PUBLIC HEALTH SIGNIFICANCE OF AFLATOXIN PRODUCING MOULD CONTAMINATION OF MAIZEAND MAIZE PRODUCTS FROM SELECTED MARKETS IN PARTS OF KADUNA STATE, NIGERIA

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DECLARATION

I declare that the work in this Dissertation entitled public health significance of aflatoxin producing mould contamination of maize and maize products from selected markets in parts of Kaduna State, Nigeria has been carried out by me in the Department of Microbiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION.

This Dissertation entitled PUBLIC HEALTH SIGNIFICANCE OF AFLATOXIN PRODUCING MOULD CONTAMINATION OF MAIZE AND MAIZE PRODUCTS FROM SELECTED MARKETS IN PARTS OF KADUNA STATE, NIGERIA BY ISAH ENYISI SULE meets the regulations governing the award of the degree of Doctor of Philosophy of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION.

By the grace of God to: My wife Mrs.HafsatAyimihi Suleiman and to our Children Hamza, Abdulhamid, Nafisat, Nimatullahiand Mohammed Hayatudeen, all for their love, support and Prayers.

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ABSTRACT

A study to determine the public health significance of mould contamination of maize and maize products in selected grain markets within Kaduna state was carried out from March 2010 - February 2011, using physical analysis, proximate composition analysis, mineral determination using atomic absorption spectrophotometer and mycological procedures.Aflatoxin production ability of isolated moulds was evaluated using the thin layerchromatography (TLC)technique and Enzyme Linked ImmunosorbentAssay (ELISA). The mean temperature, relative humidity and monthly rainfall from all the markets were 30^{o}C, 48% and 3.2mm respectively. In terms of quality, the percentage wholesomeness to broken or damaged grains was found to be high and significant differences were found to exist between percentage whole, broken and damaged grains ($P \leq 0.05$).Proximate composition of maize and maize products at four weeks post storage in a low density polythene bags were in the range of 11.6- 20 .0% (moisture), 1.10 – 2.95% (Ash), 4.50 – 9.87% (protein), 2.17-4.43 (fat), 2.10- 26.70% (fibre) and 44.60- 69.60% (carbohydrate). Statistical analysis using ANOVA showed significant differences between the mean values of the nutrient content of all the products except for moisture. Mineral elements of the maize and maize products namely: phosphorus, magnesium and potassium were found to be higher than other elements: zinc, calcium,copper, sodium, manganese and iron. The bulk of these minerals were concentrated in the maize bran. The mean levels of fungal density ranged from $\log_{10}$ 1.00 – 4.9 for old maize; $\log_{10}$ 1.17 – 2.6 for new maize; $\log_{10}$ 1.95 -4.90 for maize flour and $\log_{10}$ 1.26 -5.34 for maize bran .The effect of seasonal variation of mould count was determined and result revealed a significant high total count ($P \leq 0.05$) of mould in all the products in wet season. A total of five fungal genera namely:

Aspergillus,*Penicillium, Rhizopus,,Fusarium* and *Botrytis* were isolated*Aspergillus* was the most predominant following the orders: *A. niger*>*A. tamari*>*A. flavus*. Theaflatoxin production ability of the*Aspergillus* species indicated that, only*Aspergillusflavus*showed the ability to produce aflatoxin. Higher aflatoxin contamination (100%) and (82.70%) of the maize and maize products had total aflatoxin levels above 4ppb and 20ppb recommended by Standard Organization of Nigeria (SON) and Food and Drug Administration of United States as tolerance levels. Significant differences existed between the mean aflatoxin contaminations of old maize, fresh maize, maize flour and maize bran.Emphasis on proper harvesting, drying, handling, transportation, storage and education of the populace especially those dealing with these foods should be taken into consideration by relevant authorities. In conclusion, the high level of aflatoxin in maize and maize products may highlight the problem associated with the consumption of these products with the toxin that could lead to adverse health effects such as liver, cervical, breast or oesophageal cancer.

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CHAPTER 1

1.0 INTRODUCTION

Maize (*Zea mays, L.*) is a cereal crop grown throughout the world. It is native to America and widely grown in Nigeria. Maize plays an important role in the diet of millions of African peopledue to its high yields per hectare.Between 1992 and 1996, total area of maize production in Nigeria was more than doubled from 1.8 - 4.0 million/ha (Manyong*et al.*, 1996). The total maize production in 2004 was 5.5 million tons (FAO, 2004).Its ease of cultivation and adaptability to different agro-ecological zone, versatile food uses and storage characteristics make it the most widely grown cereal (Manyong, *et al., 1996).* On the field, as well as in store, many pests and parasites attack maize and during the storage period insects are most often considered as the principal cause of grain losses(Ominski, 1994) however fungi are also important and rank second as the cause of deterioration and loss of maize (Ominski, 1994). Due to worldwide distribution and relative lower price compared to other cereals, maize has a wide range of uses than any other cereals. It can be used for human consumption, animal feed and for many industrial uses(Serma-Sadiver*et al.,* 1990). In many developing countries such as Nigeria, maize is a major staple food and consumption per capital is very high.It can be processed into different products at traditional level as well as on industrial scale. Although most products derived from maize in developing countries are prepared using traditional methods of processing, bulk of industrial processing occurs in developed countries(Serma-Sadiver *et al.,* 1990).

In Nigeria, maize is known and called by different vernacular names depending on the locality, like *agbado, Igbado orYangan*(Yoruba), *Masara, ordawar Masara* (Hausa) *Ogbdo*

or Oka (Igbo) and *apa'apa*(Ebira)*Oka* (Bini and Isha)and*Ibokpot* (Efik (Abdulrahaman and Kolawole,2006).Maize and processed maize products such as maize flour, corn meal and corn flakes are subject to fungal attack because they are ready substrate for these agents. Maize contamination by fungi not only renders the grains unfit for human consumption by discoloration and reduction of nutritional value, but can lead to production of harmful substances called mycotoxin (COT, 1997).

Mycotoxins are natural secondary metabolites produced by fungi on agricultural commodities in field and during storage, under a wide range of climatic conditions and are of significance in food safety (Mady, 2001).Most agricultural commodities are susceptible to fungal invasion before, during and after harvesting, owing to improper handling during harvest, transportation, drying and/or storage. In countries with food legislation, mouldy foods are considered illegal and therefore unacceptable in commerce. It therefore behooves on the producers and other stake holders to avoid mould contamination of their produce as this might constitute huge losses and health hazard (Mady, 2001).Mycotoxins occur with varying severity in agricultural products all around the world (Desjardin, 2006). The estimate usually given is that one quarter of world animal feedstuffs (grains and oil pulses) is contaminated to some extent with mycotoxins (Okoli *et al*.,2006).

The Food and Agriculture Organization of the United Nations (1991) estimated that mycotoxins contaminate 25% of agricultural crops world wide.Only a few mycotoxins particularly those affecting cereals are considered to be significant to humans (FAO, 1991). Similarly, Bouraima *et al.* (1993) found aflatoxin B_1 level up to 14µg/kg and aflatoxin G1 level up to58 µg/kg in stored maize from Benin. Setamou *et al*., (1998) found that the percentage of samples contaminated with aflatoxin was 42.5% in 1994 and 30% in 1995 in

pre-harvest maize from Benin. Udoh *et al.*(2000) reported that 33% of maize samples from different ecological zones of Nigeria were contaminated with aflatoxin. Hell *et al.*(2000) reported that 9.9 - 32.2% of maize samples from different ecological zones in Benin prepared for storage had aflatoxin levels more than 5ppb and the level increased to 15 and 32.2% after six month of storage.

The food borne mycotoxins that are likely to be of great significance to human health in tropical developing countries are the fumonisins and aflatoxins (COT, 1997).Among these mycotoxins, aflatoxin has gained considerable attention because it is a more toxic and potent carcinogen even in small quantities (FAO, 1979), and it is also the mycotoxin of public health importance within the WestAfrican region (WHO, 1999).

Ingestion of high doses of aflatoxin can result in acute aflatoxicosis which manifest in hepatotoxicity (Fung and Clark, 2004). Aflatoxin contaminations of food and feed have gained global significance due to its detrimental effects on human and animal health and its importance in international trade (WHO, 1999). Crops may be contaminated by one or more of the following sub types of aflatoxin B_1, B_2, G_1 and G_2, of which aflatoxin B_1 is the most toxic and frequently detected type. The other types of aflatoxins present a significant danger, if the concentration is at high level. Aflatoxins have been implicated in human health disorder including hepatocellular carcinoma, Rey syndrome and chronic hepatitis. Animals are exposed to aflatoxin by consuming feed that are contaminated by aflatoxin producing fungal strains during growth, harvest or storage ((Fung and Clark, 2004).

The Food and Drug Administration of United States [FDA] had therefore established guidelines for acceptable aflatoxin levels in agricultural commodities to protect human and

animal health. The FDA action level of aflatoxin in agricultural products is based on the opinion made in the late 1960s that an unavoidable potential human carcinogen in important food stuffs should be controlled at the lowest practical level.With regards to products for human consumption, the current action level is 20ppb total aflatoxin in a finished product while that of milk and milk products is 0.5ppb aflatoxin M_1. When these levels are exceeded, commodities are restricted to lower value uses usually animal feeds, oil products, and alcohol production (Park and Potland, 1986).More recently, the FDA has used occurrence, metabolism and animal toxicity information to set guideline for aflatoxins in animal feedstuff. Pre-harvest aflatoxin contamination is favoured by high temperature; prolong drought conditions and high insect activity while post harvest aflatoxin production on corn is favoured by warm temperature and high humidity ((Park and Potland, 1986).

Aflatoxin in maize can be prevented by a number of ways, among which are mechanical drying, chemical treatment, planting time and field drying.In mechanical drying, a bath type system has been tested and the method found practicable; however the drying process is slow and so, batches of maize should be placed in the dryer for 48 h. In chemical treatment, only ammonia and a mixture of ammonium polypropionate and propionic acid at ratio 8:2 were economically effective in preventing aflatoxin producing fungal contamination. No government policy for controlling mycotoxin has been enacted and little attention has been paid to possible contamination by mycotoxins such as aflatoxin in Nigeria maize varieties.

1.1 STATEMENT OF THE PROBLEM.

Cereal such as maize, sorghum, millet and their products are easily disposed to mycotoxin contamination (Chelkowsh, 1988). Maize is a staple food of many countries including Nigeria and it is used for a variety of food products that are widely consumed. Maize is probably the commodity of greatest world wide concern, because it is grown in climates that are likely to have perennial contamination with moulds. Maize is more sensitive particularly because ripeness occurs under favourable condition for A*spergillus,* F*usarium* and P*enicillum* species which might produce toxin under high humidity at harvest (Reid *et al.,* 1990).

Contaminated maize with toxin has been proven to be immunotoxic and class one human carcinogen (IARC, 1993 and Turnel *et al.,* 2003). Mycotoxin induced disease lead to a reduced life expectancy in developing countries (Miller, 1996) and they have been linked with higher prevalence of hepatocellular cancer in Africa (Strosnider *et al.,* 2006). A recent epidemiological study reveals a striking association between exposures to mycotoxin and growth stunting (Gong *et al.,* 2004). Aflatoxin contamination has been linked to immune suppression by Turner *et al.* (2005) and micronutrient deficiencies in animal (William *et al*., 2004).

In poultry and livestock production, aflatoxin can cause feed refusal, loss of weight, reduced egg production and contamination of milk. The safety of food and feed for human and animal consumption should be top most priority with regards to the regulation of agricultural and food industries. According to Miller (1996), 40% of the productivity loss to diseases in developing countries is due to diseases exacerbated by aflatoxin.

1.2 JUSTIFICATION OF THESTUDY

Mycotoxins have attracted world wide attention due to the significant losses associated with their impact on health and consequent national economy (Bhat and Vashanti, 1999).The quality and safety of food are of importance, therefore markets should not be compromised for the sale of low quality or unsafe foods. The nutritional and economic importance of maize when contaminated with these toxins and consumed can be disastrous especially its cumulative effect like being immunotoxic, carcinogenic and growth stunting thus supporting the need to closely monitor aflatoxin level in food products that are widely consumed in a region particularly those that are highly prone to moulds and mycotoxins contamination such as maize. Evaluation of public health significance of mould contamination of maize and maize products will go a long way in eradicating likely cases of aflatoxicosis

1.3 AIM

The aim of this work is to assess the public health significance of mould contamination of maize and maize products.

Specific objectives

1To determine seasonal weather conditions of the study area.

2 To determine the physical quality of maize.

3 To determine the physico-chemical composition of maize, and maize products fromvarious markets at harvest and during storage.

4 To enumerate and characterize moulds isolated from maize and maize products.

5To test the ability of the mould isolates to produce aflatoxin.

6 To determine and quantify total aflatoxin levels in maize and maize products.

CHAPTER 2

2.0 REVIEW OFLITERATURE

Maize *(Zeamays L.*) is an important cereal world wide, serving as feed for growers, food for man and live stock as well as an industrial raw material (FAO, 1981; Meronuck, 1987). Unfortunately, it is also a suitable substrate for growth, development and activity of fungi(Lacey,1990).

In Nigeria, the most important cereals are sorghum, millet,rice, maize and wheat (Wudiri, 1999), of all these cereals, maize remains the most popularly grown and consumed in all ecological zones of the country. Maize as a food grain plays a significant role through out the world particularly Nigeria, Pakistan, and India. Its flour is used in preparation of nutrition and tasty meals such as snacks, cakes, pizza, bread; paste cookies, soup and as a source of dextrose. Its bran has been found very effective in decreasingfaecal transit times (Bressani and Elias,1983). In order to make maize suitable for food and Industrial product, milling is necessary.In making various food items dry milling is normally adopted whereas for industrial product like starch,corn oil and animal feeds wet milling isdesirable (Burge and Dunsing, 1989).However to make it to human consumption, it must be cooked.During cooking heat gelatinizes starch making it susceptible to starch dissociating enzyme of the digestivesystem.Fungal growth is a major problem in cereal grains throughout the world and may lead to poor quality of the product as well an adverse effect on human and animal health due to production of toxic metabolite called mycoyoxin.Microscopicfilamentous fungi can develop on a wide variety of food commodities and under suitable environmental conditions, can lead to production of highly toxic chemical substance commonly known as mycotoxins. Mycotoxinsare secondary metabolites produced byfilamentous micro fungithat

are important entities in diseases of animals and humans (CAST, 2003).They are a relatively large, diverse group of naturally occurring compound and they may have being implicated as a chemical agent of toxic disease syndromes including liver tumour and kidney tumours in humans.For centuries, these mycotoxins have alsobeen associated with quality degradation of many agricultural products which causes consideration changes in texture, flavor and colour. Toxigenic moulds are known to produce one or more these of these toxic secondary metabolites.

It is well established that not all mould are toxigenic and not all secondary metabolites from moulds are toxic. The genera of mycotoxigenic fungi are mainly represented by*Aspergillus, Pencillium*and*Fusarium*(Orsi,*et al*., 2000). Altenaria among others are alsoimportant as food contaminant or pathogen of plants.Alternaria produces mycotoxin in grain such as rice, and maize (Ramn*et al.*, 1994). Many *Aspergillus,Penicillum,* and Cl*adosporium*species are also known to produce mycotoxins.Exposure to mycotoxins occur predominantly by ingestion of contaminated food. The major mycotoxin produced by these fungal species that pose the most serious toxicological risk to both animals and humans are the aflatoxin (AF) ,ochratoxin (OTA), trichothecenes , zearalenone (ZEA), fumonisins(F)and patulin (Kumar *et al.*,2008).

These toxin accounts for million of dollars annually in losses worldwide in term of human health, animal health, and condemned agricultural products (Vasanthi andBhat, 1998). Among these mycotoxins, aflatoxin has gained considerable attention because it is more toxic andpotent carcinogen even in small quantities.Aflatoxin has deleterious effects on human beings, poultry and livestock. This made it the only toxin that has gained

prominence in scientific literature in food product in West Africa sub region(Campos, 1987).

2.1 Mycotoxins

2.1.1 Toxigenic fungi

Generally, mycotoxins are produced in the mycelia of filamentous fungi, but canaccumulate in specialized structures of fungi such as phialides, conidia or sclerotia as well as in the environment surrounding the organism (Bhatnager, *et al.,* 2002). The most important fungal genera occurring in nature in term of toxic production are *Aspergillus, Pencillium* and *Fusarium* species (Blaha *et al.,* 1990; Thompson *et al.,* 1993; Piva and Fabio, 1999).

2.1.2 *Aspergillus* species

The genus *Aspergillus* includes over 185 species.Twenty species have been reported as casual agents of opportunistic infections in man. Among these, *Aspergillus fumigatus,* is the most commonly isolated species while *A. flavus,A .niger,A.clavatus, A.glaucus* group, *A .nidulans, A. oryzae, A.tamari , A. terreus , A. Ustus,* and *A. versicolor* are among the other species less commonly isolated as opportunistic pathogen (Paterson *et al*.,2004)).

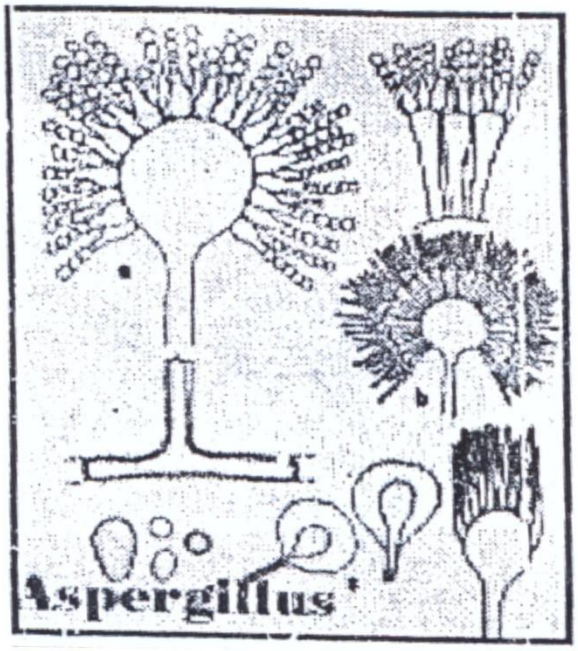


Fig.2.1: Structure of *Aspergillus* species. (Paterson *et al*., 2004)

Members of the genus *Aspergillus* have been of interest for centuries because of their positive impact as fermentation agents and their negative impact asdegraders of agricultural products as well as their toxicity andpathogenicity (Klich, 2002).Recent scientific interest in biogeography and biodiversity, as well as the increasing importance of Aspergilli as causal agents of mammalian toxicity and disease has led to an increased need to understand where these fungi occur in nature (Klich, 2002).

The severity of mycotoxin problem was realized during World War II when Russianswho ate mouldy over wintered grains suffered with severe dermal necrosis, leucopenia, hemorrhages and destruction of bone marrow. *Fusarium* was found to be the causative organism the outbreak (Forgac, 1972).The birth of mycotoxicology came about with the discovery of aflatoxins in the early 1960s (Sergeant *et al*., 1961) when they were found as

contaminants of Brazilian peanut meals. There has been long standing concern about the safety of peanuts as food and feed. The aflatoxins are produced as a result of invasion and growth of *Aspergillus flavus* and *A. parasiticus* and contamination usually occur during various stages of production, harvest, handling and storage (Diener *et al*., 1987).Mycotoxins that pose human health risks include fumonisins, ochratoxins deoxynivalenol (DON), zearalenoneand aflatoxin (IARC, 1993)

2.1.3 Fumonisns

The most important field fungi in Africa and worldwide are *Fusarium* spp. and they are known to produce over 100 secondary metabolites that can adversely affects human and animal health (Visconti 2001). Fumonisns produced by *Fusarium verticilloides* and *F.proliferatum* are one family of mycotoxins which contaminate feeds and foodstuff predominantly maize based products throughout the world (Turner *et al*., 1999). Many studies in Nigeria have found this fungus to be the most frequent in pre-harvest and stored maize (Essien 2000; Kpodo and Bankole, 2005).Fumonisns are recently receiving increasing attention in scientific literature because they have been implicated in a number of animal diseases such as Lenco enphalomalacia in equines, procure pulmonary odema, rat liver cancer, and hemorrhage in the brain of rabbits (Marasas 1995).

There is no conclusive evidence of the human health hazard associated with fumonisin contaminated food, though human health risks with fumonisn are possible (FDA, 2001).However some correlation studies have suggested a link between the consumption of maize with high incidence of *F. vertificllioides* and fumonisn and the high incidence of human oesophageal carcinoma in certain parts of South Africa and China (Yoshizawa *et al;*

1994, IPCS, 2000). Fumonisn has been demonstrated to induce apotosis in cultured human cells in rat kidneys (IARC, 1993). The United State FDA has proposed a guideline of tolerance level of 2mg/kg total fumonisns in the corn for human consumption (FDA, 2001).

2.1.4 Ochratoxin A (OTA)

The ochratoxin (ochratoxin A and ochratoxin B) are produced by *Aspergillus ochraceus, Penicillium verrucosum* and *Aspergillus carbonarium* (Frisvad and Thrane, 2000). In warm climate, such as those found in West and Central Africa, ochratoxin production is more commonly associated with *A. ochraceus* than it is with *P. verrucosum* which often produces ochratoxin A in temperate climate (Sweeney and Dabson, 1998). Ocharatoxin A is found as natural contaminant in many foodstuff including, cereal, driedfruits, cocoa, wine, poultry, egg, and milk (Sweeney and Dabson, 1998). OTA is immunosuppressive, tetratogenic, genotic and mutagenic and IARC has classified it in group 2B as possibly carcinogenic in human (IARC, 1993). Limited data exist in the occurrence of ochratoxin A in food in Africa (IARC, 1993).In Ghana, studies on fermented maize dough found ochratoxin A in 5/20 samples at levels<6.4µg/g. In Nigeria, ochratoxin A was detected in mouldy maize sample from Plateau State and the level recorded was 150µg/g (Gbodi *et al*., 1986). A total of 4outof 48 maize based gruel samples used as weaning food for children admitted to the Wesley Guild Hospital in Ilesha, Nigeria tested were to be contaminated with ochratoxin A at low level (Oyelami *et al.,* 1996). The joint expert committee on food additives of the WHO and FAO set a provisional maximum intake of 100µg/kg body weight, while scientific committee on food of the European Union proposed that maximum daily intake of OTA should not exceed 5µg/kg body weight (WHO, 1996).

2.1.5 Deoxynivalenol

Deoxynivalenol is a trichothecene mycotoxin associated primarily with *Fusarium graminearum* and *Fusarium culmorum. Fusarium graminearum* is the more common species, and may occur in warmer climate. Deoxynivalenol has been found in grains such as maize, wheat, barley, oats, rye, but it has not receive much attention in Africa (Ngoko *et al;* 2001).

The extent of infection depends on weather conditions, good agricultural practices and storage conditions of the cereal crops. Consumption of grain contaminated with deoxynivalenol has been associated with outbreak of acute diseases involving nausea, vomiting, gastrointestinal upsets, dizziness, diarrhea and headache in Asia (WHO, 2002).Only one report of deoxynivalenol in food is available from these African regions. In Cameroon deoxynivalenol was present in 12/15 maize sample at levels ranging from 100-1300µg/g (Ngoko *et al;* 2001).

2.1.6 Zearalenone

Zearalenone is produced mainly by *F. garminaerum* and *F. culmorum* and may occur in most cereals including, maize, barley, oats, and rye. Zearalenone is well known for its oeastrogenic properties in various animal species that include infertility, vulval edema and mammary hypertrophy in female (Peraica*et al;* 1999). Little information is available regarding the effect of zearalenone in humans. Zeralenone has been reported in maize and maize product from several African countries including Botswana, Egypt, Swaziland, South Africa and Zambia(Gbodi *et al*., 1986).From the West and Central African sub-region, arereport from Nigeria and Cameroun.In Nigeria, the toxin was detected in mouldy maize

samples at level of 960ng/g (Gbodi *et al*., 1986). In a study of maize beer in Nigeria, 28/46 samples were contaminated with zearalonene at level ranging from 13-200ng/g with mean of the positive samples being 82ng/g (Okoye, 1986).

2.1.7 Aflatoxins

Aflatoxins are toxic secondary metabolites that can be produced by some fungal species such as *Aspergillus flavus*, *A. parasiticus*and *A. nominus*. *A. flavus*however is the most common producer (Brandbum *etal*., 1993). Aflatoxin is the most popular and widespread mycotoxin. Adam(1978) has reported that storage fungi especially *Aspergillus, Penicillium,Rhizopus* and *Mucor* species infect grains after harvest and can grow on them during storage. These fungi occur principally in the soil and decayingvegetation.The four major aflatoxins are $B_{1,}$ $B_{2,}$ G_1andG_2. Aflatoxins M1and M_2arehydroxlylated metaboliteof aflatoxin$B_1$and B2respectively in animals.Aflatoxin B_1 (AFB_1) is a known carcinogen and is the most potent and potentially lethal metabolite. The toxigenic strains of *A .flavus* typically produceonly two aflatoxin B1 and B2 but most strains of *A .parasiticus* could produce all the four toxins (Dorner, 2004). Agricultural scientists have been studying aflatoxin for more than forty (40)years due to wide spread occurrences of contaminants and their significant effects on crops(Wildand Turner, 2002;Shepard, 2003; Fung and Clark 2004 and William *et al*. 2004).

Aflatoxins can affect a wild range of commodities including cereals, oilseeds, spices, tree nuts, milk, meat and dried fruit.Maize and Groundnut are the major sources of human exposure to aflatoxin due to their greater susceptibility to contamination and frequent consumption throughout the world. The primary target organ for toxicity and

carcinogenicityis liver in human and animals, and the toxicity is known to be mediatedby metabolism in the liver by cytocrome P 450 system to thehighly reactive AFB, -8 -9 – epoxide (Peraica*et al*.,1999; William *et al*., 2004).Aflatoxins B2and G_2 were established as dihydroxy derivatives of B_1 and G_1 respectively where Aflatoxin in M_1 is 4 hydroxyl – aflatoxin B_1 and aflatoxin M_2 is dihydroxy aflatoxin B_2 .The chemical structure of aflatoxins are shown below

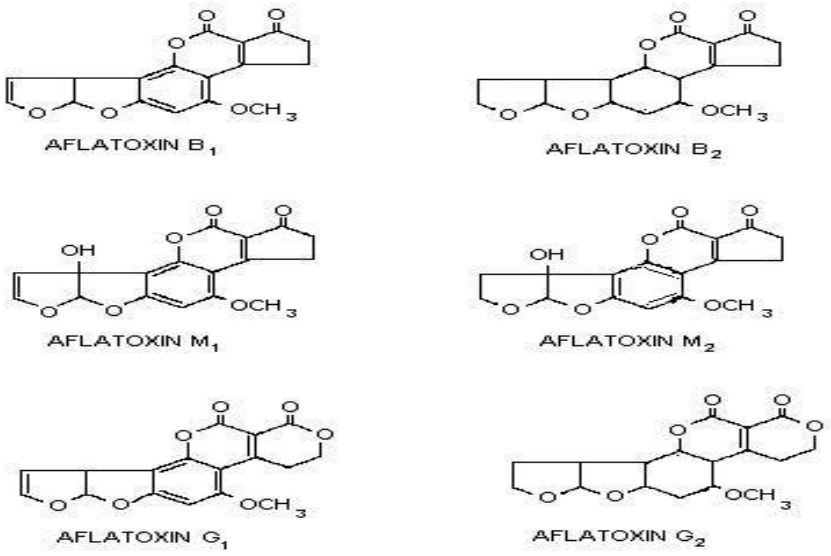


Figure 2.2 Chemical structures of aflatoxin.(Sweeney and Dobson,1998)

The maior aflatoxins have closely similar structures and form a unique group of highly oxygenated naturally occurring heterocyclic compounds. Their molecular formulas as established from elementary analyses and mass spectrometric determinations are:-

B_1: C_{17} $H_{12}O_6$ B_2: C_{17} H_{14} O_6 G_1: C_{17} H_{14} O_7

$G_{2:}$ C_{17} H_{14} O_7 M_1: C_{17} H_{12} O_7 M_1: C_{17} H_{14} O_7

Source: Cole and Cox (1981)

2.1.8 Properties of aflatoxins

Hell, (1997) stated that four major groups of aflatoxins are identified: B1, B2, G1 and G2 as shown in the Table2.1 below. These abbreviations are indicative of the colour this exhibit/fluorescence under the ultraviolet light (385 nm): thus B is for blue and G is for yellow-green. The M is a hydroxylated metabolic product of B (Bankole and Adebanjo, 2003).

2.1.9. Natural occurrences of aflatoxin

Aflatoxins occur as a natural contaminants of human and animal foods as a result of fungal contamination of food crops during pre-and post harvest (WHO, 1998). They have been occasionally detected in milk, cottonseed, sorghum, wheat, rice, beans, soybeans ,maize and a variety of other foods and feeds (Lamauaka *et al*, 2007; Reddy and Waliyar, 2009,).Agricultural commodities which have the highest risk of aflatoxin contamination are maize, peanuts (groundnuts), and cotton seed.Agricultural commodities grown in tropical and subtropical countries are moreprone to aflatoxin contamination. The exportation of such commodities to other countries spreads the contamination and makes aflatoxin a worldwide problem (Watson, 1998).

Table 2.1. Properties of aflatoxin

Properties	Aflatoxin				
	B1	B2	G1	G2	M1
Chemical formular	C_{17} H_{12} O_8	C_{17} H_{14} O_8	C_{17} H_{12} O_8	C_{17} H_{14} O_7	C_{17} H_{12} O_7
Molecular Weight	312	314	328	330	328
Melting point (^{0}C)	268-269 (D) [1]	287-289 (D)	244-249 (D)	230	299 (D)
Sorbent + Pentane	Chloroform	Chloroform	Chloroform	Ethyl Acetate	Methanol
Fluorescence (nm)	425	435 450	425	425	

D[1] = Decomposition,
Source: Cole and Cox, (1981).

2.1.10Consequences of exposure to aflatoxin

There are a range of possible consequences of exposure to aflatoxins, largelydetermined by the dose, the duration of exposure and the animal involved. In all cases, the young ones are more susceptible than the adult and nutritional status can be an important factor. The common consequences include acute illness and death, cancer, immune system suppression, and nutritional disorders (Abbas, 2005).

2.1.11 Other **mycotoxins in foods in West Africa countries.**

In addition to the five groups of mycotoxin already discussed, other mycotoxins have been detected in some foods in some countries in West Africa.These mycotoxins includes sterigmatocystin, patulin, cyclopiazonic acid, penicillic acid and tenuazonic acid from dried cassava chip in Ghana (Waering *et al.*, 2001).

2.2Fungal contamination of Maize

Most fungal contaminants in maize arise from infestations that began in the field, although some can directly infest storage grain (maize) as well as when conditions are favourable for fungal proliferation (Vieira, 2003). Aflatoxin contamination in several food stuffs in Africa has been a recurrent problem(Shephard, 2003).In many parts, maize has become the preferred cereal for food, feedand industrial use displacing traditional cereals such as sorghum and millets.However it was significantly more heavily colonized by aflatoxin – producig *Aspergillus* speciesthan either sorghum or millet (Bandyopadhay *et al*., 2007).

2.3 Maize contamination in field

The field contamination of maize is influenced by a number of factors which cannot be controlled by any producer, such factors includes temperature, evaporation, humidity, transpiration and water availability to the crop and the fungus (Widstorm, 1992). Aflatoxin contamination of maize is also promoted by stress damage to the crop due to drought before harvest, insect activity, poor timing of harvest, heavy rains at harvest and inadequate drying of the crops before storage (Hawkins *et al.*,2005).

Aspergillus species are ubiquitous in the soil, and they have disease cycle with possible infections pathways (Munkvold & Desjardins, 1997). These pathways includes infection from the seed to cob and to grain through systemic movement in stalk, infection from root to grain through stalk and cob, infection from airborne or water splashed conidia to silk and to grain and infection through wounds caused by insects that can also act as vectors of inoculums. Nelson and Desjardins(1992) reported that spoilage contributed to higher fungal contamination. The penetration of mites and insect present on the surface of the grains provides better conditions for fungal growth. Dowd(2001) noted cases where reducing insect damage also reduced aflatoxin level in maize.

2.4 Maize contamination during storage

Contamination of maize during storage is influenced by manyfactors such as specific conditions of temperature, relative humidity and moisture content of the maize grains which arise during storage may contribute to the rapid deterioration of the cereal by promoting fungal growth. Fancelli (1983) viewed maizeasan important grain for the Mexican population and other developing countries like Nigeria. In these countries, maize

is produced primarily in small farm, the farmer retain some part of the harvest for their own consumption, eventually selling the surplus to the urban population. In tropical areas where drying and storage technologies are inadequate or completely lacking stored grains are deteriorated by insects and fungi causing severe quantitative and qualitative losses which eventually has effects on food availability especially where grain storage is poorly managed owing to lack of proper knowledge andtechnology (Harein and Davis, 1992). An example of which is pooly managed can be seen in Fig.2.2.



Figure 2.2 Fungal deterioration of maize dried on bare ground

2.5Proximate compositions of maize

Maize is a multipurpose crop providing food and fuel for human being, feed for animals, poultry and livestock.Its grains have great nutritional values and are used as raw materials for manufacturing of many industrial products (Afzal *et al.*, 2009). Food composition data is important in nutritional planning and provide data for epidemiological studies (Ali *et al.*, 2008).

The major chemical composition of maize grain is carbohydrate which account for 72 – 73% of the grain (Wilson, 1987). Maize protein which ranges from 6 – 12% is regarded to be inferior because, around half of its protein ismade up of zein, which is low in two essential amino acids, lysine and tryptophan. Fortunately this deficiency nowadays has been corrected with the development of the quality protein maize which is nutritionally the most superior cereal grain.

Other composition include moisture (12 – 13 %), fat (3.8 – 4.5 %), fibre (1.9 – 3.0%) ash (1.1-1.3%) Calcium (mg per g) 6 -17%, Iron (mg per 100g8 – 4.2) and calorie per 100 g (353 – 356 (Wilson, 1987)

2.6Acceptable Aflatoxin levels in maize

The safety of food and feed for human and animal consumption should be of top priority with regards to the regulation of agricultural and food industries. In today's changing world, safety and security have generally remained basic human need, ensuring the safety of food has been a major focus of international and national action over the years. In sub-Saharan-Africa countries 70% of the populations are involved in farming and food commodities as the major items of international trade for many WestAfrican

countries(Conway and Joenniessen, 2003). The quality and safety of food is important so that market is not compromised by sale of low quality and unsafe food. Both microbiological and chemical hazards are of concern, among chemical hazards, the contamination of food and feed by mycotoxins (toxic metabolites of fungi), Fishery product by phycotoxin (toxin produced by algae) and edible plant species by their plant toxins have been recently characterized by the world health organization as a significant source of food borne illness (WHO,2002). Of these three categories of natural toxin's most attention has been directed to mycotoxins. In most part of the world, mycotoxin currently represents a major food safety issue.

Internationally, mycotoxin legislation in foodstuffs and animal feed stuffs has been carried out and the detail about its tolerances, legal basis and method for analysis and sampling are available. FAO, (1997) update on world wide regulation for mycotoxin showed that 77 countries have a specific regulation for mycotoxin, 13 countries are known to have no specific regulations, whereas no data are available for about 50 countries, 40 of these countries are in Africa where only fifteen countries have specific mycotoxin regulation. Regulatory limits or standard provide a bench mark against which effectiveness of food safety programme can be tested. Adverse health effects from the ingestion of these toxins have caused regulatory agencies throughout the world to limit the amount of aflatoxins that are permitted in food or feed that is available for sale.

The fact that some countries do not have specific regulation does not mean that the problem is ignored.Most of these countries recognize that they have problem due to mycotoxin and that regulation is necessary. Mycotoxin regulation will have limited effect in term of health protection in those countries where many farmers grow agricultural produce for their own

consumption (subsistence farming), which is the case in many African countries.Most of the existing mycotoxin regulation in Africa concerns the aflatoxin in food. All countries with mycotoxin regulations have regulation levels for AFB1 or the sum of the aflatoxin B1, B2, G1and G2 in food and feed. Only 8 African countries are known to have regulation for aflatoxin (FA0, 1997, De Vries*et al*., 2002). The concern about potential hazards posed by dietary aflatoxin started in the 1960, when more than1 00,000 young turkeys on poultry farm in England died in the course of a few months from an apparently new disease that were termed as turkey X diseases. It was soon found that the difficulty was not limited to turkeys, ducklings and young pheasants were also affected and heavy mortality was experienced. A careful survey of the early out breaks showed that they were all associated with feed namely Brazilian pea nut meat. An intensive investigation of the suspect peanut meal was undertaken and it was quickly found that the peanut meal was highly toxic to poultry and duckling with symptom typical of Turkey X disease. Speculations made during 1960 regarding the nature of the toxin suggested that it might be of fungal origin, the toxin producing fungus was identified as*Aspergillus flavus* (1961) and the toxin was given the name Aflatoxin by virtue of its origin (*A. Flavis* – Afla).

When it became evident that aflatoxin exposure caused cancer in many species, many countries including United State of America,Canada established various regulation for aflatoxin level (either total aflatoxin or for aflatoxin B1) in food and /or feed in order to limit exposure to these group of mycotoxin (Van Egmond, 1989).The initial regulations on aflatoxin were not based on the derivation of a TDIC (Tolerable daily intake), but rather on a desire to keep thelevel as low as technologically feasible (basis forregulation in some

countries), free of aflatoxin by allowing residue above the analytical detection limit (based for regulation in some other countries).

The early prudent actions regarding aflatoxin by government have been justified since aflatoxin B (AFB) have been found to be a potent genotoxic agent and carcinogen in many test systems and animal species. In rat, development of hepto cellular carcinoma proceeded by formation of liver foci and hyper plastic nodules (Wogan *et al,* 1974).

The complete elimination of aflatoxin is not possible since it is a common contaminant in many agricultural produces. The United State Food and Drug Administration (FDA) considers aflatoxin asan unavoidable food contaminant, however recommended that the amount in foods should be reduced to the lowest possible level (FAO, 2004).

In order to minimize aflatoxin contamination in food and feed, FDA established action level which specify maximum acceptable level at or above which FDA will take legal action to eliminate the producer from the market. In 1965, the Food and Drug Administration (FDA) set the first mycotoxin limit.FDA action level of 20ppb for foods including grain, rice and process fed product, 0.5ppb for aflatoxin M_1, in milk and 20ppb for peanut, peanut products and Brazilian nut. The European Union set the level of maximum aflatoxin in agricultural products at 4ppb, with AFB_1, at 2ppb (Devegowda and Castaldo, 2000).The Standards Organization of Nigeria (SON) sets standards on many food commodities, taking into account global standards as well as national production and target export markets. While it is generally recognized globally that there is no safe level of aflatoxin exposure, SON has set the maximum acceptable limit for maize grain at 4 ppb for total aflatoxins and 2 ppb for B1(SON,2003) .

The Centre for Veterinary Medicine of the FDA has being responsible for examining feed for mycotoxin. The FDA action level for aflatoxin in animal feed is 20ppb for corn and peanut product of the animal feed and feed ingredient but excluding cottonseed meal intended for immature animals, 100ppb for corn and peanut product intended for breeding beef cattle, breeding swine or mature poultry. A 200ppb for corn and peanut product intended for finishing swine of 100 pounds or greater and 300ppb for corn and peanut product intended for finishing (i.e. feed lot) , beef, cattle, and cotton seed meal intended for beef, cattle, swine or poultry (regardless of age or breeding status) FAO,2004).

2.7Aflatoxicosis

Aflatoxicosis is a disease that results from ingestion, inhalation and absorption through the skin of aflatoxin in contaminated food or feed (Wagacha and Muthomi, 2008). The primary target organ is liver and liver damage occur in many species, including birds, fish, rodent and non human primates and human (FDA, 2007). The susceptibility to aflatoxins varies significantly between different species and members of the same species and dependent on the following factors: age, sex, weight, and nutritional status of diet, environmental factors, aflatoxinexposure (dose and duration).Aflatoxicosis in animals (and possibly on humans) is categorized in two general forms namely acute and chronic. Acute aflatoxicosis is caused from the consumption of moderate to high level of aflatoxin.Symptomsinclude hemorrhage, edema, liver damage, alteration in digestion and death.While chronic aflatoxicosis is caused from consumption of low to moderate level of aflatoxins. It usually results in subclinical effects, which are difficult to recognize. Symptoms include impaired food conversion and slow rate of growth with or without production of any clear symptoms.

2.8 Effect of aflatoxicosis in human health and animal health

Humanbeing are exposed to aflatoxin mainly by eating contaminated food. The toxic effect of aflatoxin is determined by the dose and the duration. Most often, large dose or long term expose to low levels in the diet has effect. A symptom of acute aflatoxicosis includes vomiting, abdominal pain, convulsion, coma and death.

In developing world, like Nigeria, Benin, Gambia and Togo, children are malnourished as well as exposed to high level of aflatoxin from the consumption of contaminated stable foods such as maize and groundnut. Children exposed to high level of high aflatoxin may become stunted (an example of chronic malnutrion) and under weight (an indicator of acute malnutrition) susceptible to infectious diseases such as malaria, diarrhoea and respiratory infections in childhood and later in life (Bhat and Vasanthi, 2003).

In animal,aflatoxin causes a variety of symptoms depending on animal species however, in all animal aflatoxin can cause liver damage, reduced reproductive performances, reduced milk or egg production, embryonic death ,teratogenicity (birth defect), tumour and suppressed immune system function even when low levels are consumed (Van-Egmond,1989). Other effects of aflatoxin may be nutritional,which include decreased protein synthesis, altered vitamin and mineral metabolism, reduced feed utilization and efficiency, poor growth rate, reduced weight, anaemia and jaundice; whereas the immunological effects may include induced thymic aplasia, reduced T-lymphocyte function

and number, impaired macrophage function, recurrent infection,and decreased response to vaccinations.

2.9 Food borne out break (aflatoxicosis) in developing countries

Aflatoxin outbreaks are uncommon in developed countries where foods are effectively and strictly regulated. Regulations monitor of aflatoxin level in food protect human population from acute aflatoxin exposure. In developing countries, aflatoxin exposure is more common because regulation are either non existent or not stringent.Foodstuffs in these countries are usually produced, stored, prepared and consumed by the local families without any consideration for the risk of aflatoxin exposure.

In developed countries like US, the relative frequency of aflatoxicosis on human is unknown and no out break has been reported in human. In developing countries, very little information is available on aflatoxicosis outbreak because many cases go unnoticed due to inadequate medical services (FAO 2007), and most of the aflatoxin poisoning outbreak occurred in remote villages and thus the number of people affected could have being more than reported (Lewis*et al*., 2005).An outbreak of acute aflatoxin poisoning is a recurrent public health problem.

In 1960, the first outbreak of unknown diseases in England called Turkey X Disease turkeyin which over100,000 turkey poults were died was the first evidence of aflatoxin poisoning, speculation made in 1960 regarding the nature of toxin suggested that it might be of fungal origin and the producing fungus was identified *Aspergillus flavus* and the toxin called aflatoxin.

In 1967, an outbreak of aflatoxicosis occurred in Taiwan following the consumption of contaminated rice, the outbreak results in 26 cases including three child deaths. Sample of rice were found to becontaminated to a level of 200 ppb of the toxin (Angsubhakorn, 1989).

In 1974, an outbreak of hepatitis due to aflatoxicosis was reported in 200 villages in Western India (Banswada, and Panchamahass district of Rajasthan and Gujarat respectively), victims suffered rapidly progressive jaundice, high fever, emesis,pain, and edema. Hepatomegaly was evidenced on examination. This resulted in 397 cases and 106 deaths. The outbreak lasted for 2 months and was confined to tribal population whose stable food was maize, the aflatoxin level in contaminated maize was 6.25 - 15.6 parts per million (ppm). Maize samples showed that the affected people might have consumed between 2000 – 6000mg kg^{-1} (ppb) aflatoxin daily for over a period of one month (CDC, 2004).

In1981, similaroutbreak in Kenya was reported, it was suspected that an unusually high number of patient with acute hepatic failure might have acquired aflatoxicosis from eating contaminated maize, the outbreak resulted in 20 cases including 12 deaths, aflatoxin levels detected in the two liver samples was 39 and 89 ppb (Ngindu *et al.*, 1982).

In 1989 an outbreak of aflatoxicosis occurred in Malaysia, following the consumption of contaminated noodles resulted in 40 cases and 13 child death.

In 2004, one of the largest, most serve aflatoxicosis outbreak occurred in Kenya, followed by another outbreak in 2005, (CDC, 2004; CDC, 2005).Both outbreaks were due to contamination of inadequately stored, home grown maize. The outbreak resulted in 317 cases and 125 deaths. Aflatoxin level in 55% contaminated maize samples were greater

than 20ppb, of which 35 percent had levels greater than 100ppb and 7% had level greater than 1,000ppb.

Most of the outbreaks of the aflatoxicosis reported are consequences of ingestion of food contaminated with the aflatoxin. The strict control of food quality is therefore necessary to avoid such outbreak. Though there has been no reported outbreak of aflatoxicosis in Nigeria butexposure to aflatoxin is widespread in Africa (Ayalen *et al.*,2006). In the study carried out in the Gambia, Guinea, Conakry, Nigeria and Senegal, over 98% of subjects tested positive to aflatoxin markers (Wild, 1996;Moss, 2008; Turner*et al*. (2003) detected aflatoxin albumin adducts in 93% of sampled children (6-9 year) in Gambia and provided evidence that immunoglobin A (IgA) in saliva may be reduced due high dietary level of aflatoxin exposure.

In a study in China found that the level of aflatoxin of B_1 B_2 and G_2 were quite high in maize from high incidence area for human hepatocellular carcinoma. They also indicted that the average daily intake of aflatoxin B_1 from high risk area was 184 µg/ml hepatocellular carcinoma is the fifth leading cause of cancer mortality in the world and it accounts for about 70% of cancer deaths in certain part of Asia and Africa (Li *et al;* 2001,Farombi, 2006).

Aflatoxin positive "kwashiorkor" children in Togo and Benin showed much greater severity of odema, increased number of infections, lower hemoglobin levels and longer duration of hospital stay than aflatoxin negative kwashiorkor children(Adhikari *et al*., 1994).Presumably the protein deficiency characteristic symptom of kwashiorkor reduces the capacity of the liver to detoxify aflatoxin. It is assumed that aflatoxin may be a factor

responsible for the increase in morbidity of children suffering from the disease (Ramjee, 1996).Bassa et al.(2001) detected aflatoxin in 98% of dried yam chips surveyed in Benin with aflatoxin level ranging from 22 - 220 µg/kg and a mean value of 141 µg/kg.

Uriah *et al.*(2001) in their study in Nigeria found blood and semen aflatoxin levels ranged from 700 - 1393 µg/ml and 60 - 148µg/ml respectively in infertile men and were significantly higher than that in fertile men , meaning that aflatoxin may be a contributory factor to the increasing incidence of infertility in Nigeria Gong *et al.,* 2002 reported that children in Togo and Benin who ate food contaminated with aflatoxin had the syndrome of stunted growth and underweight, which are signs of malnutrition.

Oyelami *et al.,*1996a established the presence of toxin in the autopsy brain tissue of some Nigerian children with kwashiorkor. Similarly, Akano and Atanda (1990) found aflatoxin B1 concentration in the range of 20-45.5 mg/kg in groundnut cake (*Kulikuli*) purchased from markets in Ibadan, Oyo State Nigeria. Adebanjo and Idowu(1994) alsoreported that most of the corn groundnut snakes (*donkwa*) contained aflatoxin above 30µg/kg immediately after preparation.

Similarly in Oyo and Ogun State of Nigeria, aflatoxin B1 was detected in 22% of yam chips purchased from open markets (Bankole *et al*., 2003) and the presence of aflatoxin in Tiger nut (*Cryperus esculenta*) at toxicologically unsafe level was also reported(Adebanjo,1993).Atanda *et al.,* 2007, detected aflatoxin in significant quantities in breast milk, blood and semen. Theaflatoxin level in infertile and fertile man was found to be in the range of 700 - 1392 ug/ml and 60 - 148 ug/ml respectively and these values were significantly highsuggestion that aflatoxin may be a contributory factor in the incidence of

infertility in Nigeria .In other studies, 80% of maize samples from different locations in southeastern Nigeriawerepositive for aflatoxin B1 (Aja-Nwachukwu and Emejuaiwe, 1994). In Southwestern Nigeria, 45% of raw maize from farms, 80% of maize cakes and 12% of maize rolls were contaminatedwith aflatoxins at mean levels of 200, 233, and 55 ng/g, respectively (Adebanjo,*et al*., 1994)

2.10Treatment of Aflatoxicosis

Aflatoxicosis is rarely reported in humans and treatment with antibiotics or other drugs has little effects (FDA, 2007). Treatment options are mainly focused on animals and include the use of any of the following in the feed (Cassel, *et al*., 2001). Chemisorbents such as hydrated calcium aluminosilicate (HSCAS) and mineral clays such as zeolite and bentonite which bind to aflatoxin in feed and prevent their absorption and bioavailability (Cassel, et al., 2001). It is usually added to feed at the rate of 5 to 10 pounds per tone. It reduce effect of aflatoxins on the liver and aflatoxin residue in milk

Organic acids such as propionic, benzoic and sorbic acid as well as their salts.Calcium propionate, potassium sorbate, and copper sulfate inhibit mould growth in feed and increased levels of high quality protein supplements and vitamin A, D, E and K in the ration as aflatoxin in affect protein synthesis and bind to vitamins. In addition, good management practices shoud be implemented to reduce stress and to decrease the risk of secondary infectons

2.11Factors Influencing Fungal Infection and Aflatoxin Production in Maize

Contamination of food with aflatoxin is influenced by many factors and can occur at any stage of food production, from pre-harvest to storage (Wilson and Payne, 1994). Factors that effect aflatoxin contamination include climate of the region, soil type, genotype of plant planted, temperatures, daily net evaporation (Fandohan*et al.,* 2005).

Aflatoxin contamination is also promoted by stress or damage to the crops due to draught before harvest, insect activity, poor timing of harvest, heavy rains at harvest, postharvest level of humidity, aeration during drying and storage and inadequate drying of the crop before storage are all important factors (Hell *et al*., 2000, Hawkin*et al.*,2005, Turner *et al.*, 2005).The other factors that influence the aflatoxin contamination of maize are agronomic and biotic factors if maize was grown with a crop that was susceptible to aflatoxin development, these will be an increased risk of toxin metabolism (Cotty, 1994).Development of storage fungi in a post harvest commodities is also influenced by the length of time in storage (Lillehoj and Zuber, 1988).

In Nigeria, drying techniques vary among different stakeholders, the majority of the farmers dry maize on bare ground, some on polyethylene sheets or mats while other leave the crops to dry in the field. These drying methods are slow and many support the development of fungi thus increasing the potentials of aflatoxin production(Hell *et al*., 2000). Poor storage has been found to encourage aflatoxin contamination where adequate storage facilities are not available at farm level.It has been reported that majority of farmers and traders in some African countries including Nigeria store maize using woven polypropylene bagswhich do not protect the grains against aflatoxin contamination (Kaaya

et al., 2001). Grains stored or heaped on the floor (unshelled) and those store under veranda had high aflatoxincontaminations. The only method that helps to protect the grain against aflatoxin contamination was storage above fire racks (Kaaya et al., 2001) but this method can not be adopted for storage of large quantities of grain.Ahmad(1993) observed that aflatoxin contamination in storage was dependent on storage system. Level of aflatoxin were lower in closed metal bin which restricted air exchange and reduced oxygen level than in ganny bags that allowed air to flow through stored black grain seeds.

Cole *et al.*(1981) investigated the effect of peanut, maize, and soybean crop rotation on aflatoxin development. They found more aflatoxin when the maize was planted after groundnut. Griffin *el al,* (1981) also observed that when maize was cropped in rotation with groundnut, there was *A.flavus* production builds up in the soil over the year.

Barry *et al.* (1992) showed that maize cultivars that had resistance to ear-infesting insectsproduce less aflatoxin in pre-harvest grains. The incidence of *A flavus* fungi and aflatoxin contamination was comparatively higher in insects damaged maize samples from different localities in India than insect free samples (Sinha and sinha, 1992). Zuber, *et al.* (1986)reported that insect that feed in maize ears may predispose maize to fungal infection through the physical damage.

2.12Methods of Elimination/or Prevention of Aflatoxin

The possible ways of reduce exposure to aflatoxin can occur at various stages of food production and preparation. These ways vary in their cost, labor, intensiveness and applicablity and effectiveness in preventing aflatoxin development. Bankole and Adebanjo(2003)have prepared many solutions against aflatoxin production in food. Some

of preventive measures which may be applicable in West Africa include education and extention, mechanical drying, timely harvesting, storage, smoking, post harvest food preparation, physical separation, sanitation.

2.12.1Education and Extension

The problem posed to the health and economy by aflatoxin is not known to a larger percentage of the populace including even the educated one. It is therefore necessary that the National Agency in each country responsible for safety like National Agency on Food and Drug Adminstartion (NAFDAC) in Nigeria should embrace the task of creating awareness to the populace about the need to consume pathogen free or good quality food. Private own Governmental Organizations could also roped in to spread information especially to the most remote villages. Regular programmes on radio and television on aflatoxin hazards and discussion on the issues of aflatoxin should feature regularly in daily news paper and magazines.

The Ministry of Agriculture in Nigeria should educate the farmers or producers on the need to adopt good agricultural practice to produce food free of hazard. The hazard analysis critical control point (HACCP), a food safety control system based on a systematic identification and assessment of hazard in food and the identification of their control is useful in this situation. In an IdealHACCPbasedsystem aflatoxin would beminimize at every phase of production, harvesting processing and distribution. The preharvest intervention to eliminate aflatoxin production include choosing crops with resistance to draught , disease and pest and choosing a varieties that are genetically more resistance to the growth of the fungus and the production of aflatoxin(Chen *et al*., 2001; Cleveland,et

al,2003).Elimination of inocula sources such as infected debris from the previous harvest may prevent infection of the crop (Olanya *et al.,* 1992).

Bio-pesticide consisting of non aflatoxigenic strain of *Aspergillus* may competitively exclude toxic stains from infesting the crop (Cleveland *et al*., 2003)

2.12.2 Mechanical drying

Among the recommendations for solving the aflatoxin problem, rapid drying of agricultural produce to low moisture content is often emphasized because all scenario's leading to aflatoxin contamination relate to non maintenance of stored products at safe moisture content.Drygrains keep safe from insects and mould because the water activity reguired for their growth is not met.

Drying harvested maize to 15.5% moisture content or lower within 24 - 48 h will reduce the risk of fungal growth and consequent aflatoxin production (Hamiton, 2000). Siriacha *et al*1989 found that shelled grains when immediately sun dried, reduces the chance of contamination as compared with of un-dried shelled maize.

The effectiveness of drying was demonstrated in the report of Awuah and Ellis, (2002) when groundnut kernels with 6.6% moisture were free of fungi regardless of the storage protectant used for 6 months, whereas at 12% moisture, only jute bags with *S. aromaticum* effectively suppressed the cross infections of healthy maize. However when the moisture content was increased to 18.5%, the latter was not as effective. Since sundrying may be a difficult task due to high rainfall at the time of harvest, a lot need to be done in the design of solar and mechanical dryer for use by farmers in the tropic (Axtell and Bush, 1991).

Thermal inactivation of aflatoxin is achieved by cooking, roasting, frying, spray drying and baking. Different cooking methods used by Indians were compared and it was found that the pressure cooking at 15 Ps, for 5 minutes gave maximum destruction (72%) of aflatoxin than the method of ordinary cooking (50%) and cooking with excess water (50%)(Rehana *et al*., 1979). They recommended pressure cooking of rice not only because of destroying maximum amount of aflatoxin but also for preserving nutrient of rice. Microwave roasting destroy aflatoxin completely (Rehana *et al*., 1979).

Timely harvesting: extended field drying of maize results in serious losses during storage and such harvesting immediately after physiological maturity is recommended to combat aflatoxin problem. Aflatoxin levels increased for about 4 times by third of extended field during and more than7 times when maize harvest was delayed for four weeks (Kaaya *et al.,* 2006). However, after early harvesting, products have to be dried to safe levels to stop fungal growth. Leaving the harvested crop in the field prior to storage promotes fungal infection and insect infestation.

2.12.3 Storage Conditions

The process of sorting and disposing of visibly mould or damaged maize before storage is an effective method of reducing but not eliminating the development of aflatoxin. (Fandolan *et al.,*2005; Turner *et al.,* 2005). Moisture as well as insects and rodents control during storage can prevent damage to crop which will promote aflatoxin development. Aflatoxin contamination of maize is influenced by the storage structure used for storage, the length of time in storage and the form of maize stored with husk, without husk or loose grain (Hell *et al.,* 2003). Storage structures used by farmers on the farm for storage include

containers made of plant materials (wood bamboo, thatch) or mud placed on raised platform and covered with thatch or metal roofing sheets. Importantly, stores are constructed to prevent insects and rodents attack and prevent moisture from getting into the grain. This type of storage can not stand for longtime, thus making this method inadequate. However, it has been very difficult to promote new storage technologies such as the use of metal bins to small scale farmers due to their high cost. Research is needed to develop and refine suitable storage system that are not capital intensive.Community based intervention trial in Guinea(WestAfrica) focused on through drying and proper storage of groundnut in subsistence farm villages. The trial achieved a 60% reduction in mean serum aflatoxin-albumin level in people in intervention village meaning that simple and inexpensive method can have significant impact on aflatoxin prevention and control(Turner*et al.*, 2005).

2.12.4Smoking

Smoking is also an efficient method of protecting maize against infestation by fungi. The efficiency of smoking in protecting against insect infestation was found to be comparable to that of the chemical Actellics (Primiphos-methyl) (Daramola, 1986). About 3.6 – 12% of farmers in various ecological zones in Nigeria used smoke to preserve their grain and this practise was found to decrease aflatoxin levels in farmer's stores (Udoh*et al.*, 2000). The efficiency of smoke was also confirmed by Hell *et al.*,(2000) in a survey conducted in Benin. However the proplem with smoking is that if not carefully applied, it may discolor the product and change thetaste(Turner*et al*., 2005).

Other compounds used for fumigation like ethylene oxide and methylene bromide were found to significantly reduce the incidence of fungi including toxigenic species on stored

groundnuts and melon seeds.Among the chemical compounds tested in feeds , propiponic acid, sodium propionate, benzoic acid and ammonia were the best antifungal compounds followed by urea and uric acid (Gowda *et al.*,2004). The use of chemical by farmers in Africa is not always well practiced.

2.12.5Post Harvest Food Preparations

The process of food preparation or consumption involve the removal of contaminated portion of foods diluting food with uncontaminated food, neutralizing aflatoxin present in foods or altering the biodiversity of the aflatoxin consumed. Simple food preparation method, such as sorting, washing, crushing and dehulling may reduce aflatoxin level (Park 2002; Fandohan *et al* 2005). Aflatoxins are not largely affected by routine cooking temperature; but traditional methods of cooking food with alkaline compounds have been used to reduce aflatoxin exposure. Although the chemical reaction may inactive aflatoxins, the reaction may then reverse in the gastric acid of the stomach(Elias *et al* 2002;Alberas *et al*. 2004; Fandohan *et al*.2005.)

2.12.6Physical Separation

The principle of this method is based on the identification of damaged maize in the seed lots because of variations in size, shape, colour and visible mould growth. Aflatoxin contaminated seeds are usually damaged, shriveled or discoloured (Natrajan *et al.*, 1995). This could be done manually or by using electronic sorter. Udoh (1997) identified sorting of damage maize cobs and cobs with poor husk covering as a practice that led to significant reduction of aflatoxin. Hell *et al.* (2000) advocated the removal of insects damaged cobs at harvest to reduce aflatoxin contamination. Martin*et al.* (1999) recommends the final sorting

either by hand or by calorimetery as the only possible remedial technique against aflatoxin contamination of groundnut in Senegal and that the efficiency of this technique depends on the extent of seed contamination. Haddon(2002) asserted that presently there is no sorting mechanism that meets commercial needs of adequate reduction and product preservation.

2.12.7 Sanitation

Clearing the remain of previous harvest and destroying infested crop residue are basic sanitary measures against storage deterioration.Cleaning of stores before loading in new harvest was correlated to reduced aflatoxin levels (Hell *et al.*,2000). Disposing heavily damage ears, those having greater than 10% ear damage also reduced aflatoxin level in maize (Setamou*et al*.1998). Wild hosts which constitute a major source of infestation for storage pest should be removed from the vicinity of stores. In spite of the use of many preventive methods on the field, during harvest and storage, complete mycotoxin elimination is far from reality. Even the best management of agricultural strategies cannot totally eradicate mycotoxin contamination (Jouany, 2007).

2 .12 .8 Potential biocontrol agents used for management of aflatoxin contamination

There are many other control strategies of aflatoxin producing organism, and it includes biological control of insect pest and development of resistant culture have been investigated to manage aflatoxins in crop. Biological control appears to be the most promising approach for the control of aflatoxin in both pre and post harvest crops. Different organismsincluding bacteria, yeast and non toxigenic *Aspergillus* fungi have been tested for their ability in the control of aflatoxin contamination (Dornor et al., 1998).

Bacteria, several bacteria species such as *Bacillus subtilis, Lactobacilli* spp. *Pseudomons* spp. *Ralstoina* spp.*and Burkholderia*.*spp.*have showed the ability to inhibit fungal growth and production of aflatoxin by *Asperqillus* species.Palumbo et al. (2006) reported *Bacillus, Pseudomonas, Ralstonia* and *Burkholderia* strains isolated from califonia almond samples could completely inhibit *A. flavus* growth several strain of *B. Subtilis* and *P. Solanacearum* isolated from the non-rhizophere of maize soilwere able to inhibit aflatoxin accumulations (Nesei *et al.*, 2005).

Yeast: some saprophytic yeast species (such as *Candida krusei* and *Pichia anomala*) have shown promise as biocontrol agents against *A. flavus.*Similar to bacteria agent; these yeast strains were able to inhibit *Aspergillus* growth greatly in laboratory conditions (Hua *et al*., 1999: Masoud and Kaltoft, 2006). Although they were considered as potential biocontrol agents for management of aflatoxin, further field experiments are necessary to test their efficacies in reducing aflatoxin contamination under field conditions.

2.12 .9Nontoxigenic Aspergillus Strain

Greatest success to date in biological control of aflatoxin in both pre and post harvest crops has been achieved through application of competitive nontoxigenic strains of *A. flavus* and or / *A.parasiticus.*In many field experiment particularly on peanut and cotton ,significantreduction in aflatoxin contamination in the range of 70 - 90% have been observed consistently by the use of non toxigenic *Aspergillus spp*(Dorner,2004;Pitt and Hocking, 2006; Dorner, 2008). Recently two product of non toxigenic strains have received US Environmental Protection Agency (EPA) registration as biopesticide to control aflaxtoxin contamination of cotton and peanut in several states of USA (Dorner, 2004) and a

commercial product based on atoxigaenic *Aspergillus flavus* stains is being marketed (Afla Guard).In Africa, two isolates of *Apergillus flavus* have being identified as atoxigenic stains to competitively exclude toxigenic fungi in the field. These strains have been shown to reduce aflatoxin concentration in both laboratory and field trials. A mixture of four atoxigenic strains of *A .flavus* of Nigerian origin has gained provisional registration (Alfa Safe) to determine efficacy in on-farm tests and candidate strains have been selected for Kenya and Senegal.

2.12.10Breeding for resistance

Several screening tools have been developed and used to facilitate maize breeding for developing germplasm resistant to fungal growth and aflatoxincontamination(Brown*et al*., 2003). Sources of resistance to *Aspergillus* infection and aflatoxin contamination in maize have been identified and commercial hybrids have not being developed. This is largely due to the difficulty in finding elite lines that maintain high yield and maintain resistance within multiple environments (Clements and White. 2004). Brown *et al*.(2001) tested aflatoxin resistance in thirty six maize inbreed lines selected in West and Central Africa for moderate to high resistance to maize ear rot for their resistance to aflatoxin,more than half the inbreedaccumulated aflatoxin at level as low as or lower than the resistant U S lines. In 2008, six tropical maize germplasm lines with resistance to aflatoxin were registerd by the same research group (Menkir *et al*.,2008)and their distribution to national programs will start soon for the development of locally adapted hybrids.

2.12.11Control of Environmental Factor

Environmental factor that favor *A.flavus* infection in the field include high soil and/or air temperature, high relative humidity, high rate of evaporation, reduced water availability, drought stress, nitrogen stress, crowding of plants, and conditions that aid the dispersalof conidia during silking (CAST,2003). Some of these factors have been induced in a model to predict toxin contamination in peanut system in Mali weather and satellite based variables that could be used to indicate aflatoxin presence in peanut were identified.(Boken *et al.*, 2008).

2.12.12 Other Strategies

The other strategies to reduce the risk of aflatoxin ingestion in Africa are dietary change, chemoprevention, detoxification and vaccination against hepatitis B, would significantly reduce liver cancer risk (Strosnider *et al;*2006).

2.13Aflatoxin detection techniques in food and feed

The current methods used in detection of aflatoxin in food can detect very low level of aflatoxins and aflatoxin metabolites in food and biological media. The use of these methods in the developing countries is limited by practical consideration such as resource and infrastructure. Methods for testing food and biological specimens need to be adopted to fit the surveillance and epidemiologic need of developing countries. A simple method adapted for developing countries would benefit subsistence farmers and be useful to public health and agricultural institutions.Methods of detection can be classified as field methods and laboratory methods.

2.13.1Field method

Simple and inexpensive field screening methods such as portable(Aflakit), lateral flow immunochromatographic assay are available.Field method can be performed with minimal training or equipment and can be performed on site. (i.e. at a farm or grain silo). Field methods for aflatoxin analysis allow for rapid confirmation or exclusion of possible exposure at a reasonable cost, thus allowing official to quickly determine the need for further evaluation and intervention. Some of these methods are of benefit to developing countries where the remoteness of village long distance to a centralized laboratory make it impractical to take sample from village, analyze them in the laboratory and travel back to the village to delive result.

Currently, however, these lack direct applicability in developing countries, example of one field screening method uses dipstick which indicates if a sample is above or below the regulatory limit of 20ppb. This make it possible for an investigator to differentiate between sample at level above 20ppb, this method would prove the effective if they can be adjusted to action level suitable developing countries. Generally,aflakit a polyester dip strip offers a simple cost effective and reliable method of monitoring aflatoxin in food and feed and easy handling unlike the laboratory method.

2.13.2 Laboratory method

Laboratory method can be used to confirm result of field test. They are more precise, but also more labour intensive and costly, these methods require instrumentation or technique not suited to working on site. They require regular maintenance of instrumentation, training of personnels and a regular supply of reagents and materials (Trucksess, 1994).

Various analytical techniques such as Thin layer chromatograpy(TLC),High performance liguid chromatograpy(HPLC),Highperformance thin layerchromatograpy(HPTLC), radioimmunoassay, and Enzyme linked immunosorbent assay (ELISA)have been developed for quantifying aflatoxin in food, feed and biological fluids(Pestka,1988). Most of these methods require skilled manpower, sophisticated expensive equipment and laboratory set up for analysis. The food and drug administration as well as other government agencies utilize AOAC approved procedure in order to detect aflatoxin in various commodities. Two frequent chromatographic methods employed to detect aflatoxin in food commodities are HPLC and TLC.

2.13.3 Chromatography method

Traditional chromatographic techniques such on thin layer chromatography (TLC), liquid chromatography and Gas Chromatography are widely employed for separation and quantification of the mycotoxins. The enzyme linked immunosorbent assay (ELISA) and Immuno affinity column (IAC) assay are most commonly used.

2.13.4Thin layer chromatography (TLC)

Thin layer chromatography also known as flat bed chromatography or planar chromatography is one of the most widely used separation techniques. TLC separation includes sample application, plate development and visual observation and quantification (Trucksess, 2000). TLC is simple and could be economical. It is the most useful techniques for mycotoxin analysis in the developing countries. The thin layer chromatography plate can be developed in either one or two dimensional mode (Molto *et al.*, 2000).

In the analysis of most commodities such as barely, maize or other grains, one dimensional separation of compound is inadequate when the concentration of mycotoxin as low as 1 ppb are to be detected (Anon, 1980).Two dimensional TLC provides a solution for adequate separation and detection. Thus, thin layer chromatography (TLC) is a chromatographic technique that is useful for the separation, purity assessment and identification of low level of organic compound. Because of the simplicity and rapidly of TLC, it is also used to monitor the progress of organic reactions. Thin layer chromatography has found widespread use in mycotoxin determination, but has now largely been replaced by other chromatographic method (i.e. high performance liquid chromatography (HPLC) and gas chromatography (GC) because of their superior analytical performance.

In countries which do not have expensive equipment at their disposal, but which often produce and export agricultural commodities, TLC remains important, it is also in use for screening purposes prior to labour intensive and time consuming HPLC and GC methods (Betina, 1985).

2.13.5Liquid Chromatography

Liquid chromatography (LC) is similar to TLC in many respects, including analyte application, stationary phase and mobile phase. Liquid chromatography and TLC complement each other for an analyst to use TLC for preliminary work to optimize LC separation conditions is not unusual

2.13.6 Immunochemical Techniques

Thin layer chromatography and LC methods for determining aflatoxin in food are laborious and time consuming. Often these techniques require knowledge and experience of

chromatographic technique to solve separation and interference problems through advance in technology, a more rapid methods based on immunochemical techniques exist which often have the advantage of non requiring any clean up or analyte enrichment steps ELISAs have become routinely used tool for the rapid monitoring of most mycotoxins, especially for the screening of raw material (Gilbert and Ankham2002; Fremy and Usleber (2003).Although ELISA tests may show a high matrix dependence and possible over estimation of levels. The advantages of the microtitre plate, format are speed, ease of operation, sensitivity and high sample throughput.

Alternative to ELISA include a number of immunosensors as well as up coming methods using immunochemical platforms such as fluorescence polarization immuno assays (FPI) (Nqundi*et al*., 2005) or surface plasm resonance (SPR) with mycotoxin protein conjugate immobilized onto a sensor chip surface (Tudos *et al.*, 2003). Immunosensor are emerging as a cost effective alternative for screening and quantitative determination of mycotoxin (Maragos, 2004).

2.14Problems affecting aflatoxin research in Nigeria

The problem of aflatoxin research in Nigeria are diverse and numerous among which are analytical procedures, sampling, legislation and awareness of the problem by food handlers (farmers and traders) and consumers

2.14.1.Lack of Funds and other Resources

Aflatoxin analysis is expensive to conduct in Nigeria thus hindering routine analysis of food sample by individual farmers ,trader and organizations handling susceptible foods owing to supplies and reagent that are costly and some time unavailable.Aflatoxin in

Nigeria is of low priority thus very few companies import and appropriate supplies and reagents.Institutions that are into analysing aflatoxin thus charge high amount of money per sample which discourage those involved in trade to have their foods analyzed.

In Nigeria, there is a serious problem of inadequate up to date analytical equipment. There is no standing institution whose sole mandate is concern with aflatoxin research though, selected work are on going at University of Ibadan and I I T A, Ibadan. There is therefore need to upgrade laboratories with recently recommended analytical equipment like high performance liquid chromatography (HPLC), Gas chromatographs (GC) and simple presumptive or screening equipment which can produce aflatoxin presence in food samples.

Aflatoxin analytical equipment is installed together with other analytical equipments thus putting analyst and researcher in danger. Simple protective devices like gloves, glove boxes, masks and head cap are some times lacking and therefore not used during aflatoxin analysis. Lack of functioning ventilated hoods, exhaust fans and waste disposal facilities are also lacking in some laboratories(Betina,1985).

It is essential to safety handle all experimental materials associated with aflatoxin analysis thus making it important to follow mycotoxin safety precautions as stated by the International Agency for Research on Cancer (IARC) and the Association of Official Analytical Chemist (AOAC).

2.14.2Sampling

There are no proper sampling plans for aflatoxin analysis in Nigeria, Nigeria being divided into six geographical zones, there is possibilities of a non-homogenous distribution of aflatoxins. There fore different in sampling techniques could be an important contribution

to the variability in aflatoxin contents of agricultural commodities so far tested,there is need therefore to organize for proper sampling and analysis of commodities obtained from different climatic zones of the country following recommended protocols, so as to rank the zone in terms of aflatoxin contamination of produce like USA, India Cyprus and others factors promoting aflatoxin contamination of commodities in zone should subsequently be investigated and compiled, these will form the basis for designing aflatoxin control programme in the country

2.14.3 Legislation

At present, the established standards on aflatoxin contamination in Nigeria is based on Food and Drug Administration (FDA), USA standard, not on the actual aflatoxin of contents in Nigeria. There is no procedure in place for aflatoxin control, monitoring or supervision in the country. Susceptible products like maize and groundnuts are not usually inspected by qualified personnel's at buying points to separate good quality from contaminated produce; so when the produce reaches the retail market, sorting may be done, but contaminated produce may be sold to unsuspecting consumers at low prices.

There is need for full participation of government through the Ministry of Health (MOH) and that of Agriculture, together with the National Agency for Food and Drug Administration Control(NAFDAC) to put into practice regulations of monitoring susceptible produce from buying point to retail markets. Strict post harvest measures such as drying, sorting, packaging, storage and proper handling of produced should be introduced and monitored

2.14.4Awareness of the problem by food handlers

Aflatoxin is not visible neither do they have a particular flavor; therefore it is not easy to convince consumers about their existence in food. The majority of farmer's, trader and consumers in Nigeria are not currently aware of the aflatoxin contamination of food. Therefore, the need to disseminate information to these people using simplified methods about the danger and management aspect of aflatoxin and susceptible produce. The ministry of Agriculture, Health and NAFDAC should work together to develop more effective and broad training programme to farmers, traders and consumers in form of workshops.

CHARPTER 3

3.0 MATERIALS AND METHODS

3.1 Samplecollection

Maize, maize flour and maize bran for this study were obtained from sellers in grain markets.Samples were collected from eight selected markets namely, Kaduna central, Kawo, Kakuri, Giwa, Soba, Makarfi,Samara and Kasuan beichi markets(Figure3.1)Samples were collected using respective sellers sampling tools (i.e. spike and scoops) into factory low density sterile polythene bags. Collected samples were transported to the laboratory for analysis.

3.2 Sample size

The sample size was determined by using the relation:

$$Samplesize[n] = \frac{Z^2pq}{L^2}$$

Where n = sample size, L = allowable error = 0.05, prevalence rate[p] 33% (Udoh *et al*., 2000),

q = 1 – prevalence, Z = standard normal distribution at 95% confidence interval = 1.96

$$n = \frac{3.8416 \times 0.33 \times 0.70}{0.0025} = \frac{0.8814}{0.0025} = 352.5$$

A total of four hundred(400) samples were collected. Samples were collected following the standard procedure (FAO, 1982). Two hundred and fifty grammes [250gm] sample was taken from every maize bag, maize flour and maize bran. Samples were collected from bags

of maize. Multiple samples were taken from different parts of one bag or several bags belonging to one seller and combined to produce sample for the study.

3.3 Sampling Plan

Sampling was carried out for one year from March 2010 – February 2011. .Maize flour and maize bran were sampled randomly throughoutthe year.The 400samples collected were in ratio 3; 2; 1 representing 200 maize, 133maize flour and 67maize bran.The choice of market from which samples were taken was based on the following:

(a) Geographic location of the population served by the market.

(b) The number of maize sellers.

(c) Popularity of the grains markets within the study area.

3.4 Determination of seasonal weather condition

The environmental parameters such as temperature, relative humidity and amount of rainfall in the sampling areas were determined using minimum and maximum thermometer(29.092.Kata model), hygrometer(S29-102 paper hygrometer (pockettype) and rain gauge respectively. Data were collected weekly.

3.5 Determination of Maize quality

Five hundred (500g) each of the maizegrains samples were weighed into a sterile plastic tray and physically sorted out in whole (intact), broken (divided into fraction) and damaged (insect infested) maize grains using sterile hand gloves. Each part was weighed and expressed as a percentage of the total weight

Percentage of whole/broken /damaged(y) = w/b/d × 100
Total wt

Where w or b or d stands for weight of whole maize/wt.ofbroken and wt. ofdamaged grains respectively.

3.6 Determination of physico-chemical compositions

3.6.1 Moisture content

The moisture content was determined according to AOAC (2000)by drying 5g maize sample to constant weight in pre weighed moisture plate in a thermostatic oven at 130^{o}C temperature for 1h and then reweighing after cooling in a desiccator. The moisture content is expressed as percentage of the dry weight: -

Moisture content = Weight loss of sample x 100

Weight of the original sample.

3.6.2 Ash Content/Mineral content

The ash content was determined following standard methods of AOAC (2000). A procelain crucible was dried in an oven at 100^{o}C to a constant weight. Two (2g) of the dry sample was weighed in to a dry porcelain dish and then heated in a muffle furnace at 600^{0}C for 6 h. It was cooled in a desiccator and then weighed. The percent ash content was calculated using the relation:

$$\% \text{ ash} = \frac{\text{weight of ash}}{\text{Weight of sample}} \times 100$$

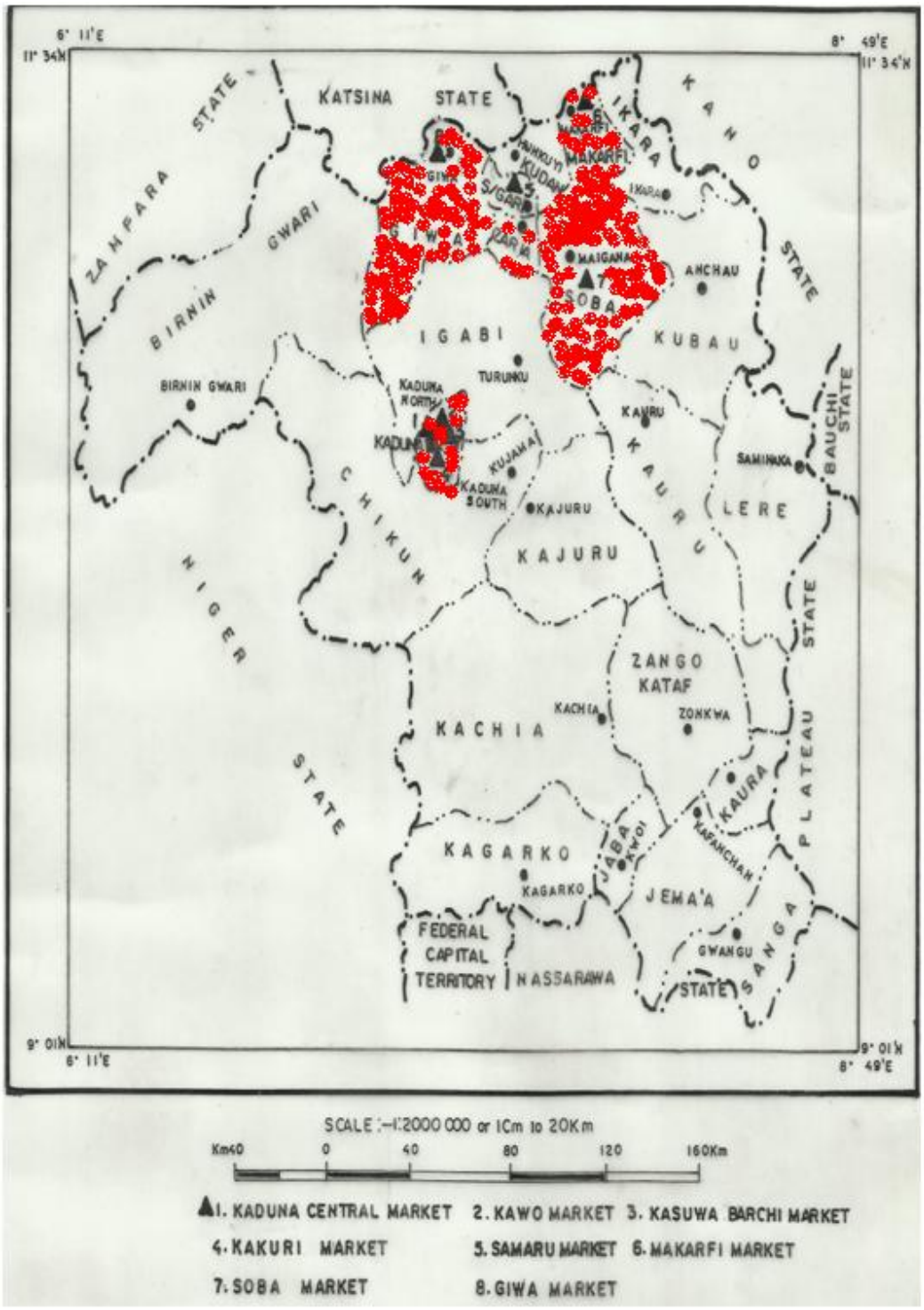


Figure 3.1 Map of Kaduna State showing selected grain markets

3.6.3 Fat content

The fat content was determined using Soxhlet method (AOAC, 2000) .Two (2g)of the sample was weighed into the Soxhlet extraction thimble. Cotton wool was used as plug to avoid loss of sample. The thimble was transferred into the Soxhlet extractor and sufficient petroleum ether was added until the latter is siphoned into the receiving flask which has been weighed. More ether was poured to cover the thimble completely and flask placed with the extractor on the electric heating mantle. The reflux condenser was heated gently for 3 h, switched off and allowed to cool for 10 minutes. Recovered solvent was transferred into dry air oven (100^{o}C) for 1hand then cooled in a desiccator and weighed. The amount of oil produced was calculated and expressed as percentage of original sample.

$$\%\text{Fat} = \frac{\text{Weight loss of sample (extracted fat)}}{\text{Weight of sample}}$$

3.6.4 Crude Protein

One (1g) of each sample was weighed into a digestion flask. Ten (10g) potassium sulphate, 0.7g mercuric oxide and 20cm^3concentrated sulphuric acid was added to the samples in the digestion flask. The flask was heated gently at an inclined angle until frothing subsides and then boiled until the solution becomes clear. This was continued for half an hour. When the frothing is in excess, a small amount of paraffin wax was added. On cooling, 90ml of distilled water was added and mixed. A small piece of pumice was also be added to prevent bumping, Eighty (8ml) of 2M sodium hydroxide solution was added while tilting the flask so that two layers are formed. The condenser unit was rapidly connected, heated and the distilled ammonia collected in 50ml boric acid / methyl red indicator. Fifty (50ml) of the

distillate was collected and titrated against 0.1M hydrochloric acid solution.The nitrogen content percent was calculated from the relation:

$$\% N = \frac{(\text{Volume of acid} \times \text{Molarity of standard acid}) \times 0.014 \times 100}{\text{Weight of sample (g)}}$$

% Crude protein content = nitrogen content x 6.25

3.6.5 Crude fiber determination

The crude fiber was determined using the method of (AOAC, 2005). Five (5g) of the sample was weighed into a beaker. Twenty (20ml) of cold sulphuric acid was slowly added followed by addition of one hundred eighty (180ml) of boiling concentrated.Sulphuric acid using a reflux condenser for

the maintenance of constant volume during the digestion, transfer of the fibre after the washig with ether, from the filter paperto a dish (previously ignited and weighed) with boilig water. Removal of th water by evaporatin on a water bath, drying at 100^{o}C,weighing the fibre followed by ashing in a furnace at 550-600 ^{O}C..The percentage crude fibre content was calculated using the formula below: -

$$\text{crude fibre} = \frac{\text{weight of dry sample} - \text{weight of ashsample} \times 100}{\text{Weight of original sample}}$$

3.6.6Total Carbohydrate

The total carbohydrate was determined by differential method. This would be achieved by subtracting the total protein, lipid, moisture and ash content from 100 as given below:

% carbohydrate (100 – (% moisture + % ash+ % fat + % protein + %fibre)

3.7 Mineral determination in maize and maize products

3.7.1 Wet digestion

The digestion of samples for metal analysis was accomplished as described by Van loon (1996). One (1g)of the pulverized maize sample was weighed accurately into 250 ml Erlenmeyer flask. Fifteen (15ml) of HNO_3 was added to the flask followed by addition of 5ml of conc. H_2SO_4. The mixture was shaken to mix and heated on a hot plate preset at 160^oC until the brown fumes disappeared and white fumes began to show.Ten (10) ml of H_2O_2 was carefully added and heating was continued to dryness.

The digest was allowed to cool and20ml de-ionized water was added to dissolve the residue. This was filtered quantitatively into 100mlvolumetric flask. The residue in the Erlenmeyer flask was washed with 10ml de-ionized water into the filtrate. The filtrate in the volumetric flask was made up to the 100mlmark and was transferred to a plastic bottle,aspirated into the machine for the trace metal analysis.

3.7.2 Analysis for minerals

Young Lin flame atomic absorption spectrophotometer model 8010 was used for the determination of Ca, Mg, Fe, Mn, Zn and Cu while K and Na were determined using Spectrum Lab 22 flame photometer by the method described by Khan and Zeb,(2007) whilePhosphorus (P) was determined using titrimetric method.

3.8 Enumerationand characterization of moulds in maize and maize products

Twenty five (25g) of each homogenized sample using stomacher was taken aseptically and weighed into a Erlenmeyer flask containing 225ml of 0.1% sterile peptone water and allowed to remain for 2 - 3 minutes with occasional stirring using sterile glass rod. Ten fold serial dilutions were prepared by transferring 1ml aliquot of the supernatant into 9ml of sterile peptone water as diluents. Further serial dilution was carried out and thereafter, 0.1ml of appropriate dilutions were plated aseptically using Spread plate method on Potato Dextrose Agar [biotech] supplemented with chlorampenicol. Inoculated plates were incubated at 28 ±2^{o}C for 5-7 days. Number of colonies after incubation were identified and counted and expressed as colony forming unit per g (cfu/g). The fungi that developed were purified by repeated subculturing. Pure cultures of fungi were examined macroscopically and microscopically and isolates were confirmed bycomparison with the existing cultures already identified by the International Mycological Institute through the use of mycological atlas. Isolates were stored at 4^{o}C in refrigerator until when required.Presumptively identifed *Aspergillus* species were sent to Commonwealth Agricuture Bureau Internatonal (CABI) Institute, London to further confirm the species.

3.9Testing the ability of the mould isolates to produce toxin

The ability of the isolates to produce mycotoxins was tested by using the procedure reported by Amadi and Adeniyi (2009).

3.9.1 Mycotoxinproduction and detection

The Potato Dextrose Broth (PDB) for this study was prepared following manufactures instruction Twenty five (25ml) of the broth was dispensed into sterile 250ml Erlenmeyer

flask. The flask was inoculated separately with 5 ml of the identified test fungi. The inoculated flasks were incubated on a shaker at ambient temperature range of (25 ±1ºC) for 12 days. The filtrates of the broth culture were obtained and assayed for the presence of aflatoxin(Amadi and Adeniyi 2009).

3.9.2 Detection of aflatoxin using thin layer chromatography

Thin layer chromatography (TLC) technique was used for detection of aflatoxin. The filtrates obtainedand the standard solutions of the toxins were spotted on prepared TLC plates coated with silica gel at a distance of 1.5 cm from the lower edge of the plate and kept for 1 minute to dry. Chloroform/methanol (97:3 and 50:50v: v) was used as solvent for the detection of mycotoxin produced by the test organism. The TLC plate was removed when the solvent front reaches a mark of 1 cm from the upper edge of the plates. The plates were kept to dry up and the solvent front was marked across the plate.

The coloredspots were marked \with pencil and visualized under U V lamp. The Retention factors R1 of the compounds/toxins detected in the filtrate were calculated as:

$$R1 = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent}}$$

3 .10Determination of total aflatoxin levels in maize and maize products were determined following manufacturers instruction of the kit (Aflatoxin plate kit (PN53012B)

3.10.1Preparation of Solutionfor extraction

Twenty (20ml) of distilled water was carefully measured and transferred to clean glass container with a tight fitting lid. Eighty (80ml)of methanol measured for each 100 mlbeing prepared was added it was then covered and shaken to mix completely and was stored tightly sealed to minimize evaporation**.**

3.10.2Sample extraction

Each test was performed with ground sample of the maize weighing 50g. This was mixed with 5.0g NaCl and 100ml of 80% methanol : water (80;20) and blended in a blender jar at a high speed for I minute with the blender covered and 10 ml was filtered through a glass fiber filter.

3.10.3 Extract dilution

Five milliliters (5 ml) of the filtered extract was collected in a clean vessel and diluted with 20ml of water and mixed thoroughly and filtered through a glass fiber filter

3.10.4 ELISA test

The total aflatoxin level in maize grains and maize products (maize flour and maize bran) were detected and quantified using enzyme linked immunosorbent assay (ELISA) kits (Helica Biosystem Inc.USA).The kit was used according to the manufacturer's instruction**.**

3.10.5 Assay principles

The aflatoxin kit is a competitive enzyme – labeled immunoassay.Aflatoxin is extracted from a ground sample by blending or shaking with methanol/water.The extract was then diluted with water, filtered and then tested in the immunoassay, Aflatoxin – HRP enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts.Aflatoxin

antibody is then pipetted into the test wells to initiate the reaction. During the 10 minutes incubation period, aflatoxin in the sample and aflatoxin-HRP enzyme conjugate competesfor binding to aflatoxin antibody which in turnbinds to the test well. Following this 10 minutes of incubation, the contents of the well are removed and the wells are washed to remove any unbound toxin or enzyme-labeled toxin. A clear substrate is then added to the wells and any bound enzyme-toxin conjugate causes the conversion to a blue colour.Following 10 minute incubation, the reaction is stopped and amount of colourin each well is read.The colour of unknown samples is compared to the colour of the calibrator and the Aflatoxin concentration of the sample is derived.

3.10 Statistical analysis

Data were subjected to statistical analysis using analysis of variance (ANOVA) using the PROC ANOVA Rothamsted Experimental Station, 1998, version8.0)to test the level of significance at $p \leq 0.05$ beingconsidered statistically significant.

Chapter 4

4.0 RESULTS

Critical Meteorological data obtained during the sampling of the markets included temperature, humidity and monthly rainfall. Table 4.1 shows a temperature range of 24-35^{o}C with a mean temperature of 30^{o}C, while the relative humidity was in a range of 35-61% with a mean of 48%. The average monthly rainfall was in the range of 2.7 – 7.0 mm with a mean of 3.2mm.

In Fig.4.1 percentage wholesomeness of the maize grain was in the range of 78.72- 98.02% while the percentage broken was in the range of 1.30% – 14.70%, The damaged grains had a range of 0.60 – 7.12% for new maize, while for old maize had percentage wholesomeness grain in the range of 90.58– 97.80%, broken 1.62 -6.24% and percentage damaged in the range of 0.74-2.98%. Statistical analysis using Anova showed that significant difference existed between the percentage whole, broken and damaged grains ($P \leq 0.05$).

The proximate analysis of maize and maize products obtained from sampled areas is as shown in Fig 4.2.The percentage carbohydrate was in the range of 44.8 – 69.6% moisture contents 11.6 – 20% , protein content 4.5 – 9.87 , fat 2.17 – 4.43 , fibre 2.10 – 26.77 and the ash content 1.10 – 2.95%.

On statistical analysis, using Anova, significant differences existed between the mean values of the nutrient content of the products except moisture

The results of the mineral composition of the maize and maize products for the study is as presented in Table 4.2 Of all the minerals, phosphorus was found to be in the range of (23.00 – 85%), followed by magnesium (29.33 – 47%), Potassium (10.67 – 15.60%) and sodium (1.50 – 4.43) while other mineral content such as calcium, manganese, zinc, iron, copper were all in low percentages.

Statistical analysis showed that statistical differences existed between the entire mineral contents of maize and maize produccts tested except calcium, iron and copper

Table 4.3 represents the mould counts of maize and maize products obtained from selected markets. A low count of $\log_{10}$ 1.00 - 1.69 with a mean of $\log_{10}$1.55for old dried maize was recorded from Kasuwa Berchi market while a count of $\log_{10}$ 4.14 - 4.6 with a mean of $\log_{10}$4.20 was recorded for kakuri market.The mould count of freshdried maize samplehad a low count of $\log_{10}$1.90 - 2.23 with a mean of $\log_{10}$2.09 from Beichi market and a count of $\log_{10}$2.23 - 2.62 with a mean of $\log_{10}$2.54 from Samaru Market.Maize flour has a connt in the range of $\log_{10}$ 2.04 – 2.6 from Samaru market with a mean of $\log_{10}$ 2.30 while a higher count of $\log_{10}$ 4.54 - 4.90 with a mean of $\log_{10}$ 5.57 was recorded for Central Market.However the mould counts of maize bran were in the range of $\log_{10}$ 1.26 – 2.43 with a mean of $\log_{10}$ 2.35 for sample from Giwa market while a higher count in the range of log10 3.28 -5.34 with a mean of $\log_{10}$ 4.97 from Kakuri market.

Table 4.1: Meteorological data obtained from sampling areas (Grain Markets) selected within Kaduna State (March 2010 -February2011)

Market Location	**Range Temperature** o_C	**Mean Temperature** ^oC	**Range of Humidity (%)**	**Mean Humidity (%)**	**Mean Monthly Rainfall (mm)**
Kad.central	26-33	31^oC	40-45	47	3.1
Kakuri	26-31	29	44-53	49	2.7
Soba	29-31	30	45-54	49	2.7
Giwa	27-35	32	35-46	42	NA
Makarfi	29-35	31	44-46	46	NA
Samaru	24-26	26	56-61	57	7.0
Kawo	25-34	32	43-48	46	5.31
Beichi	26-30	28	43-56	48	5.31

- Values of 10 replicates per market sampled.

NA –Not available

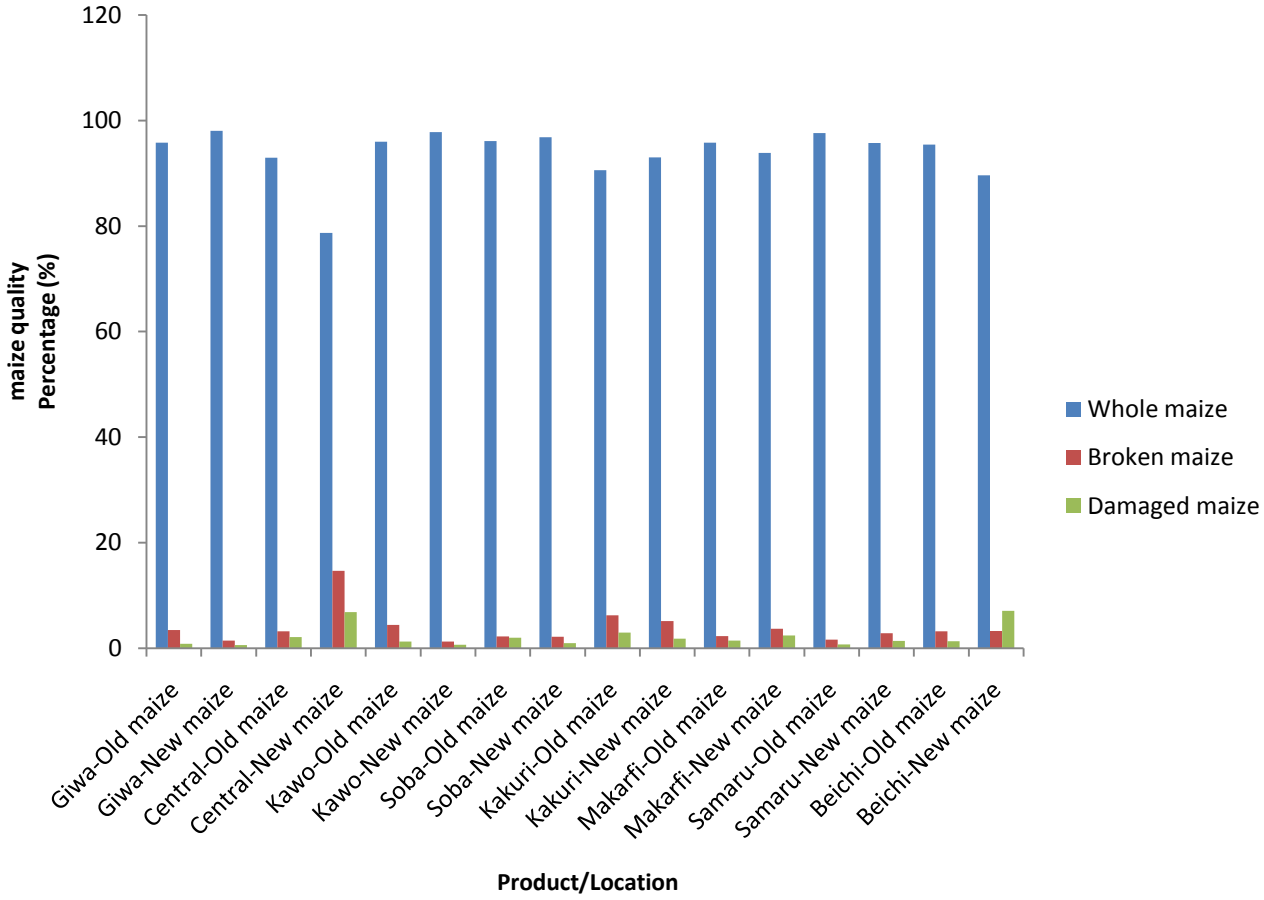


Fig. 4.1: The mean distribution of maize based on quality from different markets.

*Values are 10 replicates er market sampled.

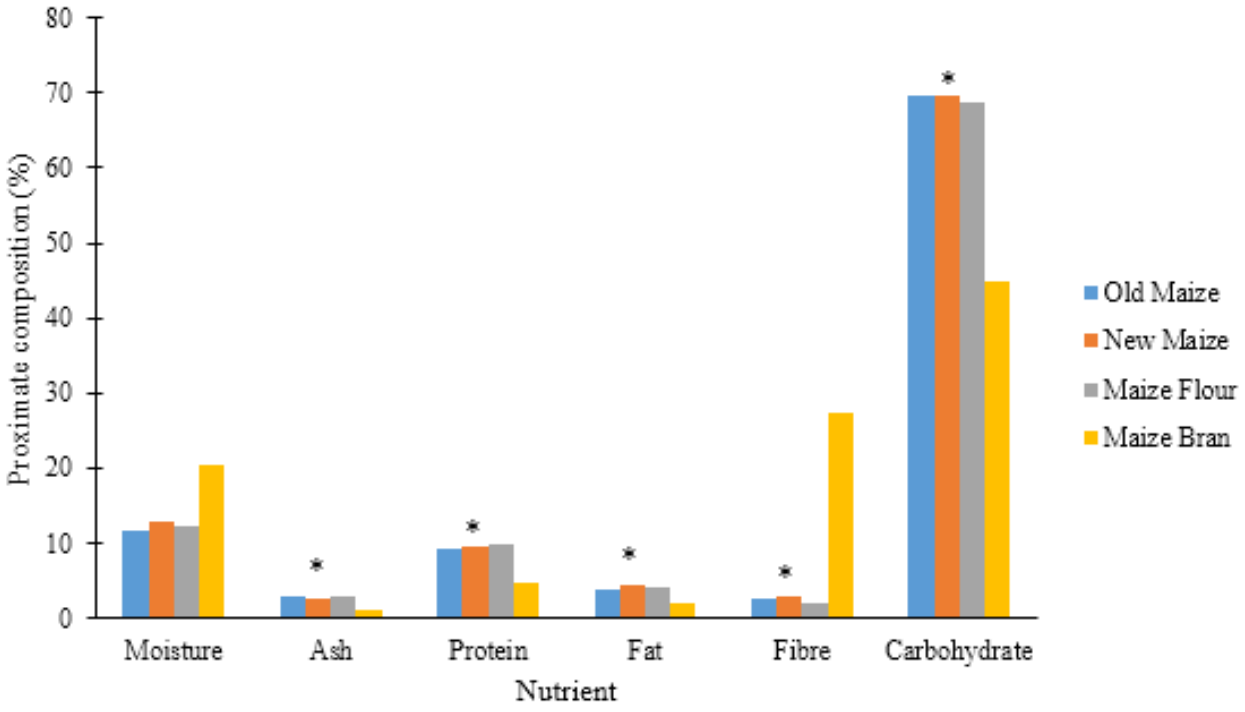


Figure 4.2Proximate composition of all maize products at 4weeks post storage

Table 4.2 Mineral composition of all maize products

Nutrient	Mean ± SEM				F	*p*
	Old maize	New maize	Maize flour	Maize bran		
Calcium	1.10 ± 0.37	0.63 ± 0.04	0.97 ± 0.53	1.75 ± 0.30	1.710	0.242
Manganese	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.11 ± 0.05	8.804	0.006*
Magnesium	35.18 ± 1.98	33.88 ± 1.50	29.33 ±4.01	47.05 ± 1.57	18.466	0.001*
Zinc	0.18 ± 0.06	0.20 ± 0.08	0.10 ± 0.04	0.47 ± 0.05	7.984	0.009*
Iron	2.05 ± 0.08	1.85 ± 0.38	1.86 ± 0.43	3.09 ± 1.84	0.373	0.775
Copper	0.61 ± 0.41	0.08 ± 0.03	0.10 ± 0.04	0.37 ± 0.08	1.472	0.294
Phosphorus	31.67 ± 6.01	55.67 ± 9.24	23.00 ±7.94	85.00 ± 7.64	12.718	0.002*
Sodium	1.80 ± 0.53	2.37 ± 0.09	1.50 ± 0.25	4.43 ± 0.15	18.781	0.001*
Potassium	15.67 ± 2.89	16.83 ± 3.63	10.67 ± 2.19	33.50 ± 3.50	10.208	0.004*

* = significant difference exists at $p \leq 0.05$

Table 4.3: Means of mould count (Cfu/g) of maize and maize products

MARKET	OLD DRIED MAIZE		FRESH DRIED MAIZE		MAIZE FLOUR		MAIZE BRAN	
	Range (Log_{10})	Mean (Log_{10})	Range (Log_{10})	Mean (Log_{10})	Range (Log_{10})	Mean (Log_{10})	Range (Log_{10})	Mean (Log_{10})
Giwa	1.6990-2.4472	2.0569	2.000-2.176	2.0792	2.3222-2.4914	2.4281	1.2553-2.4314	2.3454
Kawo	2.3222-2.4771	2.4281	1.9542-2.1761	2.0755	2.2553-2.4914	2.415	2.3414-2.5185	2.4698
Soba	1.8451-2.2788	2.1173	2.2553-2.5185	2.4249	2.1139-2.5441	2.3874	2.2303-2.5441	2.2553
Kakuria	4.1451-4.6128	4,2000	2.0000-2.3522	2.2014	2.1451-2.3424	2.7709	3.2788-5.3424	4.9685
Makarfi	1.9031-2.3010	2.0792	2.1139-2.4314	2.3979	2.2304-2.4771	2.3541	2.2041-2.4624	2.3617
Samaru	1.9542-2.2304	2.1703	2.2304-2.6232	2.5441	2.0414-2.6232	2.3010	2.1139-2.2041	2.1818
Beichi	1.0000-1.6990	1.5563	1.9031-2.2304	2.0934	1.9542-2.5441	2.2068	2.0000-2.1761	2.0792
Central	1.9138-4.9903	3.8149	1.1761-2.7243	2.2695	4.5441-4.9031	4.5798	1.7782-3.9243	3.4065

* Values are means of 10 replicates

The effect of seasonal change on mould counts is as presented in Table 4.4. A significant high count of $\log_{10}$ 2.89 ±2.25 , $\log_{10}$ 1.81 ± 1.10 , $\log_{10}$ 3.79 ± 3,23 and $\log_{10}$2.88 ± 2.59 for old maize, new maize, maize flour and maize bran respectively were recorded in wet season. While a lower count of $\log_{10}$ 1.26 ± 0.14, $\log_{10}$ 1.09 ± 0.08, $\log_{10}$1.34 ± 0.23 and log10 1.28 ± 0.21 for old maize, new maize, maize flour and maize bran were recorded in dry season respectively

The tentative identification of the fungal isolates to genus level based on colonial appearance on Potato Dextrose Agar (PDA) and microscopic characteristics using wet mount preparations in lacto phenol cotton blue stain and observed at x 40 objectives is as shown in Table 4.5Their colonial morphology on PDA after 5 days incubation was characterized by radial fissures with some having smooth and entire edges while the microscopic morphology revealed them as having long/smooth conidiophores, globose vesicles and conidia head being biseriate or uniseriate.

The frequency and distribution of mould isolate from maize and maize products is shown in Table 4 .6. A total of five mould genera were identified, *Aspergillus*, *Penicillium*, *Fusarium*,*Rhizopus* and *Botrytis*.*Aspergillus*accounted for 62% of the mould types encountered in the study .other mould types are *Fusarium*,(3%) *Penicillium* (6%),*Rhizopus* (22%) and *Botrytis* (7%). *Botrytis* occurred least followed by *fusarium*.

Within the *Aspergillus* species identified and shown in Table 4.7.*Aspergillus niger* was the most commonly isolated with a percentage occurrence of (47%),followed by *Aspergillus tamari* (30%) and *Aspergillus flavus* (23%).

Table 4.4: Effect of seasonal changes on mould counts.

Product	Mean ± SEM		t-value	p-value
	Wet season (log_{10})	Dry season(log_{10})		
Old maize	2.89 ± 2.25	1.26 ± 0.14	4.336	0.001**
New maize	1.81 ± 1.10	1.09 ± 0.08	4.042	0.001**
Maize Flour	3.79 ± 3.23	1.34 ± 0.23	3.623	0.001**
Maize bran	2.88± 2.59	1.28 ± 0.21	1.902	0.068

** = significant difference exists at $p \leq 0.05$

Table 4.5Cultural and morphological characteristics of the mould isolates

Isolates	Colour on PDA	Growth rate	Vesicle	Conidia head	Conidiophores	Possible organism
1	black	rapid	globose	biseriate	Long,smooth conidiophore	*A niger*
2	Brown	rapid	Semi globose	uniseriate	Longsmooth conidiophore	*A tamari*
3	Green	Rapid	Semi globose	biseriate	Long and smooth	*A flavus*
4.	Leafy green	Rapid	globose	biseriate	Long smooth and branded conidiophore	*Penicillium* spp.
5	Spongy wooly dark brown	Rapid	globose	biseriate	Simple, long and branched conidiophore	*Rhizopus* spp
6	Pinkish brown	Rapid	globose	biseriate	Long, smooth and branched conidiophore	*Fusarium* spp
7	Gray	Rapid	Semi globose	biseriate	Long,smooth condiophore	*Botrytis* spp.

Source of guide: Mycological Atlas (Klich, 2002).

Table 4.6. The frequency and distribution of moulds isolated from maize and maize products

	Frequency and distribution of isolated mould (%) n = 157					
Sample	***Aspergillus Spp***	***Fusarium Spp***	***Penicillium Spp***	***Rhizopus Spp***	***Botrytis Spp***	**Frequency**
Old Maize	21	1	3	6	2	33
New Maize	24	2	3	11	4	44
Maize Flour	20	ND	1	4	1	26
Maize bran	32	2	2	14	4	54
Total	97	5	9	35	11	157
Percentage (%)	62	3	622	7	100	

ND – Not Detected

Table 4.7. The frequency of occurrence of *Aspergillus* species isolated from maize and maize products.

Aspergillus species	**Maize**	**Maize products**	**Frequency**	**percentage%**
Aspergillus niger	27	19	46	47
Aspergillus tamari	22	7	29	30
Aspergillus flavus	9	13	22	23
Total	**58**	**39**	**97**	**100%**

The aflatoxin producing ability of the mould isolates is shown in Table 4.8. A solvent front of 4.5 cm was recorded and a distance of 2cm travelled by the compound was recorded with a retention factor (R_f) of 0.38, 0.53 and 0.44 for *Aspergillus niger*, A .*tamari* and *A. flavus* respectively

The mean aflatoxin concentration of the maize and maize products obtained for this study is shown in Figure 4.3. One hundred and fifty two (82.70%) of the maize products had aflatoxin contamination above the tolerance level of 20ppb fixed by World Health Organization (WHO, 1979). The old maize had a mean aflatoxin level of 177ppb while the new maize had a mean aflatoxin level of 102ppb.The mean aflatoxin level in maize flour was 174ppb while the maize bran had a mean aflatoxin level of 213ppb.

Table 4.8.Aflatoxin producing ability of mould isolates.

Organism	Solvent front	Distance travelled by compound	Retention factor (Rf)	Distance travelled by standard	Toxin produced
A. niger	4.5	1.6	0.36	2.0	ND
A. tamari	4.5	2.4	0.53	2.0	ND
A. flavus	4.5	2.0	0.44	2.0	Aflatoxin
Penicillium spp.	ND	ND	-ND	ND	ND
Fusarium spp	ND	ND	ND	ND	ND
Rhizopus spp	*ND*	*ND*	*ND*	*ND*	*ND*
Botrytis spp	*ND*	*ND*	*ND*	*ND*	*ND*

ND: Not detected.

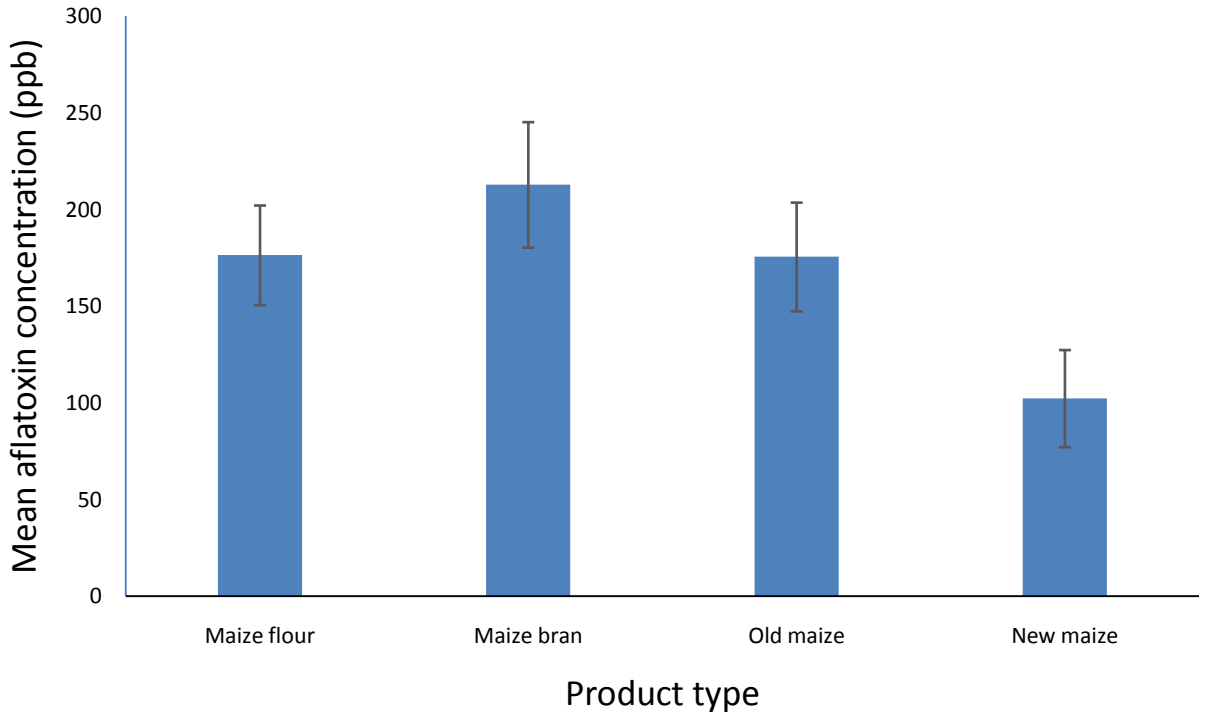


Figure 4.3: Mean aflatoxin concentration in part per billion (ppb) of maize and maize products

Chapter 5

DISCUSSION

The contamination process of maize is usually influenced by numerous factors, such as specific conditions of temperature, relative humidity, and moisture content which arise during storage. These may contribute to the rapid deterioration of cereals by promoting fungal growth (Fancelli, 1983). The main fungal species which contaminate maize are *Aspergillus flavus,Aspergillus parasiticus , Asperillus niger and Aspergillus nominus* etc .These fungal species according to Sanchis and Magan (2004) grow well in the temperature range of 19-35^{o}Cand produce maximum aflatoxin at 28^{o}C.Similarly Muthoni *et al*. (2009)in there findings put the optimum condition for growth and subsequent production of aflatoxin to include a moisture contents of 14% and temperature of 28 -30^{o}C.

Hocking (1997) reported a temperature of 32-33^{o}C as the optimum temperature required by aflatoxin producing organisms .In this study a temperature range of 24-35^{oC} recorded was in line with some other researchers (Viviane *et al*., 2008). The relative humidity recorded in this study was in the range of 35-61%. This was below the range of relative humidity of 83-85% reported as optimum relative humidity by (Christen *et al*., (1977) for growth of aflatoxin producing organism such as *Aspergillus flavus.*

Gong *et al*(2009) reported that temperature, relative humidity and amount of rainfall may jointly be responsible for the level of contamination of corn grains in China. Earlier FAO (2001) concluded that the metrological parameter recorded on different geographic region during pre-harvest and harvest period in China were found to highly influence fumonisin level in corn.

The preservation of grain at farm level is of great importance, this is a difficult task particularly in a tropical area where drying and storage technologies are deficient or completely lacking. In stored grain, insects and fungi can cause some quantitative and qualitative losses which have grave implication for the availability of food. In this study grading using quality of maize showed a higher percentage of wholesome grain (98.02%) for new maize,and (97.64%) for old maize. The percentage of broken or damaged recorded 1.48% and 0.60% for new maize and old maize and 6.24 and 2.98% damaged for old maize and new maize respectively.

The observed difference in the maize quality contamination may be attributed to prior treatments, shelling method employed, cleaning and physical sorting given to the produce before packaging for sale. Shelling involves pounding of corn cobs in a mortar, use of hands and hitting the corn cobs packed in a bag. The degree of breakage may vary depending on method used and the result obtained.Shelling of maize using manual pounding break the grains exposing them to penetration by pathogen through the broken site. Threshing by hand, drying on raised wiremesh and storage in raised and aerated stores has been found to reduce chance offungal contamination Kabaka *et al*. (2006). The use of insecticide may be responsible for low insect damaged grains recorded in this study. Studies have shown that aflatoxin contamination is concentrated in some specific maize types. According to Christesen and Kanfman (1969), injured and broken maize are more susceptible to fungal attack than whole maize. Rambo*et al*, (1974) observed a higher incidence of *Aspergillus flavus* in injured maize kernels.

The observed results in this part may possibly be responsible for the lower mould counts recorded. The percentage whole to broken or damaged in this study was similar observation reported by Mamphhuli (2007) that damaged kernels increase fungal contamination

The moisture content of the maize and maize products used in this study ranged from 1.63 – 20.33% with old maize having a moisture content of 11.63%, 12.80 % for newly harvested maize, 12.33 %for maize flour and the highest being the maize bran having a moisture content of 20.33%. Similarly, Samir *et al.*(1998) recorded a moisture content of 9 – 19%, which is consistent with the result obtained in this study.

Aisha and El-tinay (2004) found the moisture content value in 12 corn genotypes in the range of 4.6 – 6.7% which is not in agreement with this result. The observed differences may be possibly due to the varieties in maize, environmental factor and agronomic practice. The high moisture content in maize bran may be attributed to its ability to absorb water and prevent desiccation.

Percentage ash content in different maize varieties were found in the range of 0.7 – 1.3%. Maziya Duxton *et al*., 2000, found the ash content of maize in the range of 1.4 – 3.3% which is in agreement with the present study. The maize bran ash content obtained in this study was 1.16% which is lower than the findings of some other researcher who put the ash content of maize bran at 5.1% (Wilson, 1987; Mlay *et al*., 2005). Maize bran is an important source supplemental protein and energy for ruminants (Gohl, 1981).

Protein is the second largest chemical component of maize. Percentage protein in maize was found in the range of 9.27 – 9.87. IJabadenyi and Adebolu (2005) found in their study the percentage protein content of three maize varieties grown in Nigeria in the range of

10.67 – 11.25 which is not in agreement with the present study. However, the result obtained in this study is in agreement with the protein content of cereal in literature which is put at about 10% (Passmore and Eastwood, 1986; FAO, 1989).

The higher protein content of 9.87% in maize flour is not surprising as the endosperm, which is from the bulk of maize flour is the site of protein storage in maize (Woo *et al.*, 2001).

The percentage fat obtained for maize and maize products in this study was in the range of 3.83 – 4.13 with maize bran having the least constituent of fat. This finding is in agreement with other researchers (Matilda *et al.*, 1993; Ikenie*et al*., 2002). This slightly differs from the findings of Ijabadenyi and Adebolu, (2005) that found higher fat content in the range 4.17 – 5.0%. The observed differences may possibly be genetic or environmental factors.

Crude fibre was found to be the fourth largest chemical present in maize grain after carbohydrate, protein fat and moisture content. Percentage fibre was put at a range of 0.8 – 2.32%, but the result of fibre content obtained in this study wasin the range of (2.10 – 2.99).The result however is agreement with the findings of Ijabadenyi and Adebolu (2005) who reported a fibre content in the range of 2.07 – 2.97, for maize variety grains in Nigeria.

The maize bran has higher percentage of fibre content (26.77%). This result is in agreement with the findings of Mlay *et al,* (2005) who found the fibre content in maize grain to be 31.9%. Maize bran has been found to be very effective in decreasing faecal transit time.

The major constituents of maize are carbohydrate which account for 67.6 – 69.6% obtained in this study. Carbohydrate content in maize and maize products obtained for this study

varies, with old maize having the highest carbohydrate content, followed by maize flour then new maize and the least maize bran. The results in this study were similar to the findings of other researchers (Ayatse *et al.*,1983; Ijabadenyi and Adebolu (2005) who reported a similar value of 65.63 – 70.23% carbohydrate contents of maize grown in Nigeria. However Wilson *et al* (1999) had a slightly higher carbohydrate content of about 72-73% of maize kernel. The composition of maize starch was genetically controlled.

The carbohydrate content of the maize bran in this study was 44.82 – 48.67%, this was found to be lower than the findings of some other researchers. Mlay et al. (2005) recorded a higher carbohydrate content of 73.3%, the observed difference may possibly be due to the variety of maize from which the pericarp (bran) is removed.

The mineral composition of maize and maize products showed that old harvested maize is rich in magnesium (35.18%) phosphorus (31.67%) potassium (15.67) but with a low concentration of calcium (1.10%) manganese (0.03%) zinc (0.18, iron 2.05% copper 0.6 and sodium 1.80%. Whereas the newly harvested maize showed a higher concentration of these elements while low concentration of calcium, manganese, iron copper and sodium were found in low quantity. This result is in agreement with the findings of Oshodi *et al.* (1999). They concluded that these elements with high concentration are the most abundant mineral in Nigeria agricultural products. Similarly Hussaini *et al.* (2008) showed that Nitrogen fertilizer application up to 60kg/Na ha^{-1} significantly increased the concentration of nitrogen, phosphorous magnesium and potassium. This observed higher concentration of these elements in our study may possibly be due to application of fertilizer during cropping. The low concentration of calcium and zinc in recorded in this study tallies with the findings of Matilda *et al*.(1993),who found that cereal are poor in these minerals.

However, the observed differences in mineral composition in these products may be due to genetic factor and environmental factors like irrigation frequency, soil composition and fertilizer used. (Ikram et *al.,* 2010)

The maize flour which is a product of maize have a higher concentration of most of these mineral elements (phosphorous, magnesium, potassium) and low concentration of the other elements that are found to be low in old dried maize and fresh dried maize maize. The reason for this may possibly be genetic and environmental influences.

However, the maize bran used in this study was found to contain much higher phosphorus (85%) magnesium (47%) and potassium (33%). Most of the nutritionally important minerals are often located in the maize bran a factor to be reckoned with notably in milling of maize. Eating whole maize is found to be more beneficial, as the removal of the bran to make flour resulted in removing the vital component of the maize.

A low total mould count was obtained in most of the maize and maize products. The mould contamination level of most of the products was within the standard regulatory limit of (10^2–10^4 cfu/g), a tolerance limits recommended by the International Commission on Microbiological Specification (1980) and United Nation Food and Agriculture Organization (FAO, 1997).

Mould contamination were observed in all the maize and maize products. The mould counts results compararatively showed that maize bran had higher counts, followed by maize flour, the old maize and the least with freshly harvested maize. The marked differences between samples in their mould count from one market to the other may be explained by difference in weather factor during storage. It is well established that pre-

harvest weather factor influence the mycobiota of freshly harvested grain but growth and ecological succession of species of fungi in response to prevailing storage conditionis is said to dictate the kind of fungi and the extent of their contamination. Muthoni *et al.* (2009) had reported variation in fungal contamination based on agro ecological zone.

The observed low counts in this study is in agreement with Ayelow Mamed (2002) who also reported that the level of fungi counts in cereal from Ethiopia were much lower than some other tropical countries. The low mould count observed in this study may equally be attributed to the high quality of grain, following the results obtained for quality assessment of grain in this study.

A significantly higher total counts ($P \leq 0.05$) of mould were observed in all maize and maize product in wet season compared to the sample in the dry season. This might be due to the high moisture content during the wet season that encouraged growth of moulds than in the dry season with lower moisture content.Similarly the high total mould counts observed in the wet season had previously been reported (Rocha *et al.*, 2009). The finding was attributed to relatively high humidity in wet season which encouraged the germination and colonization of these moulds. Bankole and Ikotu (2002) in their finding concluded that period of sampling during rainy season (May – October) with usually high relative humidity resulting in increased moisture content could be responsible for the increased mould count during rainy season.

Fungi isolated and identified comprise of field and storage fungi. Five fungi genera comprising of *Aspergillus spp, Penicillium spp*, *Rhizopus spp, Fusarium spp* and *Botrytis spp*. were identified. The infestation by these genera of fungi in maize had previously been

reported by other researchers (Almeida *et al.*, 2000, Bankole andKpodo 2005) .Many other researchers have reported the occurrence of these fungi genera in maize. In South Africa (Marasas*et al.,* 1981), Cameroon (Ngoko, et *al*., 2001), and in Nigeria (Onyeke and Nelson 1992).

Muthoni *et al.* (2009) had also reported *Aspergillus* and *Pencillium spp.* as common storage fungi. These fungi genera are well known to be maize contaminants worldwide (Miller, 1995; Joseph and kolapo, 2008).

The distribution of fungal isolate, in this study showed that *Aspergillus* species was the most dominant of the fungi genera isolated. Three species of Aspergllus was isolated (.*Aspergillus niger (47%),Aspergillus tamari* (30%) and *Aspergillus flavus* (23). Maize infestation by these mould are common and widespread occurrence have previously been reported by Amadi and Oso (1996); Almeida *et al.,* (2000), Kpodo and Bankole(2005).This may be due to wide range growth requirement of this species and also its ability to grow on a minimal medium. This finding is in agreement with the earlier report of Bankole *et al.*,(2003). *Aspergillus* species increased over all studied sampling periods. This finding is also in agreement with Pitt and Hocking (1997) and Zimmerli and Dick (1996), who had earlier established *Aspergillus* genera predominance over other genera in tropical environments. Among the *Aspergillus* species, *A. niger* was found to be dominant mould concurring with Klich *et al*., (2007) that it was more common in some years and its incidence was higher than that of *A. flavus*. The presence of both *Aspergillus* species in the same grain is very common. Previous studies had shown that *A. niger* frequently co-occurred with *A. flavus* in the field and this is attributed to agro ecological conditions of the field. The presence of both *Aspergillus* species in the same grain is very common

According to Jelene *et al*., (2013) irrigation caused higher incidence of *Aspergillus niger* than drought while the reverse situation favours *Aspergillus* flavus.The distribution of the mould isolates in maize and maize product observed in this study may be traced to inadequate post harvest handling practices, the ubiquitous nature of these moulds and environmental factors (Ibeh *et al.,* 1991)

Similarly, Rossetto *et al. (*2005) attributed this high frequency of *Aspergillus* species to great adaptation of this fungus to the substrate, especially during storage, the high frequency of Rhizopus spp. observed in maize bran may be as a result of the contact of this substrate with the soil. Among the factors that are known to influence the distribution of these mould are moisture, temperature, relative humidity and aeration, thus the type of mycoflora associated with food and feedstuff in a locality depend very much on climate. Of all the factors moisture and temperature are the most important environmental factors.(Lillehoj, 1993)

The identified mycoflora of maize and maize products have five different fungal genera, *Aspergillus,Rhizopus Fusarium*, *Penicillium* and *Botrytis*. These genera are known contaminants of maize in Nigeria and other parts of the world (Castella,*et al;* 1996).Of these five genera, *Aspergillus species* was the most isolated. The high level of *Aspergillus species* found in this study compared well with earlier reported occurence in pre-harvest maize in Nigeria (Bankole*et al.*,2003). It is likely that pre harvest Infection greatly influenced mycoflora in storage (Hell *et al.,* 2003).On the other hand, the frequency of *Aspergillus species* can be explained by the fact that the fungus is considered as storage fungi .Besides *Aspergillus*, other species belonging to *Penicillium*and *Fusarium*species had been reported in Nigeria (Aja-Nwachukwu and Emeguaiwe, 1994), Benin (Hell *et*

al.,2003), and in Ghana (Kpodo *et al.*,2000). The co occurrence of toxigenic fungi on product in storage is commonly exacerbated as a storage condition becomes conducive for mycotoxin production. .

Many researchers have reported the occurrence of the three genera in maize South Africa (Marasas*et al.,* 1981), Cameroon (Odhay and Naicker, 2002), USA (Ngoko *et al*., 2001), Europe, Thailand and Philippines (Mills *et al.,* 1995; Miller, 1995, Castella *et al*.1996). These fungal genera are also well known maize contaminant worldwide (Miller 1995). *Fusarium* species is a field fungus (Miller, 1995).Thelow occurrence in this study may possibly be due to proper handling and adequate drying of the products.Studies carried out by Amadi and Adeniyi (2009) had also reported fungi including those found in this study. Storage fungi are normally present in large quantities before harvest, and are widely distributed.Contamination occurs through small quantities of spores contaminating the grain as it is going into storage equipment or from spores already present in storage structures (IRRI, 2006). The high prevalence rate of fungal species seen in this study supports the report of Bastiamelli and Le-Bas (2002) and Cheesbrough (2000) which stated that tropical countries such as Nigeria are more prone to fungal contamination of poultry feed raw materials of which maize is one.

The percentage frequency of*Aspergillus flavus (*23%)which is the main aflatoxin producer in maize. sharply contrasts the report from South East Asia. For instance in the report on maize from Vietnam recorded 92% of the samples analyzed in the study contained the fungi (Trung *et al.,* 2008).

The presence of *Aspergillus flavus*in maize and maize products does not imply an obvious occurrence of aflatoxin in the products. Factorsthat favour mould growth may also favour aflatoxin production but mould growth may occur with little or no mycotoxin production (Sheriff, 2004). The presence of *Aspergillus tamari* in this study is in line with finding by other researcher.Dorner (2008) reported it to be found in maize field in Nigeria.

Though *A. tamari* is not a major producer of aflatoxin, but has theability to produce aflatoxin(Goto *et al.,* 1997). In addition to aflatoxin, its ability to produce other secondary metabolites such as cyclo piazonic acid (CPA) which is an indole tetramic acid mycotoxin has also been reported (Joe, 1983). *A. tamari* is classified in the *A. flavus*group of fungi and often has been isolated from substrates that yield isolates of *A.flavus*.Manabe and Tsurata (1978) found that a high percentage of grain samples from Southeast Asia were contaminated with*A.tamari.*

A .niger which has the highest occurrence has been reported as one of the most common species of the genus *Aspergillus.*Some strains of *A. niger h*ave been reported to produce a potent mycotoxiin called ochratoxin. In addition to the production of these toxins,*Aspergillus* species can cause allergic reactionand Aspergillosis in human and animal(Stack and Carlson 2006*).*

Aflatoxin is an important naturally occurring mycotoxin in agricultural products. They are produced by several species of *Aspergillus.*Aflatoxin is not produced by other fungi (Frisvad *et al*., 2007).Not all strains of *Aspergillus* species produce aflatoxin.Aflatoxins are secondary metabolite produced mostly by *Aspergllus flavus, and A. parasiticus.* In this study, the *A. flavus* isolates were found to posses the ability to produce aflatoxin. Amadi

and Adeniyi (2009) and Yazdani*et al.* (2010) in their work also confirmed the ablity of *A flavus* to produce aflatoxin.

The ability of the *Aspegillus flavus* isolate to produce aflatoxin is widely documented by other researchers (Bradburn *et al.*, 1993). There are several reports on aflatoxin producing ablity of *Aspergillus tamari*(Klish*et al.*,2000). The *A tamari*isolate for this study did not posses the ability to produce aflatoxin concurring with the reportby Cotty, (1997); Frisvad*et al.*, (2007)..

Goto et al. *(1997*) in their finding found that *A. tamari*thoughnot a major producer of aflatoxin does produce aflatoxin meaning that the ability of the species to produce aflatoxin may depend on the strains. Although *A. tamari* did not produce aflatoxin in this study but its ability to produce other mycotoxin such as cyclo piazonic acid (CPA) is widely documented (Joe, 1983). CPA is an indole tetramic acid mycotoxin and has been reported as a natural contaminant of corn (Gallagher *et al.,* 1978). Contamination of food and feed by this toxin is a significant problem that can lead to economic loss.

Aspergillus niger isolated in this study do not posses the ability to produce aflatoxin, though the species has no known record of producing aflatoxin from any previous research but *Aspergillus niger* has a great economical and biotechnological value. It is extensively used for production of extracellular enzymes and organic acid such as citric acid (Baker, 2006). Even the*A. niger*has been granted the GRAS (Generally Regarded As Safe) status in certain industrial production processes by the Food and Drug Administration of the USA government (Perrone*et al.,* 2007). The discovery of *A. niger* strains producing ochratoxin by Abarca *et al.*,(1994)raised concerns not only for their biotechnological safety but also

for their food safety risk, due to their common presence in different commodities. Ochratoxin is a potent neourotoxin, teratogenic, immunosuppressive and carcinogenic properties.Recently, however, these concerns may have widened following several reports stating that *A.niger* can also produce fumonisin B2 (FB2) along with ochratoxin (Mogensen*et al*., 2010).

The inability of the tested isolates of *A. tamari* and *A.niger* to produce aflatoxins in this study may also be as a result of mutation as repeated process of sub culturing may lead to loss of mycotoxin producing ability of the organism (Gbodi, *et al.,* 1986) and could also be that the strains of these toxigenic fungi do not have the genetic capacity to elaborate the toxin (FAO, 1979).

The production of aflatoxin and the growth of responsible fungi are dependent upon factors such as temperature, humidity, handling during harvesting and conditionof storage (Smith and Moss 1985). The aflatoxin producing ability of the strain of *A. flavus* found in this study points to potentially heath hazard to human being and animal. In Nigeria exposure to aflatoxin through ingestion of contaminated food and inhalation of toxins has been linked to acute and chronic toxicity in animals. Effects such as liver damage, induction of tumor and tetratoxigenic and other genetic factor in animal are well documented.

Maize and maize products have been documented by several authors as an excellent substrate for mould growth and aflaxtoxin contamination in some West African countries including Nigeria.Maize is one of the high risk crops for aflatoxin contamination worldwide (Setamon *et al.,* 1987). Most countries of the world regulate total aflatoxin in all food at 20ppb (FAO, 1997).

The result obtained showed high level of aflatoxin contamination in all (100%) the sample following the acceptable aflatoxin level of 4ppm for maize set by standard organization of Nigeria and (82.7%) of the products with levels above 20 ppb, the acceptable level established by (FDA,1994) and (FAO,1997). Only 17.3% of the samples had levels below 20ppb which is considered as safe level for human or animal consumption.High level is said to be unacceptable and possible legal action to eliminate such products from market may be taken (FAO, 2000). Among staple cereals in the Nigerian diet, maize was reported to have the highest level of aflatoxin contamination (Bandyopadhay *et al.*, 2007).

The high level of aflatoxin contamination recorded in this study may be attributed to several factors, such as temperature and other environmental factors.

The high level of aflaxoxin contamination of maize and maize products has been widely reported by many researchers (FAO, 1979; Samson, *et al;* 1981., Kpodo and Bankole, 2005 and Groopman, 2008).

Aflatoxin contamination of food and feed are highly dependent on environmental conditions that lead to mould growth and toxin production. If commodities are stored in an improper dried state or under high humidity with inadequate protection; fungi will inevitably grow and possibly produce toxins.Duration of storage is an important factor when considering aflatoxin formation. Longer retention in storage, results in building up environmental condition conducive for *Aspergillus flavus* proliferation and production of aflatoxin (Kumar *et al*., 2008). Development of aflatoxin producing fungi in post harvest commodities is influenced by the length of time in storage. Lillehoj and Zuber (1988) and Ahmad (1993) observed that aflatoxin during storage was dependent on storage systems.

Level of aflatoxin were lower in closed metal bin which restrict air exchange and reduce oxygen level than in gunn/jutebags that allowed air flow through stored grains or seeds which is a common practice in Nigeria. Saleemullah *et al.,* (2006) conducted a research on effect of storage condition onaflatoxin production of paddy rice.After 2-3 months and after 12-18 months storage and reported an increase in aflatoxin content from 17.7 - 23.3 mg/kg during the storage period showing that longer storage time is an important factor in aflatoxin production.

The high level of aflatoxin observed in this study may be due to longer storage time. High level of aflatoxin was in 82.70% of the samples, while the aflatoxin producing organism *Aspergillus flavus* occurred in 34% of the samples in the present study is in agreement with earlier observation that mycotoxins are highly stable and could be detected long after the producing fungi may have died out or replaced by other fungi due to ecological succession.

Milling process to obtain products for human consumption has an important effect in mycotoxin reduction. Corn meal and corn flour which are the principal products of this process had lower content of aflatoxin than unprocessed grain. The present study is in agreement with this, but the by-product such as maize bran was found to be more contaminated. This is not surprising, as aflatoxin is known to concentrate in the maize bran (Pietri *et al*., 2009).

The lower aflatoxin contents in maize flour as observed in this study are in line with the findings of other researchers (Furlong*et al.,* 1999). The traditional method that involves the removal of pericarp from maize to make maize flour could simply mean the removal of fungi associated with mycotoxin production (Fandohan *et al*., 2005).

The overall high level of aflatoxin contamination reported in this study is in agreement with those reported from previous studies in Benin Republic and Nigeria (Setamou*et al*., 1987; Hell *et al.*, 2003). Similarly, such high level of aflatoxin in maize has been recorded in Kenya where an outbreak of acute aflatoxicosis was linked to the severe contamination level (Lewis *et al*., 2005).From our studies it is very likely that household maize may represent a significant source of exposure to aflatoxin in Nigeria as it was the case in Ghana where health concern have been raised (Kpodo ,1996). Maize samples collected from silo and warehouses were all reported to be contaminated with aflatoxin in the range of 20-355µg/kg. High level of aflatoxin contamination recorded in this study may have also been influenced by agronomic and biotic factors.Biological control of aflatoxin contamination through competitive exclusion of aflatoxin producers by toxigenic strains of *Aspergillus flavus* might provide the greatest reduction in contamination area wherefungal communities have the highest average aflatoxin producing potentials (Cotty, 2006).

CHARPTER 6

Summary, Conclusion and Recommendations

In this study, maize and maize products were contaminated by different fungal genera namely*Aspergillusspp. Rhizopus spp*., *Penicllium* spp.,*Fusarium* and *Botrytis* spp. *Aspergillus species* was the most common species.in the samples. The species of Aspergillus identified were *Aspergillus niger, Aspergillus flavus and Aspergillus tamari*. However *Aspergillus niger* was the dominant species. The degree of contamination by these toxigenic fungi was high and mycotoxins are expected to be present. The study showed that maize and maize products were contaminated by both storage and field fungi which might be due to poor storage and improper handing. *Aspergillus flavus* isolates possessed the ability to produce aflatoxin. The levels of aflatoxin contamination of maize and maize product were high thus suggesting that subsistence on the products as staple food may be at risk of aflatoxicosis.

Recommendation

Based on the major findings from this study the following recommendations are offered

1. The result of this study raises a major public health concern due to the high level of aflatoxin and means of minimizing or eliminating it by proper handling and processing should be encouraged.
2. To avert the hazard of aflatoxin contamination, the National Agency for Food, Drug Administration and Control (NAFDAC) and other relevant agencies in Nigeria should take steps to educate the masses on the dangers of aflatoxin contamination of maize and maize products.

3. Training programmes on appropriate techniques for harvesting, handling and storage should be developed for farmers and marketers.
4. Aflatoxin contaminations of other cereal crops such as sorghum and millet should be investigated.
5. Aflatoxin contamination should routinely monitored for food safety and economic loss.
6. Maize should be eaten in whole to achieve its maximum benefit since most of the mineral constituents are in the bran which are often removed by many.
7. Training programmes should be developed especially for farmers and agriculturists using appropriate techniques for harvesting, handling and storage.
8. Aflatoxin research and testing laboratories with trained personnel should be set up to Facilitate continuous mycotoxin monitoring in the six geopolitical zones.

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APPENDICES

Appendix 1. Mean distribution of maize based on quality from different markets.

Market	Product	Mean ± SEM				
		% whole	% Broken	% damaged	Temp	Rel. Humidity
Giwa	Old maize	95.83 ± 0.60	3.46 ± 0.52	0.82 ± 0.17	33.74 ± 0.25	36.40 ± 1.01
	Maize bran				32.80 ± 0.60	43.80 ± 1.50
	New maize	98.02 ± 0.19	1.48 ± 0.14	0.60 ± 0.07	28.30 ± 0.24	11.10 ± 0.28
	Maize flour				31.68 ± 0.45	45.17 ± 0.31
Central market	Old maize	92.94 ± 1.41	3.21 ± 0.37	2.10 ± 0.33	29.16 ± 0.61	46.70 ± 1.38
	Maize bran				30.00 ± 0.89	44.33 ± 1.48
	New maize	78.72 ± 2.23	14.70 ± 4.94	6.88 ± 1.29	31.29 ± 0.45	11.50 ± 0.54
	Maize flour				31.12 ± 0.36	44.91 ± 0.50
Kawo market	Old maize	95.98 ± 0.95	4.45 ±1.75	1.27 ± 0.42	31.20 ± 0.42	45.60 ± 0.40
	Maize bran				32.83 ± 0.40	46.50 ± 0.34
	New maize	97.80 ± 0.19	1.30 ± 0.19	0.68 ± 0.07	30.91 ± 0.36	11.30 ± 0.26
	Maize flour				33.13 ± 0.30	45.13 ± 0.35
Soba market	Old maize	96.08 ± 0.69	2.22 ± 0.50	2.00 ± 0.31	30.11 ± 0.16	46.90 ± 0.31
	Maize bran				30.42 ± 0.33	46.00 ± 0.32
	New maize	96.84 ± 0.50	2.20 ± 0.40	0.96 ± 0.17	31.30 ± 0.14	52.90 ± 0.35
	Maize flour				29.42 ± 0.18	48.00 ± 0.45
Kakuri market	Old maize	90.58 ± 1.18	6.24 ± 0.98	2.98 ± 0.47	29.88 ± 0.14	45.20 ± 0.14
	Maize bran				28.03 ± 0.71	45.50 ± 1.67
	New maize	93.00 ± 1.69	5.16 ± 1.19	1.84 ± 0.52	28.72 ± 0.15	51.70 ± 0.21
	Maize flour				26.68 ± 0.30	51.40 ± 0.34
Makarfi market	Old maize	95.78 ± 0.89	2.28 ± 0.49	1.44 ± 0.35	30.67 ± 0.16	47.10 ± 0.31
	Maize bran				29.94 ± 0.24	46.00 ± 0.55
	New maize	93.88 ± 1.79	3.70 ± 1.02	2.42 ± 0.79	32.83 ± 0.23	11.20 ± 0.36
	Maize flour				30.07 ± 0.08	46.17 ± 0.31
Samaru market	Old maize	97.64 ± 0.36	1.62 ± 0.18	0.74 ± 0.15	25.45 ± 0.05	58.40 ± 0.40
	Maize bran				26.28 ± 0.15	57.60 ± 0.25
	New maize	95.74 ± 0.79	2.88 ± 0.48	1.38 ± 0.33	26.09 ± 0.06	11.20 ± 0.25
	Maize flour				25.18 ± 0.09	57.30 ± 0.47
eichi market	Old maize	95.42 ± 0.89	3.21 ± 0.64	1.32 ± 0.36	28.91 ± 0.20	44.90 ± 0.43
	Maize bran				28.03 ± 0.13	46.50 ± 0.22
	New maize	89.60 ± 1.40	3.28 ± 0.62	7.12 ± 1.12	26.85 ± 0.16	53.20 ± 0.51
	Maize flour				28.82 ± 0.12	47.30 ± 0.26

CABI IDENTIFICATION REPORT

Our ref.: YN3/11/H167

Your ref:

Your number	IMI number	Identification and comments
160911/1	500957	**Aspergillus niger**. A common and widespread species. *Aspergillus niger* has been reported to produce toxins including malformin and naphthopyrones, and some strains produce ochratoxin. The species is classed in ACDP hazard group 1. This strain shows >99% homology in calmodulin gene sequence to authentic samples of the species. This material will be discarded.
160911/2	500958	**Interim report.** This sample has been processed using ITS DNA sequencing. Top matches at 100% identity were to *Aspergillus tamarii* and members of the *A. terricola* group. Species within these groups can be distinguished by sequencing of the beta-tubulin gene. Additional sequencing is in progress and a further report will follow as soon as possible.
160911/3	500959	**Interim report.** This sample has been provisionally identified as *Aspergillus* sp. Colonies were dark olive-green in colour and produced blackish sclerotia in culture. From observations of morphology, this appears close to the *Aspergillus oryzae / A. parasitic* group. Sequencing is in progress to determine the identity and a further report will follow as soon as possible.
160911/4	500960	**Aspergillus niger**. This strain has an identical calmodulin gene sequence to IMI 500957. This material will be discarded.
160911/5	500961	**Aspergillus niger**. This strain has an identical calmodulin gene sequence to IMI 500957. This material will be discarded.

CABI IDENTIFICATION REPORT

Our ref.: YN3/11/H167

Your ref:

Your number	IMI number	Identification and comments
160911/2	500958	**Aspergillus tamarii**. This sample was processed using DNA sequencing and produced multiple top matches at 100% identity to *Aspergillus tamarii*. A number of the top matches have been cited in peer-reviewed publications including: Rodrigues, P. *et al.* Species identification of *Aspergillus* section Flavi isolates from Portuguese almonds using phenotypic, including MALDI-TOF ICMS, and molecular approaches. *J. Appl. Microbiol.* **111** (4): 877-892 (2011). The morphological characteristics of this isolate are also consistent with the taxonomic description of this species as given in Klich M. (2002) *Identification of Common Aspergillus Species*, 96-97. This is a widespread species especially in the tropics, with brown to olive colonies and large thick-walled roughened conidia. It is frequently isolated from soil, seeds and other plant-based substrata. Toxic metabolites include cyclopiazonic acid and it is placed in ACDP hazard group 1. This material will be discarded.
160911/3	500959	**Aspergillus flavus.** A sequence was not obtained for this sample as the DNA failed to amplify despite a number of attempts. On the basis of morphological observations, this sample has been identified as *A. flavus*. This belongs in a group of Aspergilli which includes the closely related species, *A. oryzae*, and *A. parasiticus*. *A. flavus* has larger vesicles than *A. parasiticus* (those observed were mostly up to 40µ), and condia were observed to be globose to ellipsoidal and finely roughened. (*A.parasiticus* has more or less globose conidia which are coarsely roughened). Also a number of conidial heads were observed to be biseriate while those of *A. parasiticus* are reported to be mostly uniseriate. *A. oryzae* was discounted on the basis of the small size of conidia. Those observed were mostly 3.5-6µ wheas in *A. oryzae*, condia are larger, up to 8µ in diameter. Black sclerotia were observed in 4-week old cultures grown on CYA, a feature which can occur in all three species. *A. flavus* is very widespread and commonly isolated from indoor and outdoor environments including soil, air, food products etc. Many strains produce both B and G aflatoxins. *A. flavus* is classed in ACDP hazard group 1. For a description, see Raper, K.B. and Fennell, D.I. (1965) *The genus Aspergillus*. Williams and Wilkins, 361-36. This material will be discarded.

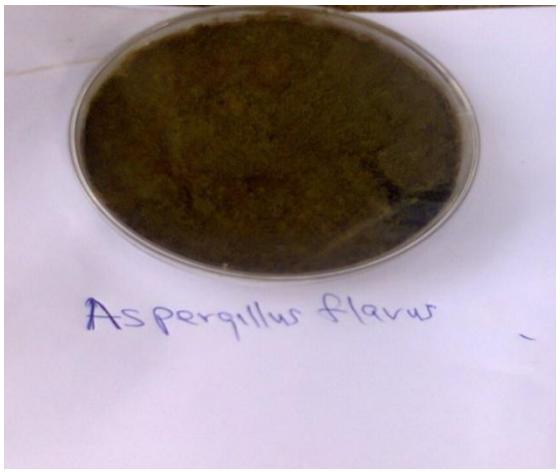
Appendix 3



Aspergillus niger



Aspergillus tamari



Aspergillus flavus

Plate1.Commparative/cultural appearance of the mould species

Appendix 4: Mean values of the proximate composition of maize and maize products

Nutrient	Mean ± SEM				F	*p*
	Old maize	New maize	Maize flour	Maize bran		
Moisture	11.63 ± 0.54	12.80 ± 0.42	12.33 ± 1.17	20.33 ± 4.53	2.961	0.098
Protein	9.27 ± 0.17	9.45 ± 0.04	9.87 ± 0.13	4.85 ± 0.09	391.728	< 0.0001*
Fat	3.83 ± 0.09	4.43 ± 0.09	4.13 ±0.07	2.17 ± 0.09	147.880	< 0.0001*
Ash	2.95 ± 0.12	2.66 ± 0.05	2.81 ± 0.02	1.10 ± 0.06	135.413	< 0.0001*
Fibre	2.70 ± 0.06	2.99 ± 0.02	2.10 ± 0.06	26.77 ± 0.15	20794.535	< 0.0001*
Carbohydrate	69.61 ± 0.42	67.71 ± 0.38	68.75 ± 1.08	44.82 ± 4.67	24.522	< 0.0001*

* = significant difference exists at $p \leq 0.05$