results in a pulmonary infection and then spreads to other areas of the body (lymphatic system, liver, spleen, and bones), and is often fatal. An indication of infection is the appearance of papules that resemble acne on the face, trunk, and extremities. *Penicillim spp.* does have the ability to produce mycotoxins. The mycotoxin known as *Ochratoxin A*, which is nephrotoxic and carcinogenic, may be produced by *Penicillium verrucosum. Verrucosidin* is another mycotoxin produced by this fungus that exhibits neurotoxity. Penicillic acid is another mycotoxin that is nephrotoxic (causes kidney and liver damage).

2.2.4 *Alternaria*

Alternaria mould is a cosmopolitan dematiaceous (phaeoid) fungus commonly isolated from plants, soil, food, and indoor air environment. The production of melanin-like pigment is one of its major characteristics. The large spore size 20 - 200 microns in length and 7 - 18 microns in sizes, suggests that the spores from this fungi will deposited in the nose, mouth and upper respiratory tract. Specimens of *Alternaria* are often found growing on carpets, textiles and horizontal surfaces such as window frames. It is commonly found in soil, seeds and plants. It is known to be a common allergen. *Alternaria* is a dry spore and is readily found in air

samples as well as on tape lift samples. *Alternaria* is commonly found in water damaged buildings, and a significant increase in its numbers compared to outdoor levels can be a sign of growth.

Commonly found in outdoor air, on many kinds of plants and food products and prefers rotting farmland manure. It may be resistant to fungicides. *Alternaria* is considered an occasional contaminant of water damaged building materials which contain cellulose. Although *Alternaria* is a notable source of fungal allergy, pathogenic infections are also reported infrequently. The genus *Alternaria* currently contains around 50 species. Among these, *Alternaria alternata* is the most common one isolated from human infections. While *Alternaria chartarum*, *Alternaria dianthicola*, *Alternaria geophilia*, *Alternaria infectoria*, *Alternaria stemphyloides*, and *Alternaria teunissima* are among the other *Alternaria* spp. isolated from infections. Among the species of *alternaria*, *Alternaria alternata* is capable of producing tenuazonic acid and other toxic metabolites which may be associated with disease in humans or animals (Javis, 1971). It may be related to bakers' asthma. It has been associated with hypersensitivity pneumonitis, sinusitis, deratomycosis, onychomycosis, subcutaneous phaeohyphomycosis, and invasive infection. Common cause of extrinsic asthma (immediate-type hypersensitivity: type I). Acute symptoms include edema and bronchiospasms, chronic cases may develop pulmonary emphysema. Alternaria mycotoxins that have been shown to occur naturally are tenuazonic acid, alternariol monomethyl ether, alternariol, altenuene and altertoxin 1. Iso-altenuene and altertoxin 11 have also been found.

2.2.5 Other Fungi

Stachybotrys spp and Cladosporium spp are another fungi genera that has the ability to produce mycotoxins, ones that are extremely toxic, suspected carcinogens and immunosuppressive. *Stachybotrys* is found indoors, water damage has gone unnoticed or ignored since it requires extended periods of time with increased levels of moisture for growth to occur. *Stachybotrys* is usually black and slimy in appearance (Dearborn et al., 1999). The most common species of *cladosporium* are *C. elatum, C. herbarum, C. sphaerospermum,* and *C. cladosporioides*. These fungi are the causative agents of skin lesions, keratitis, nail fungus, sinusitis, asthma, and pulmonary infections. Symptoms of exposure to *S.atra* include dermatitis, memory loss, balance issues, acid reflux, cough, rhinitis, nose bleeds, cold and flu-like symptoms, headache, bleeding lungs, general malaise, internal lesions, seizures, and fever (Croft et al., 1986).

Other fungi producing mycotoxins detected from cereals include species of *Bipolaris, Cryptococcus, Curvularia, Geotriculum, Gilocladium, Helminthospsrium, Mucor, Rhizopus, Rhodotorula, Trichoderma, Norcardia, Chrysosporium, Scopularia, Phoma, and Arthrium* (Makun *et al.*, 2010).

2.3 Mycotoxins

2.3.1 Origin, Nature, Chemistry and Distribution of Mycotoxins

Mycotoxins are harmful substances produced by fungi in various foods and are estimated to affect as much as 25% of the world's crop each year (CAST, 1989). Most of these mytocoxins belong to the three genera of fungi: *Aspergillus, Penicillium* and *Fusarium*. Although over 300 mytocoxins are known, those of most concern based on their toxicity and occurrence, are aflatoxin, vomitoxin, ochratoxin, zearaleone, fumonisin and T-2 toxin. They are produced in cereal grains as well as forages before,

during and after harvest in various environmental conditions. The presence of mycotoxins in feeds may decrease feed intake and affect animal performance. In addition, the possible presence of toxic residues in edible animal product such as milk, meat and eggs may have some detrimental effects on human health. Fungal contamination affects both the organoleptic characteristics and the alimentary value of feeds and entails a risk of toxicosis. The biological effects of mycotoxin depend on the ingested amounts, number of occurring toxins, duration of exposure to mycotoxin and animal sensitivity. Mycotoxins display a diversity of chemical structures, accounting for their different biological effects (Ratcliff, 2002). Depending on their precise nature, these toxins may be carcinogenic, teratogenic, mutagenic, immunosuppressive, tremor genic, hemorrhagic, hepatotoxic, nephrotoxic and neurotoxic. Mycotoxins are regulated by Food and Drug Administration (FDA).

Mycotoxins occur sporadically both seasonally and geographically. The formation of mycotoxins in nature is considered a global problem; however, in certain geographical areas of the world, some mycotoxins are produced more readily than others (Devegowda *et al.*, 1998; Ratcliff, 2002; Lawlor and Lynch, 2005).

Table 2.1, shows the mycotoxin that may be found in feeds that come from different global locations. They occur naturally in a wide variety of feedstuffs used in animal feeds. In most European countries aflatoxins are not considered to be a major problem. In contrast, vomitoxin, ochratoxin, zearalenone are found more frequently. Aflatoxins are common in humid climatic conditions like those existing in Asian and African countries and certain parts of Australia. Mycotoxins are regularly found in

feed ingredients such as maize, sorghum, barley, wheat, rice meal, cotton seed meal, groundnuts and other legumes.

Table 2.1: Geographic occurrence of mycotoxins

Location	Mycotoxins
Western Europe	Ochratoxin, vomitoxin, zearalenone
Eastern Europe	Zearalenone, vomitoxin.
North America	Ochratoxin, vomitoxin, zearalenone, aflatoxins.
South America	Aflatoxins, fumonisins, ochratoxin, vomitoxin, T-2 toxin.
Africa	Aflatoxins, fumonisins, zearalenone.
Asia	Aflatoxins.
Australia	Aflatoxins, fumonisins.

Source: Devegowda *et al.* (1998)

2.3.2 Factors Affecting Fungal Growth and Mycotoxins Production

The main factors affecting fungal growth and mycotoxin production on a given food are moisture, temperature, pH, and the environment. Production of mycotoxins is optimal at relatively high temperatures, so contamination is most acute and widespread in warm, humid climates. Warm temperatures (32° to 38°C) favour the infection of grains more than cool temperatures (21° to 26°C) (Ominski *et al* 1994). Fungi will only grow when the moisture content exceeds 9%, at 80-85% relative humidity and above (Diener and Cole; 1982). It grows best between 10°C and 45°C at a relative humidity of 75% or more (Panasenko; 1967). Fungi may invade agricultural products during plant growth (preharvest), harvest and afterwards (postharvest).

Mycotoxins contamination of foods and feeds highly depends on environmental conditions that lead to mould growth and toxin production. Insect and bird damage, drought, stress and excessive rainfall encourage preharvest mould growth and mycotoxin production. Contamination of grains by moulds occurs primarily in the field.

At preharvest stage, drought-prone soils in which crops like groundnut is grown year after years are hot spots for aflatoxin contamination. Prolonged drought/moisture stress (3-4 weeks) during grain/seed filling and maturation stages triggers aflatoxin contamination. In USA, maize exposed to drought stress in the field had more *Aspergillus flavus* infected kernels than maize in irrigated plots.

Over maturity of the crop has been identified as the potential factor for mycotoxin contamination. Delayed harvest not only leads to yield loss but also reduces crop quality. Mechanical damage during harvesting is a big problem in crops like groundnuts, sweet potato and cassava. In most instances, mycotoxins are formed after harvest, particularly when harvesting takes place during unseasonal rains.

The drying stage is all-important to reduce attack and damage from insects and fungi. Traditional drying techniques involving bare ground drying are a major source of fungal contamination. They are slow, time consuming and labour intensive involving lots of crop handling, and due to rains that normally persist at harvesting, it is difficult to achieve the recommended moisture level for safe storage. In addition, the crop is persistently exposed to soil contamination which is the source of fungi. Inefficient and slow drying process under the humid conditions enhances mycotoxins contamination greatly.

Mechanical damage to foodstuff during shelling, threshing and winnowing makes them much more vulnerable to invasion by storage moulds, including *A. flavus*. Under any given environmental conditions fungal growth may be several times faster in damaged compared to intact produce. Cracks and breaks in maize or groundnut pods and testa are caused mainly during shelling by beating, although insect feeding may also be responsible for breaks in the pericarp.

Duration of storage is an important factor when considering mycotoxins formation. The longer the retention in storage the greater will be the possibility of building up environmental conditions conducive to fungi proliferation and production of mycotoxins. Storage structures, whether traditional or modern, should maintain an even, cool and dry internal atmosphere; they should provide protection from insects, rodents, and birds; should be easy to clean and should be water proof and protected from flooding to avoid fungal contamination and mycotoxins production.

2.3.3 Types of Mycotoxins

2.3.3.1 Ergots of alkaloids

Ergot is often used as a name for any condition which causes for the production or accumulation of ergot alkaloids either in hardened masses of fungal tissue (sclerotia), the most typical association, or liberated into host plant tissue by endophytic fungi. Ergot alkaloids are a large group of compounds produced by fungi that attack a wide variety of grass species, including small grains, during the growing season. The major ergot fungus is *Claviceps* which produces sclerotia in several grass species with *C. purpurea* being the most commonly found species, however, *C. fusiformis* has produced ergot in pearl millet and *C. paspali* has been associated with problems in

dallis grass poisonings (CAST, 2003). The sclerotia are brown to purple–black in colour and contain the ergot alkaloids. The fungus gains entry into the host plant from sclerotia that have been in the soil. The infecting fungal elements (ascospores) are ejected forcibly but also are assisted by wind and splashing rain in gaining access to the host plant where the florets are invaded with subsequent sclerotial development. The sclerotia are harvested with the grain and if not eliminated by screening or some other process they can end up in feed or food made from the contaminated grain.

Ergotism is one of the oldest mycotoxicoses with ancient records of its occurrence. The major outbreak of the disease was in Central Europe, and it became more endemic in the Middle ages where it was known as St. Anthony's fire (Matossian; 1989). In these cases, signs of gangrene, central nervous system and gastrointestinal effects were observed. Animals are affected similarly. In swine, agalactia has been attributed to ergot alkaloids. The loss of ears and other appendages is a common effect of ergot in animals.

Two types of ergotism have been described; gangrenous and convulsive. The differences may be due to the different kinds of alkaloids present in the ergot as variations in amount and kinds of alkaloids can occur in the sclerotia. In more recent years, outbreaks have occurred in human populations and the effects included gangrene and loss of limbs and nervous signs including giddiness, drowsiness, nausea and vomiting (Demeke et al., 1979; Krishnamachari and Bhat, 1979). Several different alkaloids were found in these outbreaks. Because some of the ergot alkaloids are vasoconstrictive and have beneficial pharmacological properties, they have been used therapeutically. Fescue toxicity caused by mycotoxins, produced by *N.*

coenophialum in the United States, has caused severe economic losses in the beef cattle industry as well as in the dairy and equine industries. There are no regulatory actions for ergot in grain but the USDA grain grading agency, GIPSA, classifies grain containing 0.05% or more sclerotia as "ergoty."

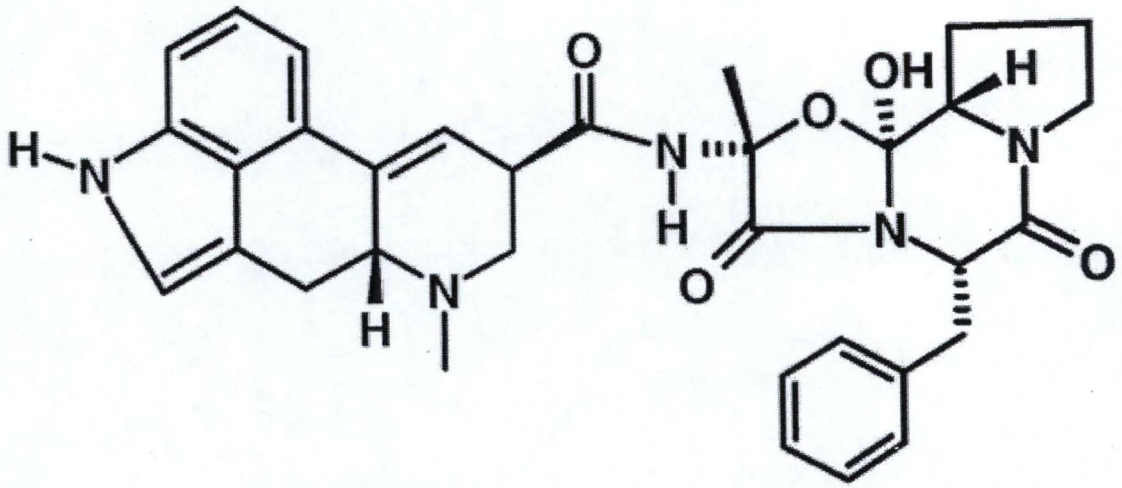

Fig. 2.1. Structure of ergotamine H as a representative of the ergot alkaloids.

2.3.3.2 Aflatoxin

Aflatoxins are a family of extremely toxic, mutagenic, and carcinogenic compounds produced by *Aspergillus flavus* and *A. parasiticus* (Deiner et al., 1987; Kurtzman et al., 1987). Aflatoxin contamination of corn, peanuts, tree nuts, cottonseed, and other commodities is a continuing worldwide problem. Toxigenic *A. flavus* isolates produce aflatoxins B_1, and B_2 and toxigenic *A. parasiticus* isolates produce aflatoxins B_1, B_2, G_1, and G_2 (Cotty et al., 1994). A number of closely related compounds namely aflatoxin M_1, parasiticol and aflatoxicol are also produced by *A. flavus*. Aflatoxin $M_1$and M_2 are major metabolites of aflatoxin B_1 and B_2 respectively, found in milk of animals that have consumed feed contaminated with aflatoxins.

Aflatoxins are fluorescent compound, they are chemically classified as difurocoumarolactones and their biosynthesis by the producing fungi is via the polyketide pathway (Smith and Moss, 1985). The most potent and the most frequently occurring of the four compounds is aflatoxin B_1. Infection and production of aflatoxins in field crops by these species is often associated with drought stress and insect damage (Richard *et al.*, 1993). While young animals are most susceptible to the effects of aflatoxin, all ages are affected; and clinical signs include gastrointestinal dysfunction, reduced productivity, decreased feed utilization and efficiency. Nursing animals may be affected by exposure to aflatoxin metabolites secreted in the milk.

Aflatoxin causes a variety of symptoms depending on animal species. However, in all animals, aflatoxin can cause liver damage, decreased reproductive performance, reduced milk or egg production, embryonic death, teratogenicity (birth defects), tumors and suppressed immune system function, even when low levels are consumed (Jones *et al.*, 1994). Aflatoxin contaminated feed is detrimental to the swine industry (CAST, 1989). Reduced feed intake, lowered gains and in some cases reduced feed efficiency has been observed for swine fed contaminated feed (Coffey *et al.*, 1989). The physiological effects of aflatoxin consumption include liver damage characterized by enlargement, release of enzymes into the blood (for example, asparatase aminotransferase and alkaline phosphatase) and impaired protein synthesis (CAST, 1989). Aflatoxins M appears in milk of sows consuming aflatoxin contaminated feeds and may affect piglets nursing those sows (Jones *et al.*, 1994).

Aflatoxin affects all poultry species. Although it generally takes relatively high levels to cause mortality, low levels can be detrimental if continually fed. Young poultry, especially ducks and turkeys are very susceptible (Jones *et al.*, 1994).

Food products contaminated with aflatoxins include cereal (maize, sorghum, pearl millet, rice, and wheat), oilseeds (groundnut, soybean, sunflower, and cotton), spices (chilies, black pepper, coriander, turmeric, and zinger), tree nuts and milk.

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by the fungus *A. flavus* and *A. parasiticus* on variety of food products. Among 18 different types of aflatoxins identified, major members are aflatoxin B_1, B_2, G_1 and G_2. Aflatoxin B_1 (AFB_1) is normally predominant in amount in cultures as well as in food products. Pure AFB_1 is pale-white to yellow crystalline, odourless solid. Aflatoxins are soluble in methanol, chloroform, acetone, acetonitrile.

Aflatoxins refer to the group of difuranocoumarins and classified in two broad groups according to their chemical structure; the difurocoumarocyclopentenone series (AFB_1, AFB_2, AFB_{2A}, AFM_1, AFM_2, AFM_{2A} and aflatoxicol) and the difurocoumarolactone series (AFG_1, AFG_2, AFG_2A, $AFGM_1$, $AFGM_2$, $AFGM_{2A}$ and AFB_3). The aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of AFB_1 > AFG_1 > AFB_2 > AFG_2 as illustrated by their LD50 values for day-old ducklings. Structurally the dihydrofuran moiety, containing double bond, and the constituents liked to the coumarin moiety are of importance in producing biological effects. The aflatoxins fluorescence strongly in ultraviolet light (ca. 365 nm); B_1 and B_2 produce a blue fluorescence where as G_1 and G_2 produce green fluorescence. Aflatoxins contamination in grain poses a great threat to human and livestock health as well as international trade. Aflatoxins have been implicated in human diseases including liver cancer, Reye's syndrome, Indian childhood cirrhosis, chronic gastritis, kwashiorkor

and certain occupational respiratory diseases in various parts of the world, particularly in African and Asian countries. In China, the Philippines, Thailand, Kenya, Swaziland and Mozambique, higher levels of aflatoxins in the food supply have been correlated with aflatoxins and their derivatives in human fluids which may be associated with liver cancer (Palmgren and Hayes, 1987). They have been reported cases of aflatoxic hepatitis in India and Kenya; a common feature in all these outbreaks has been the involvement of staple foods such as corn, wheat or pearl millet, following unseasonable rains or drought during either the growing season or harvest. Aflatoxins may cause decreased production (milk, eggs, weight gains, etc.), are immunosuppressive, carcinogenic, teratogenic and mutagenic (Miller and Wilson, 1994). Aflatoxins can be present in milk of dairy cows, meat of swine or chicken eggs if the animals consume sufficient amounts in their feed. Aflatoxin B_1 is a human carcinogen but may be only part of the total answer to human liver cancer (Robens and Richard, 1992). Pet foods contaminated with aflatoxins have been involved in disease and death of animals consuming sufficiently contaminated food (Garland and Reagor, 2007). According to FAO estimates, 25% of the world food crops are affected by mycotoxins each year. And also crop loss due to aflatoxins contamination costs US producers more than $100 million per year on average including $ 26 million to peanuts ($69.34/ha).

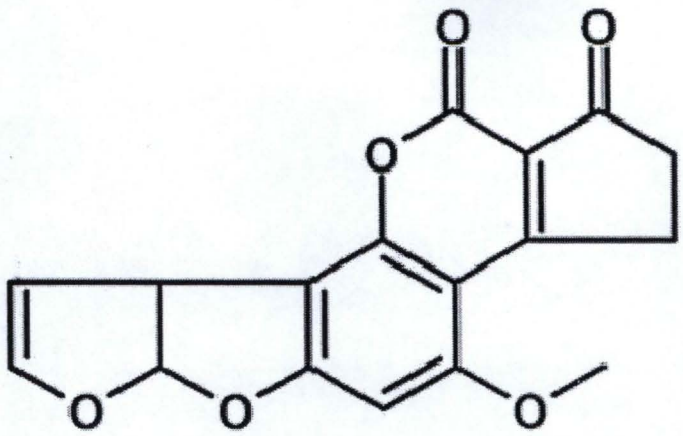

Fig. 2.2. Structure of aflatoxin B_1 as a representative of the aflatoxins.

Aflatoxin causes metabolic activation/modification of DNA leading to cell deregulation, cell transformation and cell death (Riley and Norred, 1996). Guanine in the DNA is the principal target for the attack of activated aflatoxin forming covalent binding to DNA which may lead to cancer (Lin *et al.*, 1977).

Aflatoxin B_1 causes decrease in RNA content and in RNA polymerase in the nuclei of the liver (Sporn *et al.*, 1966). Aflatoxin B_1 also causes decrease in liver glycogen level, by inhibiting biosynthetic enzymes such as glycogen synthetase and by increasing the activity of enzyme metabolizing glycogen precussors e.g the NADP reducing enzyme glucose -6- phosphate dehydrogenase. Aflatoxin give rise to accumulation of hepatic lipids by increasing the cytosolic level of NADPH necessary for fatty acid synthesis in the liver and inhibits triglcerides, phospolipid and cholesterol transport causing fatty liver (Tung *et al.*, 1972). Aflatoxin B_1 inhibits electron transport in mitochondria (Doherty and Campbell, 1973) and with aflatoxin M_1 could act as uncoupler of oxidative phosphorylation (Pai *et al.*, 1975).

2.3.3.3 Fumonisins

The fumonisins are a group of compounds originally isolated from *Fusarium moniliforme* (Gelderblom *et al.*, 1988). Different fumonisins (FA_1, FA_2, FB_1, FB_2, FB_3 and FB_4) have been reported, the A series are amides and the B series have a free amine (Gelderblom *et al.*, 1992).

In most animals fumonisin impairs immune function, causes liver and kidney damage, decreases weight gains, and increases mortality rates. It also causes respiratory difficulties in swine (Jones *et al.*, 1994).

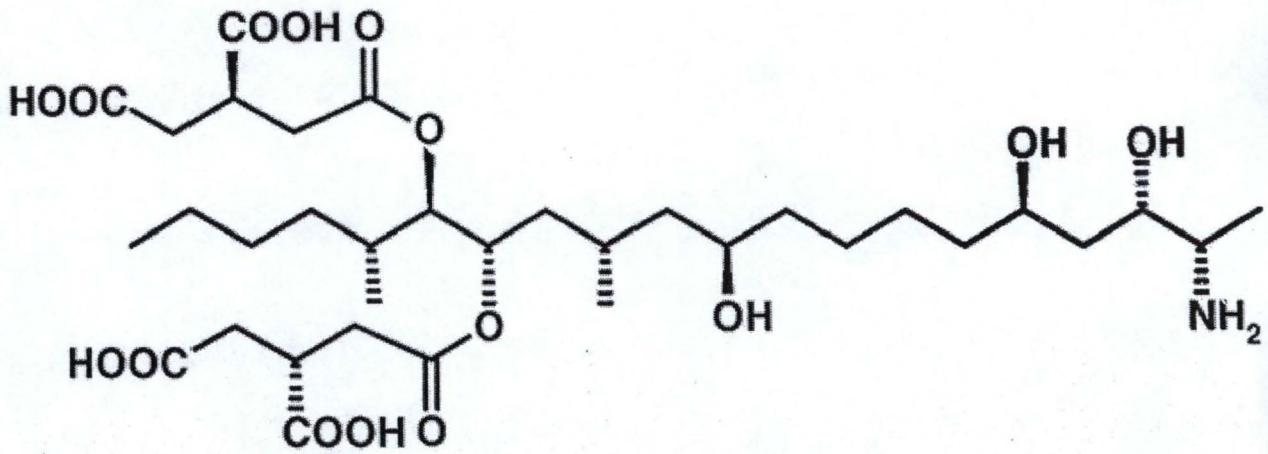

Fig. 2.3. Structure of fumonisin B_1 as a representative of the fumonisin

The fumonisins (FB_1 and FB_2) were isolated from *Fusarium moniliforme* cultures and found to promote cancer in rats (Gelderblom *et al.*, 1988). These toxins occur naturally in corn and have been associated with equine leukoencephalomalacia (Ross *et al.*, 1990). Liver damage and elevated serum liver enzymes occur in all livestock. However, this liver damage is temporary, and liver function returns to normal when fumonisin exposure ends. Toxin concentration is typically highest in broken grain. FDA guidance levels for total fumonisins in animal feeds indicate that with corn and corn by-products not to exceed 50% of dietary dry matter, fumonisin levels in corn

and corn by-products should not exceed 30 ppm and in finished feeds should not exceed 15 ppm (Federal Register., 2000).

Fumonisin B_1 was first isolated in South Africa where *Fusarium moniliforme* has long been associated with animal problems (Gelderblom et al., 1988). Fumonisin has been shown to cause leucoencephalomalacia in horses (Marasas et al., 1988), pulmonary oedema in swine (Harrison et al., 1990) and hepatoxicity in rats (Gelderblom et al., 1991). This family of mycotoxins is produced by the species of *Fusarium* in the Liseola section. *F. verticilloides* (formerly *F. moniliforme*), a species that is almost ubiquitous in corn, and *F. proliferatum* are the main species producing high yields of fumonisins. Fumonisins B_1, B_2, and B_3 (FB_1, FB_2, and FB_3) are fumonisins in fungal cultures or found in naturally contaminated corn samples (Cawood et al., 1991). Feed infected with *F. verticilloides* has long been associated with outbreaks of blind staggers, equine leucoencephalomalacia (ELEM), in equines (Wilson et al., 1985).

Fumonisins are structurally similar to sphingosine, a component of sphingolipids. Sphingolipids are in high concentrations in myelin and in certain nerve tissues. Fumonisin toxicity is thought to result from blockage of sphingolipid biosynthesis (Diaz and Borermans, 1994).

Fumonisin activates sphinganine N-acyl transferase which disrupts lipid metabolism causing cell deregulationwhich leads to apoptosis/cell death (Riley *et al.*, 1996).

2.3.3.4 Ochratoxin

The ochratoxins are metabolites produced by certain species of the genera *Aspergillus* and *Penecillium* (Wood, 1992). Ochratoxin A was discovered in 1965 by South

African Scientists as a toxic secondary metabolite of *Aspergillus ochraceus* (Van der Merwe *et al.*, 1965). Other species of *Aspergillus ochraceus* group and several P*enicillium* species, including *Penicillium viridicatum*, have been shown to form ochratoxin A (Harwig *et al.*, 1974). Ochratoxin A is the major metabolite of toxicological significance and it is mainly a contaminant of cereal grains (corn, barely, wheat and oats). It has also been found in beans (soyabeans, coffee, cocoa) and peanuts and meat in some countries (Krogh, 1987). Ochratoxin A is teratogenic in rat, hamster and chick embryo and is an inhibitor of hepatic mitochondrial transport systems.

Ochratoxin A has also been reported to cause damage to the liver, gut, lymphoid tissue and renal tubular damage (Harwig *et al.*, 1974).Ochratoxin, a suspected carcinogen, causes increased water consumption and urination and can lead to permanent scarring of the kidneys. At least nine ochratoxins have been identified, but ochratoxin A is the most common and has the greatest toxicological significance.

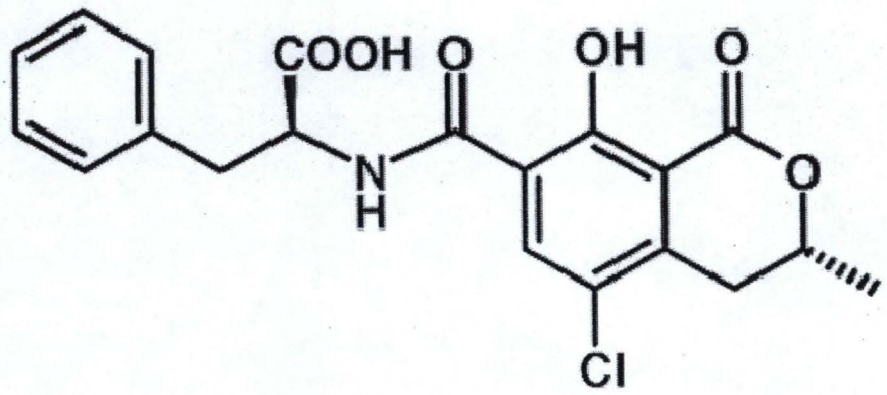

Fig. 2.4 Structure of ochratoxin A

Ochratoxin A (OTA) is a causative agent of kidney disease in pigs that has been referred to as mycotoxin porcine nephropathy (Krogh, 1979). OTA can reduce weight

gains and performance in swine (Cook et al., 1986) and poultry (Huff et al., 1988). Other symptoms include diarrhoea, increased water consumption, diuresis and dehydration (Krogh et al, 1979). OTA is rapidly degraded in the rumen and thus thought to be of little consequence unless consumed by young pre-ruminant calves (Sreemannarayana et al., 1988). Ochratoxins have been associated with Balkan endemic nephropathy and urinary tract tumours (Berry, 1988).

Ochratoxin disrupts phenylalanine metabolism thereby causing reduced gluconeogenesis leading to cell death (Marquardt and Frohlich, 1992). Ochratoxin A decreases liver glycogen especially in adrenalectomised rats (Suzuki *et al.*, 1975). It acts as acompetitive inhibitor of mitochondrial transport carriers by competitively inhibiting the mitochondria uptake of the bicarbonic acids, succinate and malate, of ADP and of inorganic phosphate (Meisner, 1974). Ochratoxin causes metabolic activation and inhibition of protein/DNA synthesis, alters membrane permeability, disrupts calcium transport which leads to cell deregulation and death (Benford *et al.*, 2001).

2.3.3.5 Trichothecenes

The trichothecenes are a very large family of chemically related toxins produced by various species of *Fusarium, Myrotecium, Trichoderma, Cephalosporium, Verticimonosporium,* and *Stachybotrys* (Richard 2000).

They are markedly stable under different environmental conditions. The distinguishing chemical feature of trichothecenes is the presence of a trichothecene ring, which contains an olefinic bond at C_9, C_{10}; and an epoxide group at C_{12}. All trichothecenes are mycotoxins, but not all mycotoxins are trichothecenes. This family

of mycotoxins causes multiorgan effects including emesis and diarrhoea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostatic derangements, skin toxicity, decreased reproductive capacity, and bone marrow damage. T-2 mycotoxin, a highly toxic trichothecene that, together with some closely related compounds, has been the causative agent of a number of illnesses in humans and domestic animals. During the 1970s and 1980s, the trichothecene mycotoxins gained some notoriety as putative biological warfare agents when they were implicated in "yellow rain" attacks in Southeast Asia.

Trichothecenes are a family of 200 - 300 related compounds that apparently exert their toxicity through protein synthesis inhibition at the ribosomal level. Several species of *Fusarium* and related genera produce trichothecenes. T-2 toxin, diacetoxyscirpenol (DAS), and deoxynivalenol (DON), are commonly found in agricultural commodities. (Desjardins et al.,1993). However, except for DON, it appears that most contamination with T-2 toxin and DAS occur post-harvest.

The toxic effects of trichothecenes include gastrointestinal effects such as vomiting, diarrhea, and bowel inflammation. Anemia, leukopenia, skin irritation, feed refusal, and abortion are also common. The trichothecenes, as a group, are immunosuppressive (Sharma, 1993).

Deoxynivalenol (DON, vomitoxin) causes feed refusal in swine. It is produced by *Fusarium* species (*F. graminearum*) and is commonly found on wheat, barley, rye, and oats. DON occurrence is most frequently reported in cool, temperate regions of the Northern U.S. and Canada.

Wet, rainy, and humid weather at flowering promotes infection by *Fusarium*. The results are ear rot in corn and scab or head blight in sorghum, barley, wheat, oats and rye (Tuite et al., 1974). Minimum tillage and no tillage production are believed to increase the amount of disease in small grains and corn/wheat rotations because of increased inoculum survival on crop residue (Trenholm et al., 1988). DON occurs in cereal grains worldwide and can increase in stored grain with kernel moisture contents of 22 –25%. One ppm or more of DON results in reduced feed intake in swine, resulting in lower weight gains. Two independent Midwestern field studies (Vesonder et al., 1978 and Côté et al., 1984) showed DON to be the primary mycotoxin associated with swine disorders including feed refusals, diarrhea, emesis, reproductive failure, and deaths. Vomiting has been reported in some outbreaks. Diets containing pure DON decrease feed consumption on a dose related basis (Marasas et al., 1984).

Other mycotoxins co-occur with DON and Foster et al. (1986) found that DON concentration was not a good predictor of grain toxicity. Smith and McDonald (1991) have indicated that fusaric acid interacts with DON to produce the symptoms previously attributed to just DON. Chickens and turkeys apparently are not very susceptible to the effects of DON. Leghorn chickens showed no effect on weight gain from dietary levels of DON at 18 ppm (Kubena et al., 1987).

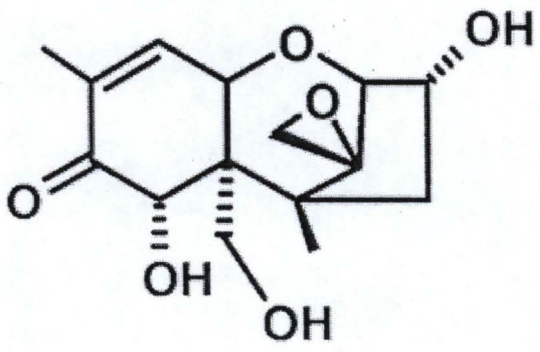

Fig. 2.5. Structure of deoxynivalenol.

T-2 toxin (T-2) is produced primarily by *F. sporotrichioides* and *F. poae*, but is also produced by other species of *Fusarium* (Marasas et al., 1984). T-2 (and DAS) is often found in barley, wheat, millet, safflower seed, and in mixed feeds. Unthriftiness, reduced feed intake, reduced gain, low milk production, reproductive failure, gastrointestinal hemorrhage, and increased mortality can occur when cattle consume rations contaminated with these trichothecenes.

Effects of T-2 on swine include infertility accompanied with some lesions in the uteri and ovaries. Drastic and sudden decreases in egg production in laying hens have been shown to be caused by T-2 toxin in the parts per million ranges. Other effects in chickens include decreased shell quality, abnormal feathering, mouth lesions, and reduced weight gain. Pier et al. (1980) reported that egg production and shell quality were decreased at 20 ppm of dietary T-2 toxin.

Turkeys fed T-2 exhibited reduced growth, beak lesions, and reduced disease resistance (Christensen et al., 1988). Mouth lesions were caused by DAS and other trichothecene toxins in broiler chickens (Ademoyero and Hamilton, 1991). In cattle, dietary T-2 toxin at 0.64 ppm for 20 days resulted in death and bloody feces, enteritis, and abomasal and ruminal ulcers (Pier et al., 1980).

T-2 toxin is a very potent mycotoxin and in cattle has been associated with gastroenteritis, intestinal hemorrhages (Petrie et al., 1977; Mirocha et al., 1976) and death (Hsu et al., 1972 and Kosuri et al., 1970). Weaver et al. (1980) showed that T-2 was associated with feed refusal and gastrointestinal lesions in a cow, but did not show a hemorrhagic syndrome. Kegl and Vanyi (1991) observed bloody diarrhoea, low feed consumption, decreased milk production and absence of estrus cycles in

cows exposed to T-2. Serum immunoglobulin and certain complement proteins were lowered in calves receiving T-2 toxin (Mann et al., 1983). Gentry et al. (1984) demonstrated a reduction in white blood cell and neutrophil counts in calves. A calf incubated with T-2 developed severe depression, hindquarter ataxia, knuckling of the rear feet, listlessness and anorexia (Weaver et al., 1980).

2.3.3.6 Zearalenone (ZEN)

Zearalenone, a non–steroidal estrogenic mycotoxin and it major metabolic products (α -zearalenol and β-zearalenol) occurs in corn, wheat, barley, and sometimes oats. Zearalenone is insoluble in water and heat-stable and it persists in both animal feeds and human food prepared from contaminated grains (Shipchandler, 1975).

Fusarium graminearum (*Gibberella zeae* and *Fusarium culmorum* are the major zearalenone producing species and are distributed worldwide with high humidity and low temperatures favouring its production (Marasas *et al.*, 1984). Zearalenone mimics the effect of the female hormone estrogen and at low doses, increases the size or early maturity of mammary glands and reproductive organs. At higher doses zearalenone interferes with conception, ovulation, implantation, foetal development and the viability of newborn animals. (Jones *et al.,* 1994). Zearalenone causes oestrogenic responses in dairy cattle, and large doses of this toxin are associated with abortions.

Other responses of dairy animals to zearalenone may include reduced feed intake, decreased milk production, vaginitis, vaginal secretions, poor reproductive performance and mammary gland enlargement in heifers. It is recommended that zearalenone should not exceed 250 ppb in the total diet (Jones *et al.*, 1994).

Zearalenone is the cause of hyperestrogenism, the oestrogenic syndrome, in swine. Zearalenone has been reported to occur in corn, other grains, and silage in many areas of the world. Weathered soybeans have also been reported to be contaminated with ZEN (Hagler et al., 1989). ZEN is also found in wheat, barley, oats, sorghum, sesame seed, hay, and silages. Conditions exacerbating ZEN accumulation in corn include weather which holds moisture contents at 22-25%, or delayed harvest (Abbas et al., 1988).

Swine appear to be most susceptible to ZEN (Diekman and Green 1992). In pre-pubertal gilts, swollen vulvae appear; this can progress to vaginal or rectal prolapse (Friend et al., 1990); internally, enlarged, swollen, distorted uteri, and shrunken ovaries are observed. Litter size may also be reduced. Hyperestrogenism occurs when contamination of ZEN is as low as 0.1 ppm (Mirocha et al., 1977). Young male pigs exposed to ZEN undergo symptoms of "feminization", such as enlarged nipples, testicular atrophy, and swollen prepuce (Newberne, 1987). The binding of zearalenone to estrogen receptors is specific as regards structure and allows the 6'—ketone and 6' —hydroxyl derivative to compete with 17 —estradiol at the receptor sites (Kiang, 1978). According to Boyd and Witthiff (1978) zearalenone competitively inhibits association of 17 —estradiol with its specific receptor sites.

Zearalenone is rapidly transformed into α— and —β zearalenol by 3α—hydroxysteroid dehydrogenases in the liver (Hurd, 1977). The formation of the two metabolites, α— and β-zearalenol, has been confirmed by Tashiro and Ueno (1980), Tashiro et al., (1980), Mirocha et al., (1976). As α-zearalenol is a three to fourfold

more active estrogen compound than zearalenone, zearalenol formation involves activation.

The reduction of zearalenone by means of 3α—hydroxysteroid dehydrogenases may involve another mechanism of action of the toxin. Zearalenone is rapidly reduced to α—zearalenol in pig and conjugated with glucuronic acid in vivo (Olsen et al., 1985). Soon after ingestion, conjugated zearalenone and α—zearalenol — but no free substances — can be detected in plasma. Consequently zearalenone, continuously administered in the feed and rapidly and constantly reduced to zearalenol, could constitute a serious obstacle to the metabolism of steroids catalysed by 3α— (and 3—) hydroxysteroid dehydrgenases. *Fusarium* toxins have been suspected to have a role in diseases such as Kashin Beck syndrome in the USSR, China and Viet Nam; Mseleni joint disease in southern Africa; endemic familial arthritis in India; alimentary toxic aleukia in the USSR; and oesophageal cancer in southern Africa.

2.3.3.7 Miscellanous Mycotoxins

Patulin

Patulin is a toxin produced by the *P. expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species. *P. expansum* is especially associated with a range of mouldy fruits and vegetables, in particular rotting apples and figs (Moss, 2008; Trucksess and Scott, 2008). It is destroyed by the fermentation process and so is not found in apple beverages, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals (Moss, 2008). In 2004, the European Community set limits to the concentrations of patulin in food products. They currently stand at 50 μg/kg in all fruit juice concentrations, at 25

µg/kg in solid apple products used for direct consumption, and at 10 µg/kg for children's apple products, including apple juice (Moss, 2008; Trucksess and Scott, 2008).

Patulin is an antibiotic produced by a number of moulds. It occurs in rotten apples contaminated by *Penicillium expansum* and, consequently, may occur in apple juice and other apple-based products (Moss 2008). Experimental studies have demonstrated that patulin is a neurotoxin and that it produces marked pathological changes in the viscera. Patulin causes non protein sulphurhydryl depletion, alter ion permeability, causes oxidative stress and inhibition of macromolecular biosynthesisleading to cell death (Speijers, 2004). Although patulin has been reported as inducing local sarcomas, no mutagenic activity has been discernible in most short-term tests.

JECFA (JECFA, 1996b) has established a provisional maximum tolerable daily intake of 400 ng/kg body weight for patulin.

Citrinin

Citrinin is a mycotoxin originally isolated from *Penicillium citrinum*. It has since been found to be produced by a variety of other fungi which are used in the production of human foods such as grain, cheese, sake and red pigments. Citrinin acts as a nephrotoxin in all species in which it has been tested, but its acute toxicity varies (Bennett and Klich, 2003). It causes mycotoxic nephropathy in livestock and has been implicated as a cause of Balkan nephropathy and yellow rice fever in humans (Benneth and Klich 2003). Citrinin is used as a reagent in biological research. It induces mitochondrial permeability pore opening and inhibits respiration by interfering with complex I of the respiratory chain. Citrinin is produced by a variety

of fungi including: *Aspergillus niveus, Aspergillus ochraceus, Aspergillus oryzae, Aspergillus terreus, Monascus ruber, Monascus purpureus, Penicillium citrinum Penicillium camemberti.*

Table 2.2: Incidence of aflatoxin B_1 contamination of groundnut and some groundnut related commodity in certain developing countries

Country	Commodity	Contamination rate	Reference
Argentina	Groundnut	20-200 µg/kg	Park and Njapau;1989
	Cotton seed	20-200 µg/kg	Park and Njapau;1989
Bangladesh	Groundnut	65 µg/kg	Dawlatana *et al.*, 2002
Botswana	Peanut	to 64 µg/kg	Siame *et al.*, 1998
	Peanut butter	0.3 to 23 µg/kg	Siame *et al.*, 1998
Brazil	Peanuts and products	43-1099 ppb	Freitas *et al.*, 1998
China	Corn	9-1396 ppb	Li *et al.*, 2001
Egypt	Haze nut	25-175 ppb	Williams *et al.*, 2004
	Soybean	5-35 ppb	el Kadt *et al.*, 1993
	Wall nut	15-25 ppb	Williams *et al.*, 2004
Gambia	Ground nut source	162 ppb	Williams *et al.*, 2004; Hudson *et al.*, 1992
Ghana	Kernels	5.7-22,168 ppb	Awuah and Kpodo, 1996
	Groundnut	216µg/kg	Mintah and Hunter, 1978
India	Pistachio nuts	15 to 259 µg/kg	Candlish et al 2001

	Groundnut	33-440 µg/kg	Singh et al;1982
Kenya	Groundnut	0 to 20ppb	Mutegi et al 2007
Malaysia	Peanut	1-378 µg/kg	Ali et al; 1999
Nepal	Peanut, corn flakes Peanut butter Vegetable oil	>30 ppb	Koirala et al; 2005
Nigeria	Shelled melon	5-20 µg/kg	Bankole et al; 2004
	Groundnut	Up to 2000 µg/kg	McDonald; 1964
	Peanut cake	37-455 µg/kg	Akano and Atanda; 1990
Senegal	Peanut	40 ppb	Ndiaye et al; 1999
	Groundnut	20-200 µg/kg	Park and Njapau;1989
Sudan	Peanut butter and peanut	87.4 - 197.3µ/kg	Omer et al; 1998
Thailand	Peanut oil	102 µg/kg	Lipigorngoson et al; 2003
Turkey	Hazenut cream	0.126-5 µg/kg	Vural et al; 2007
Uganda	Groundnut	80ppb	Kaaya and Warren; 2005

2.3.4 Aflatoxins Research in Nigeria

The serious concern about mycotoxins began in the early 1960s after it was discovered in the United Kingdom that Turkey "X" disease is caused by aflatoxins. This leads to the research on mycotoxin in Nigeria in order to protect its export of groundnut. Since then many mycotoxin has been isolated from food and feed stuff in Nigeria. Different cases of human and animal mycotoxicosis have been reported in

Nigeria. Different fungal genera have been isolated from many Nigeria food of which include *Aspergillus, Penicillium, Fusarium* and they produce possible mycotoxins such as aflatoxin, ochratoxin, zearalenone, trichothecenes, fumonisins, patulin, deoxynivalenol, citrinin, moniliform. The presence of the detected mycotoxins in our food system and tissues has enormous public health significance because these toxins are nephrotoxic, immunotoxic, teratogenic and mutagenic which are capable of causing acute and chronic effects in man and animals ranging from death to disorder of central nervous, cardiovascular, and pulmonary systems and intestinal tract (Bhat and Vasanthi, 2003). Of greatest concern is the relevance of these toxins in human hepatoma and oesophageal cancer, increased susceptibility to diseases especially in children and childhood pre-five mortality and reduced life expectancy (Marasas, 2001). Akano and Atanda (1990) found aflatoxin B_1 concentrations in the range of 20-455μg/kg in groundnut cake ('Kuli Kuli') purchased from market in Ibadan, Oyo State, Nigeria. Similarly Adebanjo and Idowu (1994) reported that most of the corn-groundnut snacks, ('donkwa') contained aflatoxins above 30μg/kg immediately after preparation. A correlation was established between the incidence of *Aspergillus flavus* and aflatoxin contamination detected in 55% of tiger nuts with concentrations ranging from 10-20μg/kg collected from different parts of Nigeria (Bankole *et al*., 1996). In a recent study in Nigeria Uriah et al. (2001) found that blood and semen aflatoxin levels ranged from 700 to 1393 ng/ml and 60 to 148 ng/ml, respectively in infertile men and were significantly higher than that in fertile men. Again, a recent survey, 27% of melon seed samples from farmers' stores contained aflatoxin B_1 with mean levels of 14μg/kg in the forest and 11μg/kg in the savanna of Nigeria (Bankole *et al*, 2004). Gbodi (1986) investigated the mycoflora and mycotoxins in acha (*Digitaria exilis stapf*), maize (*Zea mays*) and cotton seed (*Grossypium spp linn*) in Plateau State and

aflatoxins B_1, B_2, G_1 and G_2 were detected. In a large investigation undertaken on 327 babies with jaundice and 80 matching controls in Nigeria, it was found that the occurrence of glucose-6-phosphate dehydrogenase (G6PD) deficiency together with the presence of aflatoxins in the serum are significant risk factors for the development of neonatal jaundice (Sodeinde 1995).

The health impact is no less enormous. In 1988, some primary school pupils died and this was traced to their consumption of aflatoxin–contaminated ground nut cake called 'kulikuli' in south western Nigeria. Also posthumous autopsy of some children suffering from kwarshiorkor revealed significant levels of aflatoxin in their brains. This was equally traced to the consumption of contaminated maize–based gruel regularly fed infants in Nigeria (Oyelami *et al* 1996).

Cases of death due to unknown etiology may be due to mycotoxicoses.

2.3.5 Cultivation and Consumption of Groundnut

Groundnut is cultivated on about 26.5 million ha in the world, with an average annual production of 35.7 million Mt in the Year 2003 (FAO, 2003). The average yield world over is 1348 kg/ha. Asia and Africa accounts for 97% of the groundnut area. It has been recognized around the world by an assortment of colourful names. While Americans call it peanut, it is known by several other names such as African nut, Chinese nut, Manila nut, kipper nut, hawks nut, jar nut, earth chestnut, monkey nut, goober pea, ground pea, and ground bean (Johnson, 1964). Although peanuts have gained importance relatively recently, the origin of this crop dates back to 350 BC (NPC, 1990). With a humble beginning, groundnuts have gained prominence for their economic importance and nutritional value on a global scale. Groundnuts have

become a substitute for costly nuts such as cashews. Now, they are widely regarded as poor man's cashews. Groundnuts are now cultivated throughout the world. Some grow groundnuts for consuming directly as food or snacks, while in other places they are important source of vegetable oil. For instance, groundnut production and use in the United States and India are of great contrast. In India they are cultivated entirely manually while its production is heavily mechanized in the United States. Often it is hard to believe that hundreds of acres of groundnut crop operated by small farm owners in India are cultivated and harvested without using machines. Also, people in India like to consume baked and fried peanuts as opposed to the boiled peanuts preferred by Americans. Obviously, tremendous variations occur in the way groundnuts are produced and used in different parts of the world.

Major countries with more than a million hectares under groundnuts are India, China, and Nigeria. Countries where more than a half a million hectares of land is used for growing groundnuts are Senegal, Sudan, Zaire, Indonesia, and USA. While in countries like Myanmar, Chad, Vietnam, Mozambique, Burkina Faso, Mali, Uganda, Argentina, Zimbabwe, Cote Divore, South Africa, Pakistan, and Thailand groundnuts are grown in an less than five hundred thousand hectares. India is the world leader in area under groundnut production. China, the second most important country, has almost doubled its area under groundnuts steadily in the last three decades. Nigeria, the third most important groundnut growing country has regained its edge as an important groundnut growing country after a decline in the 1970s and 1980s. During this period, groundnut area in Nigeria declined to almost half of the existing levels of 1.7 million ha (NPC, 1990). The groundnut production in Africa has suffered from fluctuations and downward trend. Low yields in the Eastern Africa have been attributed to the unreliable rains with recurrent droughts, lack of high-yielding

cultivars, pests and diseases, as well as low inputs used in groundnut cultivation (Mahmoud et.al., 1992). Major factors attributed to the increase in groundnut production in China include agricultural reforms started in the late 70s, development of a market economy, increase of inputs into groundnut production, and application of improved varieties with improved techniques (Zeyong, 1992). Traditional commercial peanut production areas in Nigeria encompass the Sahel (12°-13°N), Sudan (10° - 13°N), the Northern half of the Northern Guinea Savannah (8°-11°N), and most part so the southern Guinea savanna (6°- 8°N) vegetation zones. The major peanut-producing areas are located in the Sudan and Northern Guinea ecological zones where the soil and agro climatological conditions are favourable (Misari *et al.*, 1980). Major areas of production have changed over the years. In the sixties, 33 to 50 % of the production came from Kano state. Sudan Savannah zone receives adequate rainfall for peanut production. The rainy season lasts between 70 to 200 days with rainfall ranging between 500-1600 mm. Peanut productions is mostly concentrated in the northern states. The southern states accounted for less than 1 % of the total production. The crop is grown usually as a component of a variety of crop mixtures including sorghum, millet, cowpea, and maize (Misari *et al.,* 1988). There are two main varieties grown in Nigeria. Long season varieties (Virginia types) mature in 130 to 145 days; and short- season varieties (Spanish and Valencia type) maturing in 90 to 129 days. Groundnut cultivation in Niger State is in some Local Government areas such as Agwara, Borgu, Chanchaga, Edati, Gbako, Kontagora, Lapai, Lavun, Magama, Mariga, Mashegu, Muye, Rijau, Shiroro, Suleja, Tafa and Wushishi (RMRDC, 2008).

Most peanut farmers grow them in order to sell them for cash. It is cultivated for kernels, the oil, and hay for livestock feed. Groundnut cake is often deep fried or dried

to make a snack locally called *kuli-kuli.* Groundnut flour is used as an ingredient in soups, sauces, sweets, confectioneries, and puddings. The husks are used as fuel, roughage, litter for livestock, mulch, manure, and as soil conditioners.

Table 2.3: Major groundnut growing countries

Country	Area(000 ha)	Production (000 mt)	Yield (kg/ha)
China	5,125	13,448	2,623
India	8,000	7,500	938
Indonesia	683	1,377	2,016
Myanmar	730	730	1,270
Pakistan	100	106	1,060
Thailand	132	132	1,517
Vietnam	240	400	1,665
Chad	480	450	938
Ghana	350	450	1,286
Malawi	206	158	767
Nigeria	2,800	2,700	964
Senegal	900	1,900	1,000
Sudan	211	900	632
Uganda	1,200	150	711
Argentina	156	316	1,348
Brazil	85	177	2,082
Mexico	62	75	1,204
US Of America	531	1,880	3,540

Computed from FAOSTAT (2008)

2.3.6 Fungi and Mycotoxins Contaminating Groundnut

Groundnut harbour large number of fungi under certain conditions. The major fungi associated with groundnut are *Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochraceus, Fusarium moniliforme, Penicillium citrinum and Penicillium expansum* (CAST; 2003). Species of *Aspergillus, Rhizophus, Penicillium, Curvularia, Fusarium and Mucor* have been isolated from groundnuts in different storage systems in Nigeria (Kutama and Aliyu; 2007). *Aspergillus, Rhizopus* and *Mucor* were the most frequently occurring taxa found. Adebajo and Diyaolu; (2003) reported the isolation of species of *Aspergillus* and *Penicillum* in nuts sold in markets around the south west part of Nigeria. *Aspergillus flavus* and *Aspergillus Parasiticus* was reported to contaminate groundnut and produce aflatoxin in groundnut marketed in some part of Nigeria (Odoemelam and Osu; 2008). The commonest mycotoxins found in groundnuts are Aflatoxin, Ochratoxin A, patulin, trichothecenes and stergmatocystin (Keenan and Savage., 1994).

Fungal growth and mycotoxin production on groundnut is a worldwide phenomenon. Aflatoxin has been found to contaminated peanuts in USA (Wood, 1989), peanut butter in UK (Jelinek, 1987), peanut in Philippines (Diener, 1987), groundnut in Ghana (Mintah and Hunter, 1978), groundnut in Kenya (Mutegi et al., 2007), groundnut in Senegal and most part of Africa (Coulibaly, 1989).

2.3.7 Economic Implications of Mycotoxins

International trade in agricultural commodities such as wheat, rice, barley, corn, sorghum, soybeans, groundnuts and oilseeds amounts to hundreds of millions of

tonnes each year (FAO, 1988). Many of these commodities run a high risk of mycotoxin contamination.

Economic losses from mycotoxicoses in agriculture are due to effects on livestock productivity, losses in crops, and the costs and effects of regulatory programs directed toward mycotoxins. It was estimated in 1989 that about 2% of the U.S. corn crop is affected by mycotoxins, and the impact may be devastating in some areas (CAST, 1989). The estimated 25% contamination of the world's annual crops (CAST, 1989) would extrapolate to billions of dollars (Trail et al., 1995a). However, these estimates were generated before information about the widespread occurrence of fumonisin in corn (Anon., 1995).

Numerous reports focusing on different countries/ regions, commodities, toxins, and cost categories (e.g., costs of regulations, testing, production loss, trade losses) offer some indication of these losses. For example, the Council for Agricultural Science and Technology (2003) estimated that crop losses (to corn, wheat, and peanuts) from mycotoxin contamination in the United States amount to $932 million annually, in addition to losses averaging $466 million annually from regulatory enforcement, testing, and other quality control measures.

Wilson and Otsuki (2001) estimated that, for a group of 46 countries—including the United States—the adoption of a uniform aflatoxin standard based on international (Codex Alimentarius) guidelines would increase trade of cereals (grains) and nuts by more than $6 billion, or more than 50 percent, compared with the divergent standards in effect during 1998. Potential export gains to the United States amounted to $700 million. Also, since less developed countries generally have less stringent mycotoxin standards, those that conduct trade with one another will lose more export opportunities than will developed countries.

Sometimes mycotoxins occur at concentrations high enough to cause major losses in health and performance of animals. However, mycotoxins are more usually at lower levels that result in interactions with other stressors to cause subclinical losses in performance, increases in incidence of disease and reduced reproductive performance. To the animal producer, these subclinical losses are of greater economic importance than losses from acute effects.

The exact figures for world economic losses resulting from mycotoxin - contamination may never be available. Apart from the obvious losses of food and feed, there are losses caused by lower productivity; losses of valuable foreign exchange earnings; costs incurred by inspection, sampling and analysis before and after shipments; losses attributable to compensation paid in case of claims; farmer subsidies to cover production losses; research, training and extension programme costs; costs of detoxification; etc. When combined, these costs may be staggering (Coulibaly, 1989).

2.3.8 Mycotoxin prevention and treatment

Pre-harvest control has involved using agronomic practices, which minimize mycotoxin accumulation in the field. These include proper irrigation, pesticide application in some cases, resistant or adapted hybrids, tillage type, and proper fertilization. Unfortunately, breeding for mycotoxin-resistant hybrids has been only partially successful. Fungicides have shown little efficacy in controlling pre-harvest aflatoxin contamination in corn (Duncan *et al.*, 1994).

Post-harvest approaches for management of mycotoxin contamination include: mycotoxin analysis of feedstuffs and diversion of contaminated lots; ammonization of corn and cottonseed to destroy aflatoxin; dilution; and storage technology (Trail *et al.*, 1995b). Mycotoxin-contaminated grains can be used for ethanol production, and in some cases mycotoxin-contaminated grains can be diluted with clean feeds (Desjardins *et al.*, 1993). The FDA does not allow dilution of aflatoxin-contaminated feeds, which is considered adulteration. The best strategy for postharvest control of mycotoxins is proper storage and handling of feed grains.

The potential for effective treatments has improved. Certain feed additives can reduce mycotoxin exposure of animals and thus minimize their negative effects. Some additives may be beneficial in reducing mycotoxin formation because they are effective in reducing mould growth.

Ammonia, propionic acid, microbial, and enzymatic silage additives have all shown some effectiveness as mold inhibitors. Additives to enhance fermentation can be added at ensiling.

Mould growth inhibitors such as propionic acid may be helpful as a surface treatment when capping off the silo or daily after silage feed-out to reduce molding of the exposed silage feeding face.

If unacceptably high levels of mycotoxins occur, dilution or removal of the contaminated feed is preferable; however, it is usually impossible to replace all of a major forage ingredient.

While dilution is sometimes a viable practice to reduce exposure, reduced feeding of silage could result in such a slow feed out, those mycotoxin problems within the

about 10% of total 1242 ppb of aflatoxin B_1 decreased in naturally contaminated peanut by heating at upto 100°C (Songpan, 1989). Since aflatoxin resists to higher temperature upto 260°C, long-time cooking and overheating would destruct essential vitamins and amino acids in treated foods.

Ionizing radiation such as gamma-rays can stop growth of food spoilage organisms, including bacteria, moulds and yeasts. It also inactivates pathogenic organisms including parasitic worms and insect pests. It has been reported that gammairradiation (5-10 M-rad) caused reduction of aflatoxin (Sommer and Fortlage, 1969). The irradiation, however, could not completely destroy the toxin and its mutagenicity. In the laboratory, only about 30% of total 600 ppb at aflatoxin B_1, either pure toxin or in contaminated peanuts was destroyed by 1 and 5 Mrad or gamma irradiation (Chipley and Uraih, 1980). The treatment combination of gamma irradiation and ammoniation should therefore be attempted for more aflatoxin decontamination (Park *et al.*, 1988).

Chemical treatment has been used as the most effective means for the removal of mycotoxins from contaminated commodities. The method should be sure that the detoxification system is capable of converting the toxin to a nontoxic derivative (s) without deleterious change in the raw product. Mutagenicity of the treated products should be assessed. The toxicity may be checked by feeding animals including bouts, egg embryos, chicken, ducklings and rats. Many common chemicals have been brought to test the effectiveness in detoxification of aflatoxin. These chemicals include the followings: acetic acid (C_2H_5OH), ammonia gas (NH_3) or NH_4OH or ammonium salts, 3-5%, calcium hydroxide ($Ca(OH)_2$), Formaldehyde, hydrogen peroxide (H_2O_2), methylamine (CH_3-NH_2), ozone gas (0_3), phosphoric acid (H_3PO_4),

phosphine gas (PH_3), very highly toxic, sodium bicarbonate ($NaHCO_3$), sodium bisulfite ($NaHSO_3$), sodium hydroxide (NaOH), sodium hypochlorite (NaOCI).

Certain conditions such as moisture content, heat, ultraviolet or gamma irradiation, sunlight and pressure at different treatment-periods have been simultaneously combined with the chemicals for the enhancement of detoxification.

Inactivation methods can be achieved by mixing, packing, fumigation and immersion with the chemical used.

2.3.10 Control of Mycotoxins

Careful control of mycotoxins should be started and administered by the government of each country through ministries and organizations such as the Ministry of Health, the Ministry of Agriculture, Food and Drug Administration, National Environment Committee Board and Consumer Protection Committee Board. The control programme may be set up by a special administrative committee and the legislative body who regulate the national policy of food safety and the maximum tolerance limits for mycotoxins. Farmers, middlemen, food and feed factories and exporters will be well educated about mycotoxins and encouraged to prevent and control the contamination of microflora and their health-hazardous mycotoxins in their commodities as much as possible.

International cooperation for the mycotoxin regulation in trading products or commodities is also needed. The countries should establish quality control limits for certain commodities intended for export or import. The producer countries would be stimulated to be aware of mycotoxin contamination in their exported susceptible commodities. For example, the European Economic Community (EEC 2002) has

already established certain maximum tolerance limits of aflatoxins for animal feeds, i.e. not more than 20 ppb for complete feed for pigs, poultry and feed supplements for dairy animal; 50 ppb for produce to be processed into mixed feed and complete feed for cattle, sheep and goats.

International organizations such as Food and Agricultural Organisation (FAO), World Health Organisation (WHO) and United Nations Extended Programme (UNEP) in the UN system are engaged in providing essential information on various aspects of prevention and control of mycotoxin problems to all the countries. Guidelines for international trade include: a) procedure of sampling and analysis, b) surveillance and food control inspection systems, c) use of contaminated produce in feeding of different animals, d) protocols for detoxification and the quality control of the products. Conferences, symposiums, trainings and workshops on current informations of mycotoxins should be promoted. Low-cost technology for assessment, prevention and control of environmental mycotoxins could be then transferred from developed countries to developing ones.

Table 2.4: Maximum tolerated levels of aflatoxin B_1 in groundnut and some groundnut related commodity in certain countries

Country	Commodity	Level (ng/g)
Argentina	Groundnut	20
Australia	Peanut butter, nuts	15
Colombia	Oil seeds	10
Denmark	Peanut products	2
Egypt	Peanut products, oil seed	10
France	Peanuts, Pistachenut, oil seeds	1

Kenya	Peanut products	20
Malawi	Peanuts	5
Newzealand	Peanut butter, shelled nuts	15
Nigeria	Groundnut	20
Senegal	Peanut products	50
South Africa	All foods	10
USA	Peanut products feed	100
Zimbabwe	Groundnut	5

Computed from FAOSTAT (2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

All chemicals used were of Analar grade and manufactured by May and Baker LTD Dagenham England unless otherwise stated. Chloramphenicol (Sigma, Aldrich) , Potato dextrose agar (PDA), Silica gel 60-120 mesh, petroleum spirit (60-80^{o}C), n - Hexane, Orthophosphoric acid, methanol, sodium sulphate anhydrous, D- glucose monohydrate, sulphuric acid, sodium hydrogen carbonate, methylene chloride. Mycotoxin standards of aflatoxins (B_1, B_2, and G_1) and OTA standards were obtained from Sigma, St. Louis, Mo., USA. HPLC was fitted with ZORBAX Eclipse XDB-C18, 4.6mm X 150mm, 3.5µm column.

3.2 Collection of samples

Table 3.1: Niger State Local Government Areas (LGAs) according to pattern of rainfall

Zone	Annual Rainfall Range (mm)	Local Government Area
1 (wettest)	> 1400	Suleja and Tafa
2 (Wet)	1200 – 1400	Borgu and Magama
3 (Dry)	1000 – 1200	Agaie, Agwara, Bida, Lapai, Lavun, Mashegu,Munya Minna, Gbako, Gurara,Paikor, Katcha, Kontagora, Mokwa,Rijua,Shiroro
4 (Driest)	<1000	Mariga, Rafi and Wushishi

Samples of groundnuts were collected during the rainy season (August-September), from the twenty five Federal Government recognized local government areas of Niger State according to pattern of rainfall in the state (Table 5). In each of the zones, farm, stored and roasted samples were collected and the field samples were only taken during harvest period. The stored samples were collected from locally built mud barns called "rumbu" in Hausa and the roasted samples were collected from the markets. About 0.5kg of each sample were collected, labeled, packaged in small container and taken to the laboratory. In the laboratory, each sample portion was ground and used for mycotoxin and mycological analysis.

3.3 Isolation and Identification of Fungi.

Fungi were isolated and cultured according to the method described by Smith and Moss, (1985) and Halfon-Meiri and Barkai-Golan (1990). About 1g of the grains was washed with ten successive 9ml of sterile distilled water and surface sterilized using 5% sodium hypochlorite solution. 1ml were placed at random in each of the Petri-dishes containing potato dextrose agar (PDA) and chloramphenicol (500mg per litre). The dishes were incubated at room temperature and examined daily for five days. Fungi from plated grains were transferred to PDA slant media bottles and fresh PDA in Petri-dishes for identification.

Identification of isolates was carried out at the Microbiology Department of Federal University of Technology, Minna.

3.3.1 Identification of Fungi

Each of the fungi culture was aseptically placed on a sterile slide in universal bottles using a forcep. The fungi on the sterile slide were stained with dye (Lacto phenol

blue) and viewed under x 40 objective lens of the microscope. Each pure culture was characterized and identified on the basis of their morphological and microscopic characteristics using the keys of Pitt and Hockings (1997).

3.3.2 Sub-cultuting of Fungi isolated

The pure culture of different isolate (identified fungi) were aseptically sub-cultured in potatoes dextrose agar slant and incubated at room temperature and kept in the refrigerator for further analysis.

3.4 Analysis of mycotoxins.

The samples were screened and analyzed for aflatoxin B_1, B_2, G_1, G_2 and ochratoxin A. The AFB_1, B_2, G_1, $G_2$and OTA standards were obtained from Makor Chemicals Ltd, Jerusalem, Israel. A multimycotoxin assay method (Ehrlich and Lee, 1984) was used for the mycotoxins analysis. In the method, methylene chloride and phosphoric acid are used for the simultaneous extraction of AFB_1, B_2, G_1, G_2 and OTA. A separate portion of the initial methylene chloride/phosphoric acid extract was subjected to a specific clean-up procedure for each mycotoxin. Each of the procedures was a modification of a published procedure for each toxin as described below:

3.5 Extraction and Identification of Mycotoxins

50g portion of pulverized groundnut samples was weighed into 500ml Erlenmeyer flask and 25ml 1M-phosphoric acid and 250ml of methylene chloride were added. The flask was shaken for 30 minutes using a shaker and the content filtered under pressure on Buchner funnel fitted with 18 cm circle rapid filter paper. About 200ml of the filtrate was collected and from this, 50ml aliquot each was placed in separate 100ml Erlenmeyer flasks with glass stoppers, for AFB_1, B_2, G_1, G_2 and OTA assay.

3.5.1 Ochratoxin analysis

Ochratoxin A was analyzed using Paulsch *et al*, (1982) method, 50ml of the filtrate was taken and put in a separating funnel, 70ml $NaHCO_3$ solution (4gm $NaHCO_3$/100ml distilled water) was added and shake for 1min.

After phase separation (about 15mins), upper layer ($NaHCO_3$) was transferred into a beaker, the lower methylene chloride(CH_2Cl_2) was transferred to a 2nd separating funnel and rewash with 35ml $NaHCO_3$ for two times. The $NaHCO_3$was combined in a beaker and acidify it to pH 2 with H_2SO_4.

The acidify solution was transferred to a clean 250ml separating funnel, and 50ml methylene chloride was added and shake carefully, there was CO_2 evolution.

The lower methylene chloride layer was drained through sodium sulphate (Na_2SO_4) in a funnel into a beaker. The aqueous phase in the separatory funnel was washed with an additional 50ml methylene chloride (CH_2Cl_2) and drain through Na_2SO_4. The $Na_2SO_4$was washed with about 5ml methylene chloride. The extract was then evaporated to near dryness on a steam bath.

The extract was transfered quantitatively with methylene chloride CH_2Cl_2 into 4ml glass vial. The extract was analysed using High Pressure Liquid Chromatography.

3.5.2 Aflatoxins analysis

Aflatoxins were analyzed using the method of the Association of Official Analytical Chemists (AOAC, 1990). A column was set up with glass wool, 150ml methylene chloride was poured in column and then emptied half way, anhydrous sodium sulphate (Na_2SO_4) was added, the sides was washed with methylene chloride (CH_2Cl_2).Silica gel was added to green line of column and 80ml methylene chloride

(CH_2Cl_2) added, and then allowed to settle half way. 3 scops sodium sulphate (Na_2SO_4) was added and drained off to top of column.

50ml filterate sample was added, drain off to top of column, 130ml hexane was then added, drained and dump. 130ml ether was added and drained. 130ml ether: Methanol: H_2O (96:3:1) was added and then collected off column in a new beaker. The extract was evaporated to near dryness and put into vials and analysed using High Pressure Liquid Chromatography method.

3.5.3 HPLC analysis

The mycotoxins were separated by HPLC using a mixture of water, methanol and acetonitrile and detected using UV 365nm, the base line separation was achieved in less than 5.5 minutes.

The operating conditions for the determination of aflatoxins and ochratoxin by HPLC were; the mobile phase, Water/ Methanol/ Acetonitrile 50:40:10 (V/V/V) injected at a flow rate of 0.8mL/min with injection volume of 10µL (0.044mg/mL) at room temperature of about 21 degrees celsius. The detection wavelength was at UV of 365nm while Column is 4.6mm x 150mm and detection limit was 0.001µg/ml (Coral *et al.*, 2005).

The quantitation of the mycotoxins was done by calculating the area of the peaks from the High Pressure Analysis Plot of the standards and samples. This involved the comparison of the area values of the mycotoxins in the samples with those of corresponding standard and extrapolates the concentration from the standard plot. The corresponding concentrations were then recorded and the concentrations of the mycotoxins in the samples in µg/kg were then calculated.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Fungi Isolated from Groundnut Samples

Table 4.1 shows the list and incidence of fungi isolated from farm, store and roasted groundnut collected from different local government area of Niger State. Ten fungal species were isolated from a total of eighty two samples studied. The results indicate that the genera of fungi contaminating groundnut in Niger State in order of frequency were *Aspergillus, Rhizopus, Mucor, Penicillium,* and *Fusarium*. The most frequent fungi species infecting groundnut in the State were *A.niger, A.ochraeus, A.flavus, Rhizopus spp* and *Mucor spp*.

During the season May – October, *A.ochraeus* (72/82) was the most common fungi followed by *A.flavus* (43/82), *Rhizopus spp* (43/82), *Mucor spp* (42/82) and *A.niger* (36/82). Fungal contamination increases from field to store while roasting decreases it. There was higher fungal contamination in the stored samples (37.5%) while the least isolates were from the roasted samples (27.4%). The common field fungi were *A.ochraeus*, *A.flavus, Rhizopus spp* and *A.niger* while the predominant storage fungi were *A.niger, A.ochraeus, A.flavus, A.parasiticus* and *Mucor spp. Mucor spp, A.niger, A.flavus* and *Rhizopus spp* were the most frequent contaminant of roasted groundnut samples.

Table 4.2 presents the incidence of fungi according to the micro climatic zones. Fungal contamination increased with humidity. More fungi were obtained from the wettest zone (88), followed by wet zone (69), dry zone (65) and driest zone (61)

respectively. The incidence rates were wettest zone (29.8%), wet zone (28.8%), dry zone (21.8%) and driest zone (20.5%).

Table 4.3 presents the incidence of fungi in groundnuts collected from some local government area of Niger State and it shows that the highest fungal contamination was observes in Suleja (26), Madalla (23),Sabowuse (22), Wushishshi (21) and Bida (20) respectively while the least contamination was observed in Bosso (10), Tafa (13), Minna (16), Borgu (19) and Paiko (19) respectively.

4.2. Mycotoxin contamination of Groundnut

Aflatoxin B_1: The result of AFB_1 determination in groundnut samples is presented in Table 4. The toxin was found as a contaminant of groundnut with highest incidence 72/82 out of the other aflatoxins. More farm samples (28/32) were contaminated with AFB_1 than the stored (25/28) and roasted (19/21).

AFB_1 was also a common contaminant of groundnut in the four micro climatic zones (Table 4.5), and the incidences decreases with moisture content, with higher values observed in samples from dry, driest, wettest and wet zones in decreasing order. The concentrations of the mycotoxins were significantly ($p>0.05$) higher in the dry zone when compared to the wet zone.

Table 4.6 indicates that AFB_1 was detected in samples from the representative towns of the local government areas sampled with the highest concentration in Bida while the lowest is in Borgu.

Aflatoxin B_2: AFB_2 was detected in sixty one of the eighty two samples analysed. The maximum level recorded was 38.40µg/kg. The incidence and concentration of the toxin were highest in roasted as compared to farm and stored samples (Table 4.4).

Wettest zone had the highest AFB_2 content (10.54µg/kg) followed by dry zone (9.06µg/kg), driest zone (6.62µg/kg) and wet zone (5.76µg/kg) (Table 5). AFB_2 was found contaminating groundnut in all the ten local governments sampled (Table 4.6).

Aflatoxin G_1: AFG_1 was detected in fifty five of the eighty two samples analysed. The maximum level recorded was 516.00µg/kg. There was no significant difference ($p<0.05$) between the concentrations of AFG_1 detected in roasted, stored and farm samples (Table 4.4). Wet zone had the highest concentration of AFG_1 (175.24µg/kg) while the wettest zone had the least concentration (96.06µg/kg) but the concentration of AFG_1 between the zones were not significantly different ($p<0.05$) (Table 4.5). Concentration of AFG_1 in Bosso (286µg/kg) was significantly higher than the concentration found in Madalla (59.2µg/kg) (Table 4.6). Only fifteen samples out of the eighty two samples analyzed qualitatively showed the presence of aflatoxin G_2.

Ochratoxin A: Of the eighty two samples analyzed for OTA, seventy three contained the toxin. In the samples analyzed (Table 4.4), the toxin had higher incidence in farm samples (30/32) than roasted (19/21) and stored samples (24/28). OTA was also a common contaminant of groundnut samples in the four micro climatic zones (Table 4.5). The concentration was higher in the wet zone (15.80µg/kg) but there was no significant difference ($p<0.05$) in the concentrations of OTA in all the zones. The highest concentration of OTA was found in Borgu (45.60µg/kg) while the lowest was detected in Wushishi (0.8µg/kg) local government areas respectively (Table 4.6).

4.3 Co-occurrence of Mycotoxin Isolated

This studies has shown that Ochratoxin A (73/82) was the predominant mycotoxin of the four toxin contaminating groundnut in the state and was followed by AFB_1 (72/82), AFB_2 (61//82) and lastly AFG_1 (55/82). Co-contamination with two types of mycotoxins occurred in virtually all the samples, with AFs and OTA found in thirty seven samples, thirty eight were contaminated with $AFB_1/AFB_2/AFG_1$. AFB_1/AFB_2 was found in sixty samples while only thirteen samples contained both AFG_1 and AFG_2. These multiple contaminations were detected across all the ten local government areas analyzed.

4.4 Unsafe Samples

This study shows that aflatoxins B_1, B_2 and G_1 were found to be unsafe in (71/72), (19/61) and (54/55) samples respectively which is 98%, 31% and 98% of the analyzed samples. This number of unsafe samples was according to European Union Standard 2002 for the highest concentration of mycotoxins expected in feed and feedstuff before it is safe for consumption both for animals and humans. According to Nigerian Standard for Aflatoxin B_1 in 2010, the number of unsafe samples found in this study is eleven out of seventy three which gives only 15%.

Similarly when considering EU regulation for OTA in foods, samples from the market, store and field were contaminated above the maximum acceptable limits of 5µg/kg. On the overall about 55% of the samples analyzed for OTA in this study had concentration above the EU regulation of 5µg/kg.

The number of unsafe samples found in this study poses a serious concern and can be a serious challenge on exportation of groundnut from Niger State.

Table 4.1: Incidence of fungi in roasted, stored and farm groundnuts in Niger State

Fungi	Roasted 22)	Stored (28)	Farm (32)	Total incidence (82)	Frequency (%)
A. niger	12	17	7	36	12.9
A. flayus	12	18	13	43	15.5
A. fumigates	-	7	4	11	3.9
A. ochraeus	6	20	36	62	22.3
A.parasiticus	4	6	14	24	8.6
Fusarium	1	4	3	8	2.9
Mucor spp.	6	17	19	42	15.1
Penicillium	2	5	6	13	4.7
Rhizopus spp.	4	10	19	43	11.9
Yeast	2	4	-	6	2.2
Total	79	108	101	278	
	27.4%	37.5%	35.1%		

Table 4.2: Incidence of Fungi in groundnuts in Niger State in accordance of rainfall pattern

Fungi	Driest (22)	Dry (20)	Wettest (22)	Wet(19)	Total incidence (82)	Frequency (%)
A. niger	10	8	7	11	36	12.7
A. flayus	8	8	10	17	43	15.2
A. fumigates	-	3	4	4	11	3.9
A. ochraeus	17	19	18	18	72	25.4
A. parasiticus	4	6	9	5	24	8.5
Fusarium	2	1	2	3	8	2.8
Mucor spp.	10	6	17	9	42	14.8
Penicillium	3	3	3	4	13	4.6
Rhizopus spp.	6	10	14	13	43	15.2
Yeast	1	1	4	-	6	2.1
Total	61	65	88	69	283	
	20.5%	21.8%	29.8%	28.2%		

Table 4.3: Incidence of Fungi in groundnut samples collected from representative towns of the Local Government Area of Niger State

Fungi	Tafa	Madalla	Suleja	Sabowuse	Borgu	Bosso	Bida	Paiko	Minna	Wushishi
A.niger	1	2	2	2	3	1	4	2	1	2
A.flavus	1	4	3	2	4	1	3	2	2	2
A.fumigatus	-	2	2	-	1	-	1	-	2	-
A.ochraeus	4	5	5	4	5	4	7	3	5	4
A.parasiticus	1	2	2	4	2	-	1	2	3	1
Fusarium	1	1	-	-	1	-	-	-	1	1
Mucor spp.	3	2	4	3	1	1	1	3	1	5
Penicillium	-	-	2	1	1	-	1	2	-	1
Rhizopus spp.	2	4	5	3	1	3	2	4	1	4
Yeast	-	1	1	3	-	-	-	1	-	1
Total	13	23	26	22	19	10	20	19	16	21

Table 4.4: Incidence and concentration (µg/Kg) of AFB_1, AFB_2, $AFBG_1$ and OTA in roasted, stored and farm groundnuts in Niger State.

Mycotoxin	Incidence &Conc (µg/Kg)	Roasted	Stored	Farm	Total
Afla B_1	Incidence	19/21	25/28	28/32	72/82
	mean±SE	64.78^{a}±5.11	42.78^{ab} ±4.27	54.32^{abc}±4.36	53.06±2.57
	Range	27.00 – 188.00	4.80 – 165.60	4.00 – 181.60	4.00 –188.00
Afla B_2	Incidence	16/21	19/28	26/32	61/82
	Mean±SE	11.60^{a}±1.46	5.08^{ab} ±0.81	8.10^{abc}±0.99	8.08.±0.63
	Range	0.40 – 36.40	0.80 – 32.80	0.40 – 38.40	0.40 – 38.40
AflaG_1	Incidence	18/21	18/28	19/32	72/82
	Mean±SE	107.94^{a}±12.67	156.88^{ab} ±15.63	146.76^{abc}±17.25	137.38±8.83
	Range	16.00 – 340.00	20.00 – 508.00	8.00 – 516.00	8.00 – 516.00
Ochra A	Incidence	19/21	24/28	30/32	73/82
	Mean±SE	13.30^{a}±0.81	13.60^{ab} ±1.17	14.46^{abc}±1.08	13.86±0.62
	Range	5.20 –32.80	0.80 – 40.00	1.60 – 45.60	0.80 – 45.60

Note: Values with different superscripts were significantly different from each other ($P < 0.05$)

Values are Mean ± Standard error for n replicate

Table 4.5: Incidence and concentration (µg/Kg) of AFB_1, AFB_2, $AFBG_1$ and OTA in groundnuts in Niger State in accordance of rainfall pattern.

Mycotoxin	Incidence &Conc (µg/Kg)	Driest	Dry	Wettest	Wet	Total
Afla B_1	Incidence	17/21	19/19	19/22	17/19	72/82
	Mean±SE	56.10^{a}±5.11	74.02^{ab} ±5.94	45.10^{abc}±4.13	35.44^{acd}±4.25	53.04±2.57
	Range	8.00 – 165.60	5.60 – 188.00	6.40 – 141.60	4.00 – 13.60	4.00 – 188.00
Afla B_2	Incidence	14/21	16/19	16/22	15/19	61/82
	Mean±SE	6.62^{a}±1.10	9.06^{ab} ±1.23	10.54^{abc}±1.47	5.76^{abcd}±1.23	8.08.±0.63
	Range	0.80 – 32.40	0.80 – 32.80	0.40 – 36.40	0.40 – 38.40	0.40 – 38.40
$AflaG_1$	Incidence	14/21	12/19	16/22	13/19	72/82
	Mean±SE	134.42^{a}±18.94	154.72^{ab} ±19.91	96.06^{abc}±12.49	175.24^{abcd}±20.28	136.38±8.83
	Range	12.00 – 508.00	16.00 – 424.00	8.00 – 340.00	20.00 – 516.00	8.00 – 516.00
Ochra A	Incidence	17/21	19/19	18/22	19/19	73/82
	Mean±SE	13.50^{a}±1.4	12.64^{ab} ±0.94	13.56^{abc}±1.23	15.80^{abcd}±1.43	13.88±0.62
	Range	0.80 –38.00	1.20 – 30.40	1.60 – 40.00	4.00 – 45.60	0.80 – 45.60

Note: Values with different superscripts were significantly different from each other ($P < 0.05$)

Values are Mean ± Standard error for n replicate

Table 4.6: Incidence and concentration (µg/Kg) of AFB_1, AFB_2, AFG_1 and OTA in groundnut from representative towns of the local government area in Niger State.

Mycotoxin	Occurrence Concentration (µg/Kg)	Tafa	Madalla	Suleja	Sabowuse	Borgu	Bosso	Bida	Paiko	Minna	Wushishi
Afla B_1	Occurrence	5/6	5/5	5/7	4/4	17/19	4/4	4/4	5/5	6/6	17/21
	Mean ± SE	44.4±3.9	51.0±5.2	38.6±4.6	46.8±2.4	35.4±4.2	88.8±5.6	90.8±6.5	67.8±3.6	58.2±4.4	56.0±4.6
	Range	10.4-81.6	10.4-141.6	6.4-109.6	33.6-57.6	4.0-137.6	45.6-181.6	6.8-188.0	42.6-104.8	5.6 - 101.6	8.0-165.6
Afla B_2	Occurrence	6/6	3/5	4/7	3/4	15/19	4/4	4/4	4/5	4/6	14/21
	Mean ± SE	7.8±1.2	6.8±1.8	7.4±2.5	24.0±3.0	5.8±1.2	2.6±0.2	13.6±2.6	12.4±2.5	7.8±1.6	6.6±1.1
	Range	1.52–15.6	2.4 – 14.0	0.4 - 26.2	3.4-36.4	0.4-38.4	1.6 -3.6	1.6-32.8	0.8-30.4	0.8-16.0	0.8-32.4
$AflaG_1$	Occurrence	4/6	5/5	4/7	3/4	13/19	2/4	¾	4/5	3/6	14/21
	Mean ± SE	115.2±38.8	59.2±4.9	67.0±5.7	170.6±43.7	175.4±20.3	286±69	142.3±42.4	84.0±23.4	173.6±43.7	134.4±18.9
	Range	8.0-34.0	26.0-80.0	36.0-88.0	32.0-332.0	20.0-516.0	148.0-424.0	53.6-316.0	16.0-220.0	82.4-348.4	12.0-508.0
Ochra A	Occurrence	4/6	5/5	5/7	4/4	19/19	4/4	4/4	5/5	6/6	17/21
	Mean ± SE	10.6±1.7	21.6±1.2	11.6±3.6	9.0±1.5	15.8±1.4	6.6±1.0	12.8±2.0	15.4±2.3	14.4±1.7	13.6±1.4
	Range	2.8-192	14.4-29.2	1.6-40.0	2.8-17.2	4.0 - 45.6	1.2-10.4	4.0-23.2	4.4-30.4	2.8-21.6	0.8-38.0

NB: Values are Mean ± Standard Error for n replicates.

Table 4.7: Frequency occurance of AFB_1, AFB_2, AFG_1 and OTA in groundnut from representative towns of the local government area in Niger State.

Mycotoxin	Tafa	Madalla	Suleja	Sabowuse	Borgu	Bosso	Bida	Paiko	Minna	Wushishi
Afla B_1	5	5	5	4	17	4	4	5	6	17
%Incidence	6.9	6.9	6.9	5.5	23.6	5.5	5.5	6.9	8.3	23.6
Afla B_2	6	3	4	3	15	4	4	4	4	14
%Incidence	9.8	4.9	6.6	4.9	24.6	6.6	6.6	6.6	6.6	23.0
AflaG_1	4	5	4	3	13	2	3	4	3	14
%Incidence	7.3	9.1	7.3	5.5	23.6	3.6	5.5	7.3	5.5	25.5
Ochra A	4	5	5	4	19	4	4	5	6	17
%Incidence	5.5	6.8	6.8	5.5	26.0	5.5	5.5	6.8	8.2	23.3

4.5 DISCUSSION

Mycological examination of groundnut samples revealed that ten fungal genera were found to contaminate groundnut in the state. Many of these families of fungi have also been shown to cause spoilage to groundnut in other parts of the globe. As observed in this study (Okoye, 1992) identified the following fungal genera as the main contaminants of groundnut in Northern Nigeria: *Aspergillus, Fusarium and Penicillium* etc. the other genera represented in their reports were *Rhizopus* and *Mucor*. Diener *et al* 1987; Pitt *et al* 1998; Pitt and Hucking 1997; Horn *et al* 1995 reported the incidence of the above mentioned fungi in other parts of the world.

Fungi are a cause of deterioriation and loss of grains and seed and their invasion in groundnut decrease the quality, grade and market value of these agricultural products which in most instances are rendered unsafe for human and animal consumption. Fungi contamination in this study shows that contamination increases with humidity and roasting decreases it. Conditions favouring fungal growth have been understood to include hot humid conditions (Umoh, 1997). These conditions which are more prevalent in the rainy season in Niger State may account for the higher fungal contamination recorded in the wettest and wet zones (Tafa, Madalla, Suleja, Sabowuse and Borgu), than the dry and driest zone of the same season. The higher fungal growth in groundnut may also be due to other factors such as longer storage time which may account for the higher fungal contamination in stored samples and this may also be as a result of the prevalent conditions like humidity in these areas and also the level of crop protection awareness of the farmers in the areas.

Aspergillus spp occurs with higher frequencies in the subtropical warm temperate zones between 26-35°C (Klich 2002) and climate of Niger State falls within this range which

is conducive for the growth of *Aspergillus spp* that produces aflatoxins and ochratoxin A which are the predominant mycotoxin contaminant of groundnut in the state. The abundance of *Aspergillus spp* in the groundnut samples correlated with the high incidence of AFB_1, AFB_2, AFG_1 and Ochratoxin A found in this study.

Toxigenic *A.flavus* isolated from this study has been found to produce aflatoxins B_1 and B_2, and toxigenic *A.parasiticus* isolates produce aflatoxins B_1, B_2, G_1 and G_2 (Cotty *et al.*, 1994), this also contributes to high incidence of the mycotoxins which correlates to the fungi isolated. Other studies including this study have detected aflatoxin and ochratoxinA in groundnut in Nigeria and other parts of the world (Bhat *et al* 1996; Zhang *et al* 1996; Freitas and Brigido 1998; Ali *et al* 1999; Arim 2000; Okano *et al* 2002).

Penicillium spp, a fungal contaminant of groundnut produces mycotoxin including ochratoxin A (Wood, 1992). *Fusarium spp* produce zearalenone, fumonisin, trichothecenes and many other minor toxins (Marasas *et al.*, 1984, Mehan et al., 1985, Gelderblom *et al.*, 1988). *Rhizopus* and *Mucor spp* isolated from this study are known to produce toxins which have deleterious effects to liver. Diseases caused by these toxins have been documented (IARC 1993).

Mycotoxins are considered unavoidable contaminants in food and feedstuffs because agronomical technology has not yet advanced to stage at which preharvest infection of grains by fungi can be eliminated. Aflatoxins and ochratoxin A are among the agriculturally important mycotoxins which are found in foods especially grains including groundnut and pose a wide range of problems and challenges to food production, public health and international trade (Muzaffer and Perez 2010).

As observed in this study, (Darling 1963; Peer 1965, McDonald and Harkness 1965, Abalaka and Elegbede 1982) also extracted aflatoxins from groundnut from different part of Northern Nigeria above permissible limit at concentrations of 30-100ppb and 20-455µg/kg. The incidence of aflatoxins was also reported from other part of Nigeria (Okoye 1992, Opadukun 1992) at concentrations of 44-1250µg/kg. These incidence correlates with the occurance and incidence of the toxins found in this study which is at concentrations of 4-188µg/kg. Mycotoxins concentration in groundnut may contribute to the occurance of the toxin in groundnut products like groundnut cake (kulikuli) (Abalaka *et al.*, 2004).

Aflatoxin B_1 recieves greater attention than other mycotoxins because of its carcinogenic effect in animal and its acute toxic effect in humans (Bressac *et al* 1991). Foods contaminated with aflatoxins have been involved in diseases and death of animals consuming the contaminated food (Garland and Reagor 2007). The content of aflatoxin in this study varied from 4-188.0µg/kg and such high content is alarming, as the maximal level allowed by the Food and Drug Administration is 2µg/kg for European Union and 50µg/kg for Nigeria. Similar results were reported by (Farid *et al* 2010) who detected aflatoxin B_1 in examined samples of groundnut at levels above permissible limit. The mean AFB_1 contamination level recorded in this study are lower than what was reported by Abalaka *et al* 2011 (5-1000µg/kg) but was a direct agreement to the findings of Abalaka et al 2004, Adebanjo and Idowu 1994 (10-160ppb). Akano and Atanda 1990 found AFB_1 concentrations in the range of 20-455 µg/kg in groundnut cake purchased from market in Ibadan, Oyo State and this range is also in agreement with findings from this report (4-188µg/kg). There was slight variation in concentrations of aflatoxins in different samples which might be due to the differences in localities from where they were collected and in storage conditions. The high concentration of B_1, B_2

and G_1 aflatoxins in this study indicate the extent of poor storage conditions to which they are exposed and the associated health risks posed to the consumers, many of which are not aware of the health implications of ingesting mycotoxins.

Ochratoxin A is known to be produced by mainly *Aspergillus ochraeus, Penicillium verrucosum* and *Penicilium nordicum* (Van der Merwe *et al.*, 1965, Harwig *et al.*, 1974, Wood, 1992) and this specie of *Aspergillus* were isolated in this study. *A.ochraeus* produce ochratoxin A between 0-30°C (Ominski *et al* 1994) and this temperature range is usual during rainy seasons in the state. Ochratoxin A is one of the world's most important mycotoxin, rated third of the top six, primarily due to the documented deaths of humans, primarily in Europe (FAO Food and Nutrition Paper No 81 2004). Ochratoxin A is nephrotoxic, teratogenic, carcinogenic and immunosuppressive in animals, and in humans it has been associated with a chronic renal failure (Muzaffer and Perez 2010). Ochratoxin A was isolated as a natural contaminant of maize with concentration range of 0.2-40µg/kg (Shotwell *et al* 1981) and has also been isolated from many agronomical important crops like peanuts at concentration range of 0.8-65.3µg/kg (Abarca *et al* 1994, Palencia *et al* 2010).

The incidence and concentration of the ochratoxin A were higher in the wettest and wet zone of the state which correlates with the fungi contamination.

Groundnuts in the farm can be infected by fungi due to some mechanical damage (during shelling, threshing, winnowing) and insect infestation and this may account for the higher incidence in the farm samples. Bad storage conditions and longer storage time also are likely to be associated with the incidence in the stored samples while contamination and handling practices are likely to be the cause of high concentration and incidence observed in roasted samples.

The more enhancing hot humid conditions of the wettest and wet zones of the rainy season than the driest zone may be responsible for the higher fungal and mycotoxin contamination of the samples.

The danger in co-occurrence of mycotoxins is that they can act synergistically to potentiate their effects. Penicillic acid is often times co-produced with ochratoxin A by strains of *A.ochraceus* (Riley *et al* 1993) and by numerous other species of *Aspergillus* and *Penicillium* (Lindenfelser and Ciegler 1977). The co-occurrence of mycotoxins can affect both the level of mycotoxin production and the toxicity of the contaminated material (Miller 1994). Co-occurrence of AFs and OTA exhibited antagonistic interaction between the toxins with regards to teratogenic effects (Wangikar *et al* 2005). Sedmikova *et al* 2001 reported that OTA can increase the mutagenic ability of AFB_1 in the case of the two simultaneously occurring in the same crop. OTA and AFs assayed in fatty livers of poultry was reported to reduce aflatoxin effect for the fatty liver, synergistic for growth weight and nephropathy. AFB_1 and OTA also cause mild antagonism in renal interstitial fibrosis of swine. The multiple contaminations found in this study may also contribute to the further mycotoxicoses.

According to European Union Standard 2002, the highest concentration of aflatoxins that are expected in feed and feedstuff before it is safe for consumption both for animals and humans are 2µg/kg for aflatoxin B_1, 4µg/kg for aflatoxins B_2, G_1 and G_2 and 5µg/kg for Ochratoxin A. Based on these standards, the number of unsafe samples found in this study pose a serious concern and will be a serious challenge on exploitation of groundnut from Niger State.

The season of sample collection may also affect the concentration of the mycotoxins and needs to be taken into account in the further studies. Results from zonal comparisons may be compromised by seasonal effects.

The presence of mycotoxigenic fungi and the studied mycotoxins in groundnut in Niger state as shown in this study implies that groundnut could be an agent of human and animal mycotoxicoses discussed both in Nigeria and on a global scene. The consumption of contaminated groundnut therefore has adverse consequences on livestock production, human health and economy.

The prevention of mycotoxin contamination of human foods could have a significant effect on public health and therefore deserve significant attention. This will lead to improve economic sustainability of the food industry, enhance food safety efforts, enhance international trade efforts and improve public health.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

In conclusion, although the numbers of groundnut samples analyzed in this study were limited, the high frequency and high levels of aflatoxins and ochratoxin A found in these samples clearly show the necessity for regular surveillance. Limiting mycotoxin occurrence in groundnut before harvest can be achieved by limiting high temperature stress, controlling weeds, reducing insect damage, using effective harvesting technique and crop rotation. Genetic engineering may offer ways of limiting the pre-harvest contamination of crops. Also mycotoxins can be controlled by controlling factors which affect fungal growth example water activity and temperature by drying and proper storage.

5.2 RECOMMENDATIONS

i The pathogenic expression of these fungi and other unknown species results in poor quality nuts that are rated low for human consumption, the production of aflatoxins and ochratoxin A on this important crop underlines the need for more comprehensive studies on fungi and mycotoxins contaminating groundnuts in the state.

ii The prediction of rising global temperatures will influence the growth of fungi and add to the mycotoxigenic potential of the different species in the state therefore surveillance should be targeted on groundnuts and other grains in the state.

iii The stakeholders in the production chain particularly farmers should be made aware of the importance to reduce mycotoxin contamination.

iv Training programmes for the development of practical control and management strategies should be conducted in order to set up strong mycotoxin management programmes.

v Mycotoxin management should involve farmers, traders, processors and policy makers especially those involved in inspection and regulatory control.

vi Careful control of mycotoxins should be started and administered by the government of the state and country through ministries and organizations such as the Ministry of Health, the Ministry of Agriculture, Food and Drug Administration, National Environment Committee Board and Consumer Protection Committee Board.

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APPENDICES

Appendix A

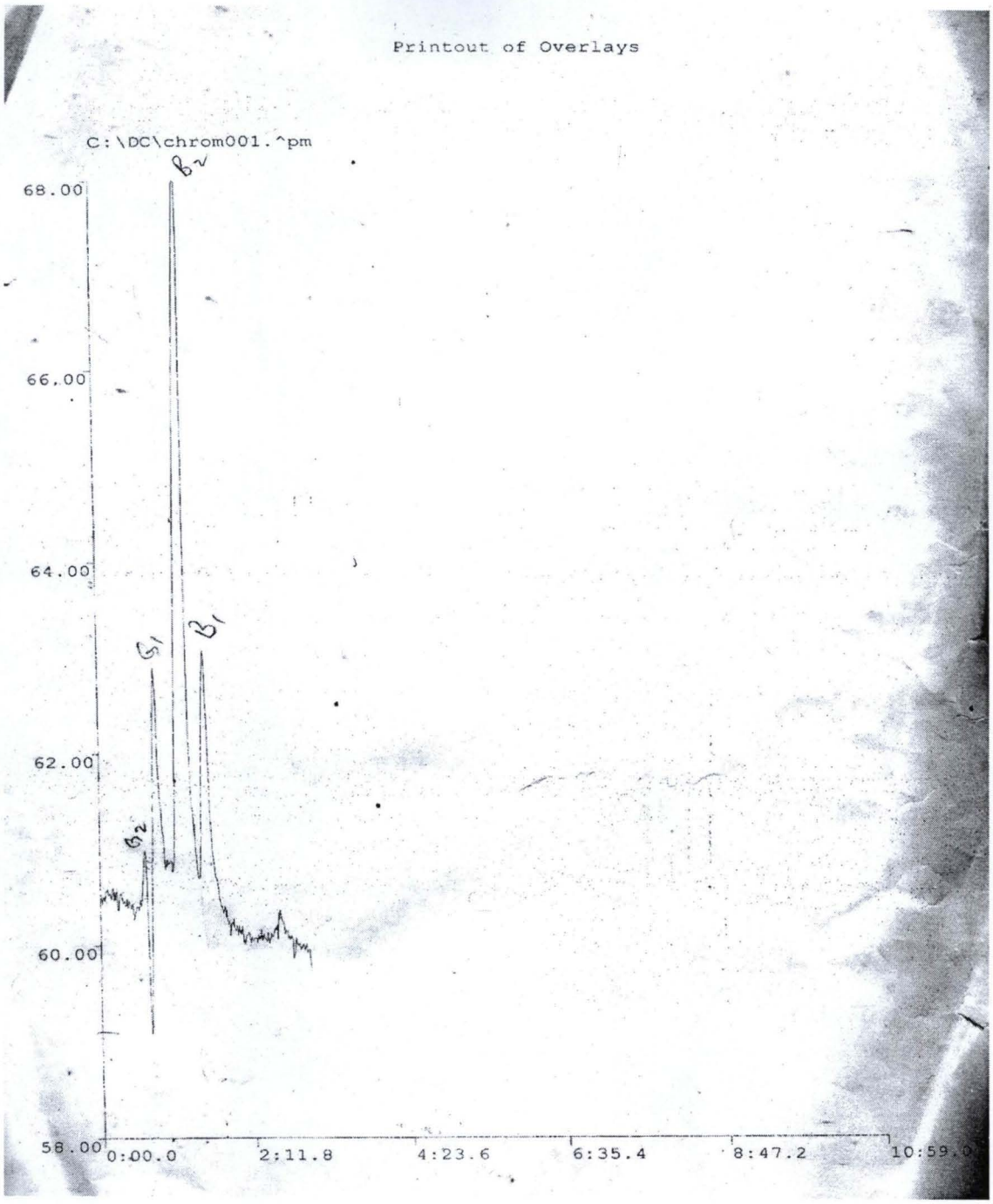

Fig 1 : Standard Chromatogram of aflatoxins (B_1, B_2, G_1 and G_2)

Appendix B

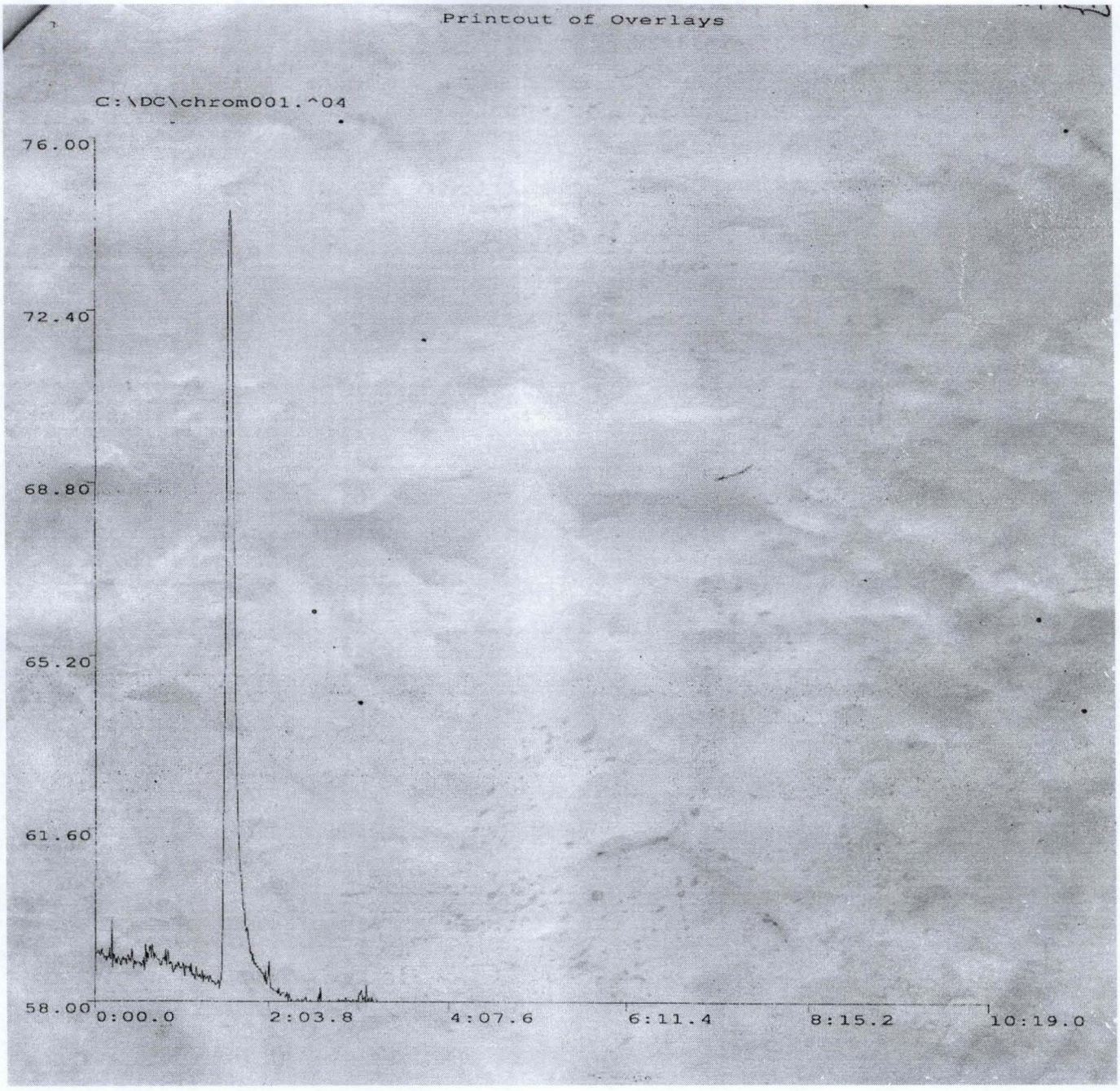

Fig 2: Standard Chromatogram of aflatoxins B_1 at 20µl/ml concentrate

Appendix C

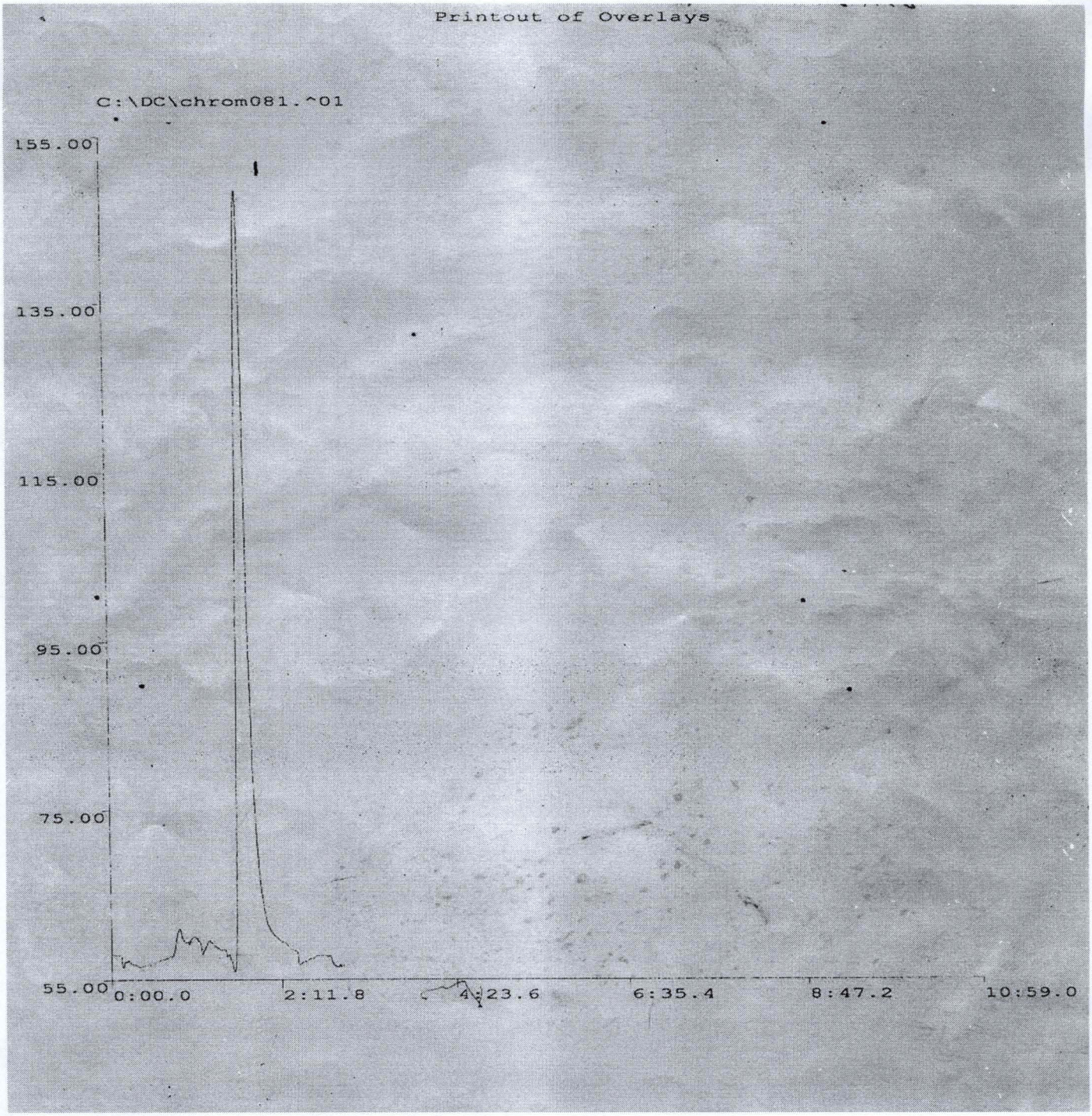

Fig 3: Standard Chromatogram of aflatoxins B_2 at 20µl/ml concentrate

Appendix D

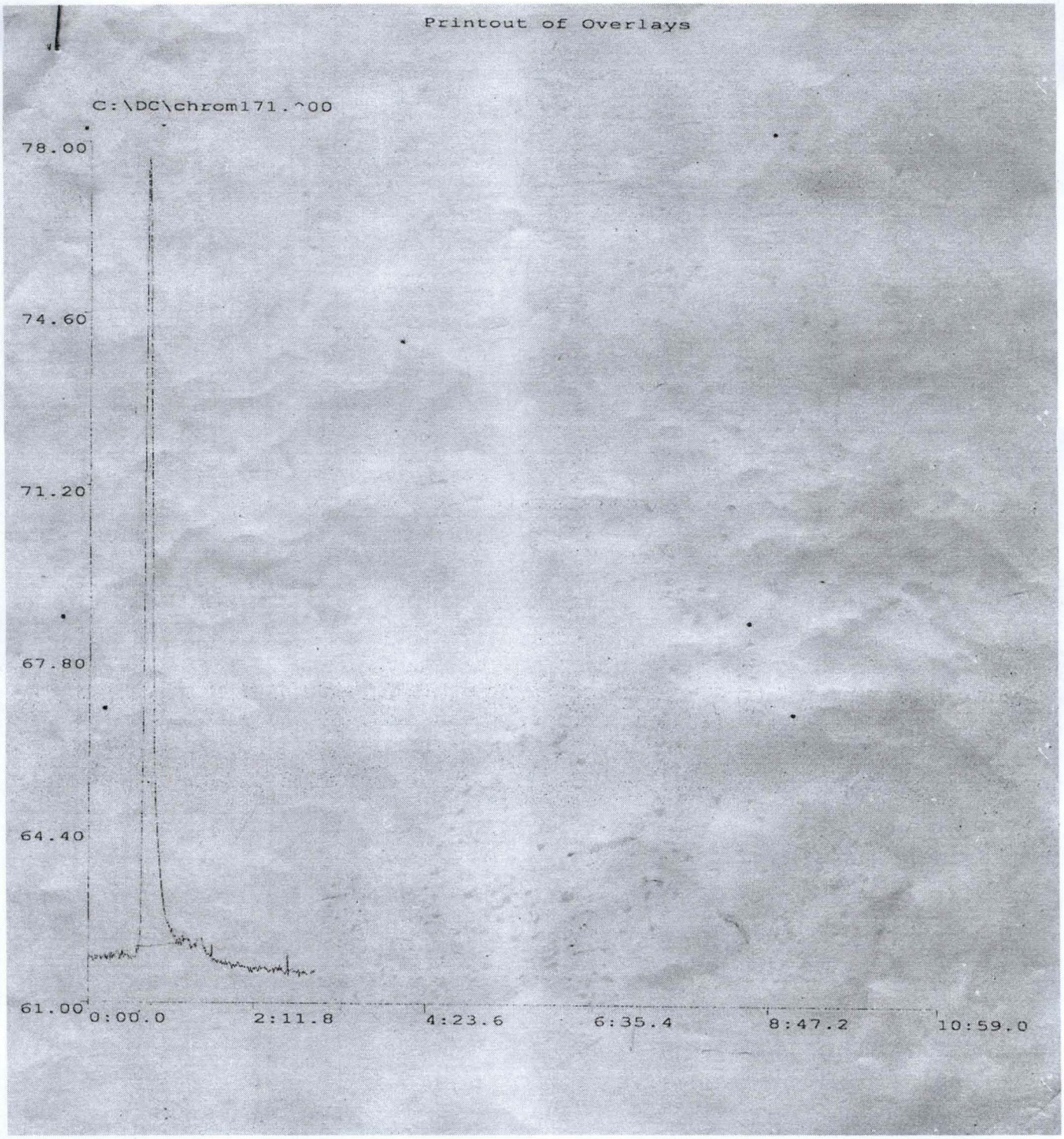

Fig 4: Standard Chromatogram of aflatoxins G_1 at 20µl/ml concentrate

Appendix E

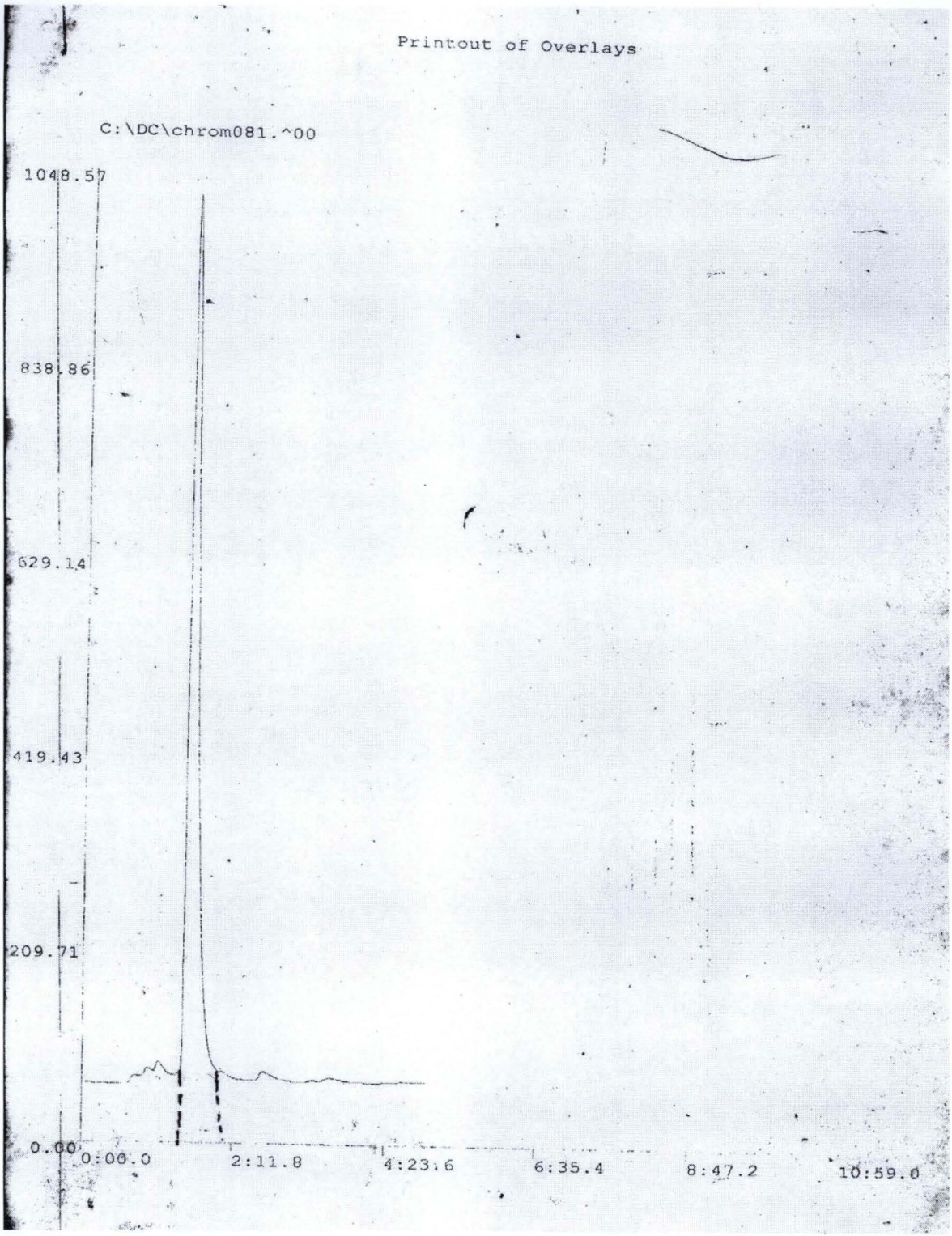

Fig 5: Standard Chromatogram of ochratoxin A at 20µl/ml concentrate

Appendix F

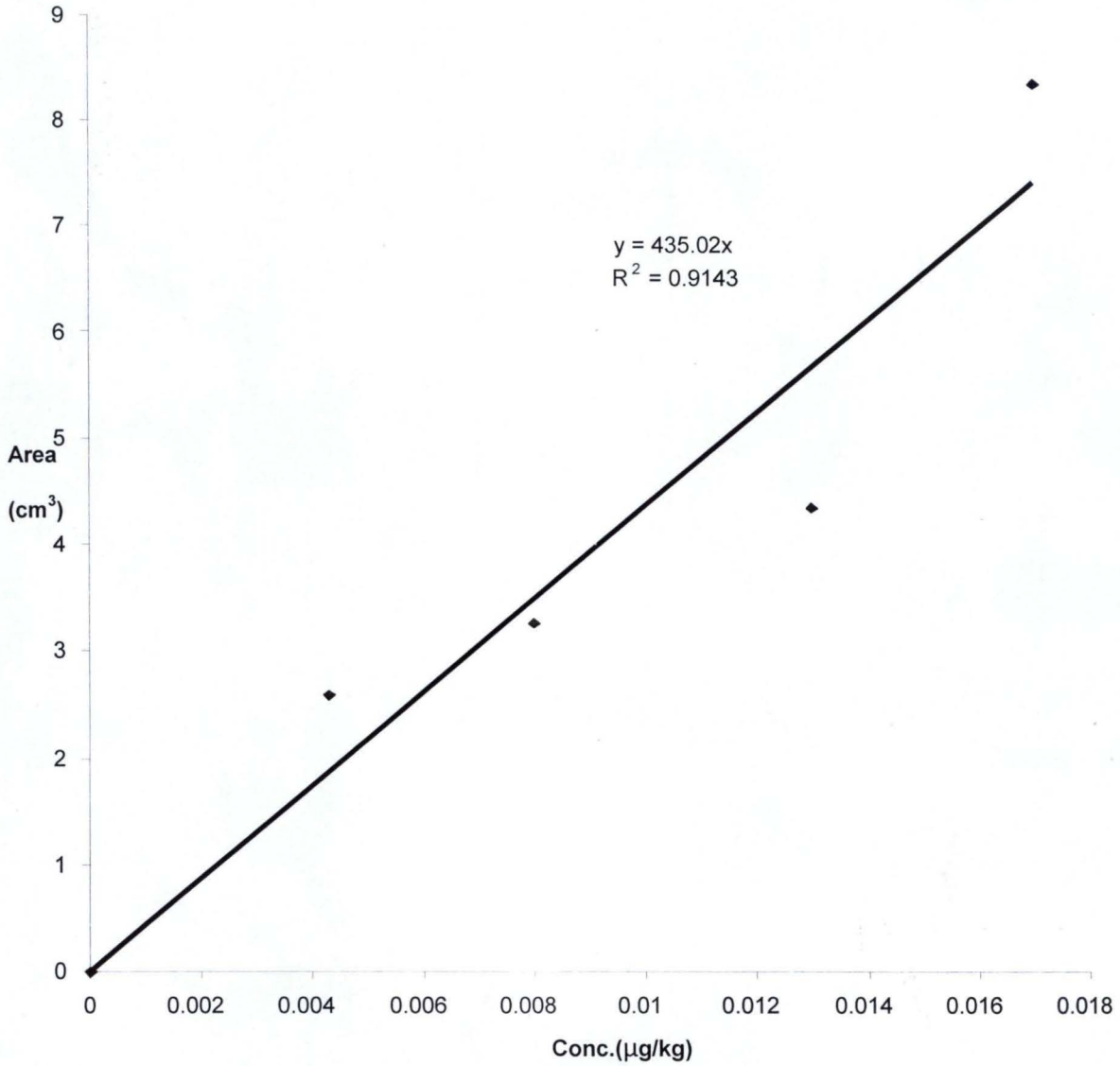

Fig 6: Standard Graph of aflatoxins B_1

Appendix G

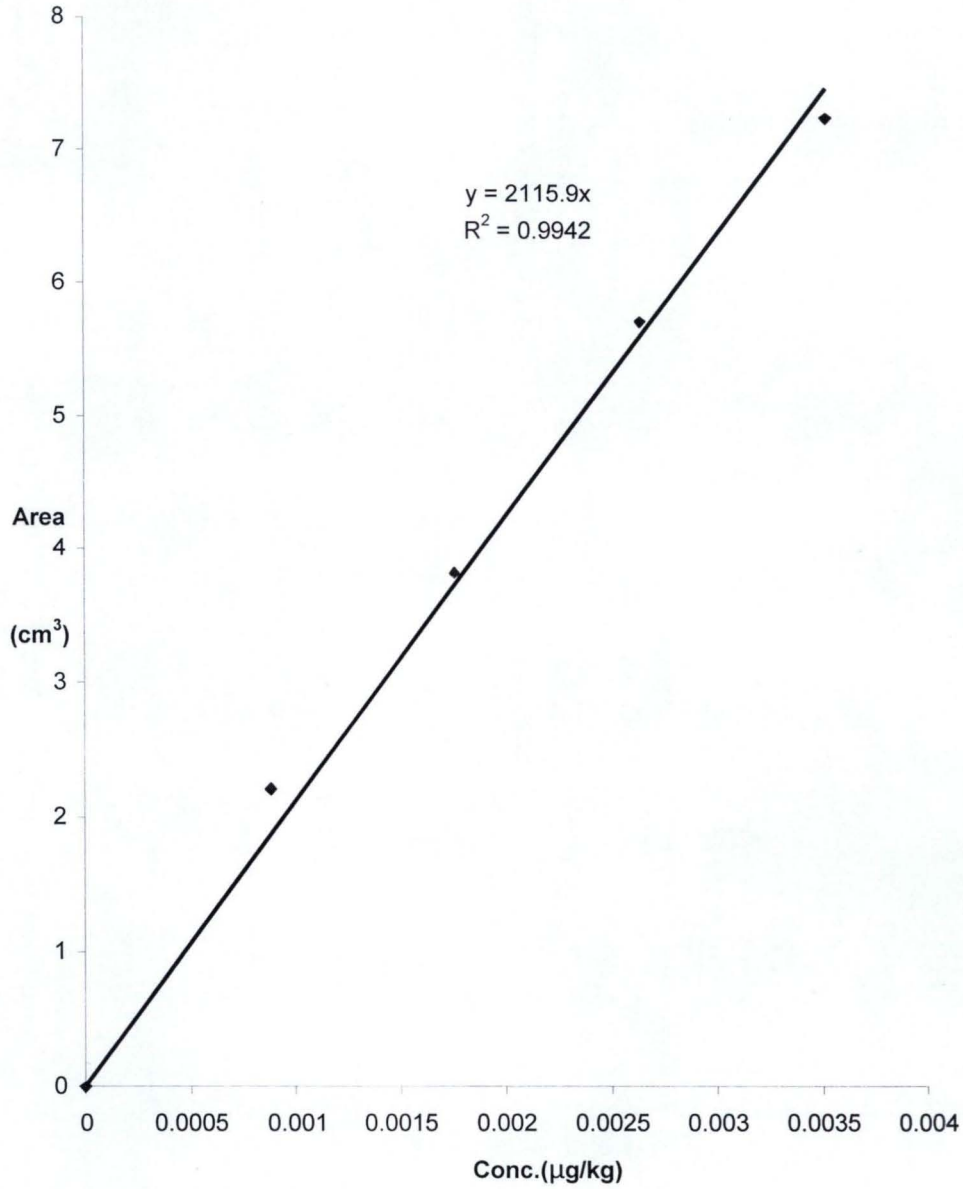

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Fig 7: Standard Graph of aflatoxins B_2

Appendix H

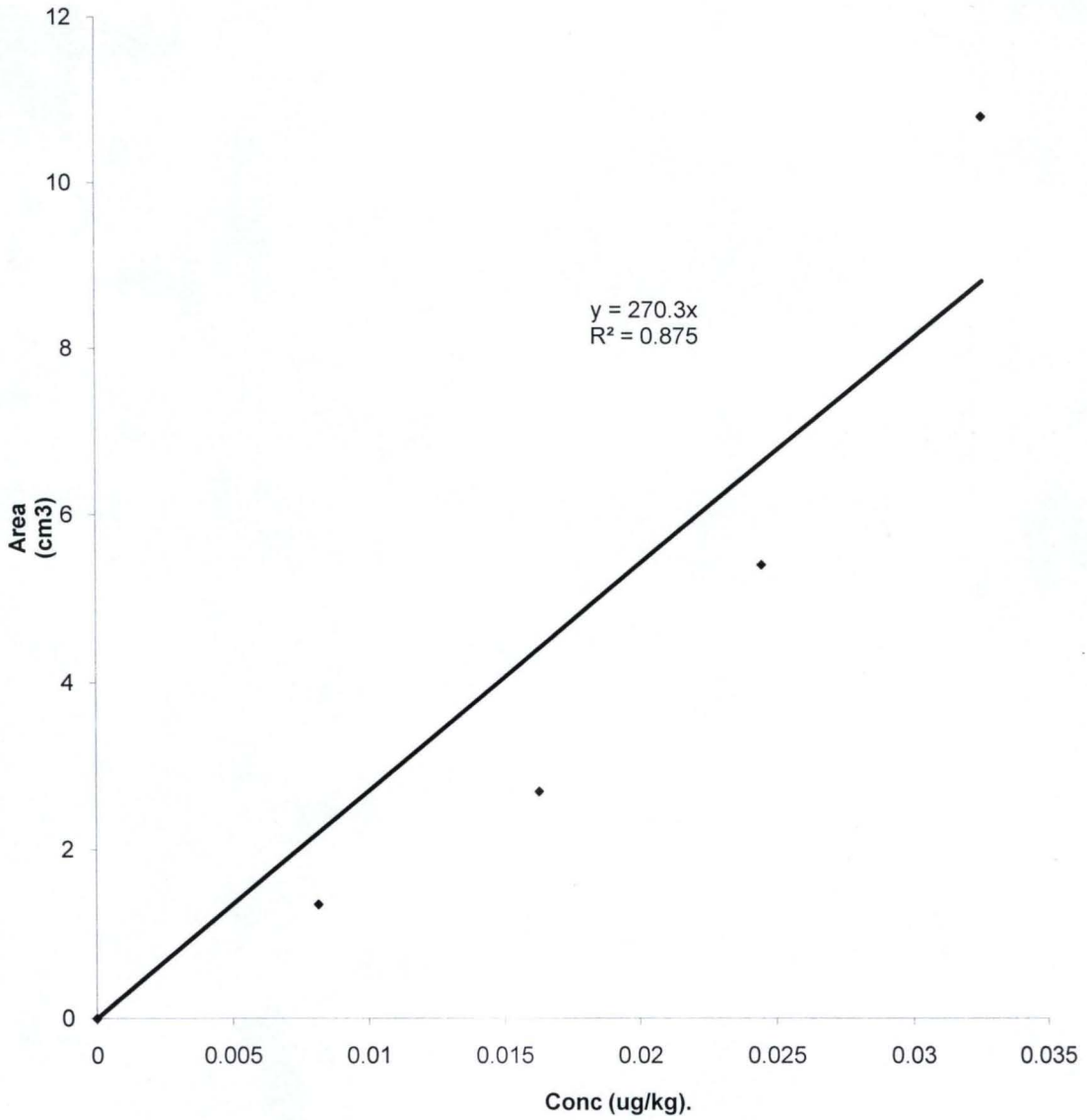

Fig 8: Standard Graph of aflatoxins G_1

Appendices I

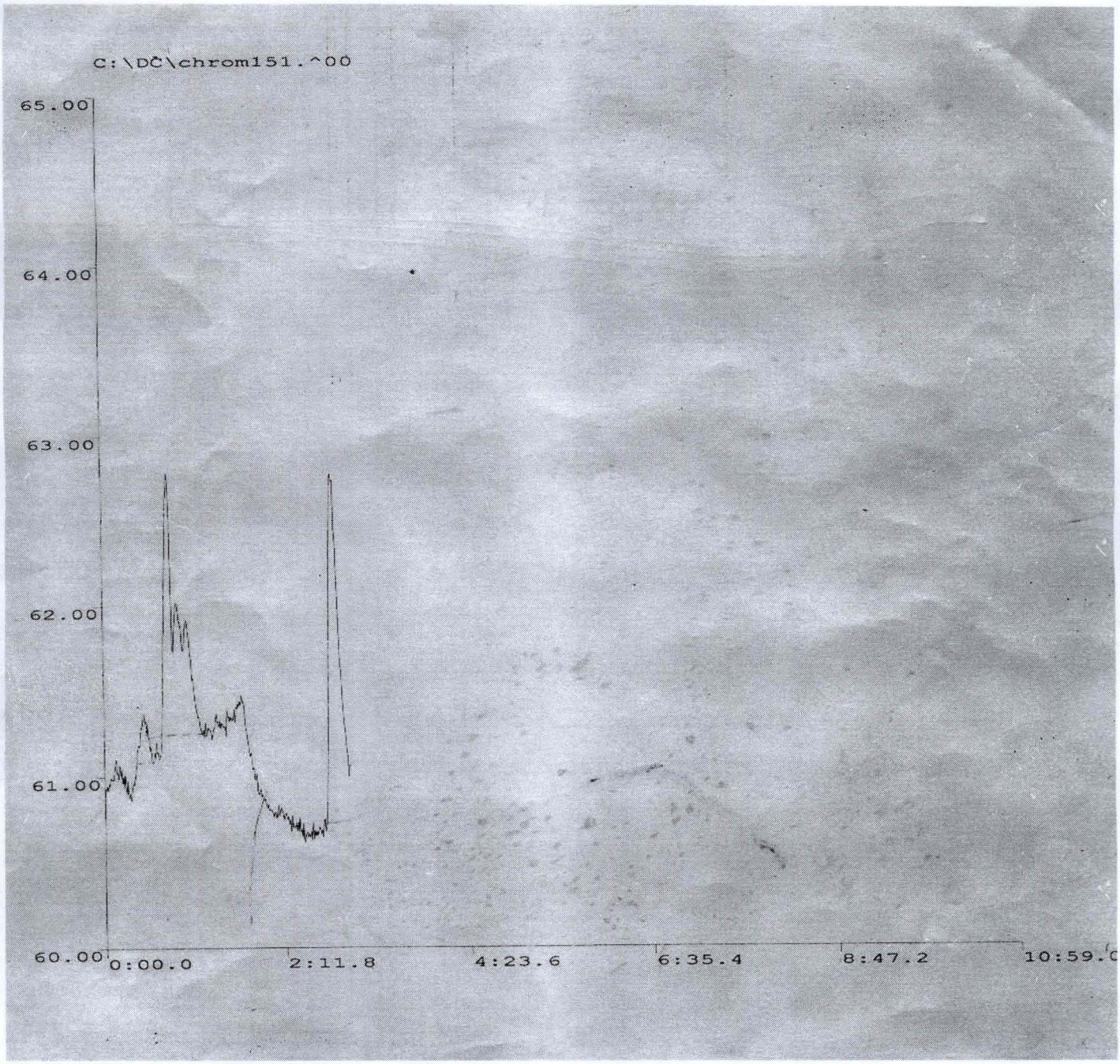

Fig 9: **Chromatograms of some selected samples of aflatoxins (B_1, B_2, G_1 and G_2)**