FUNGI AND AFLATOXIN CONTAMINATIONS IN SOME SPICES SOLD IN SELECTED MARKETS IN KATSINA STATE, NIGERIA

BY

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BOTANY.

DEPARTMENT OF BIOLOGICAL SCIENCES,
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NIGERIA.

AUGUST, 2015

DECLARATION

I declare that the work in this Thesis entitled "FUNGI AND AFLATOXIN CONTAMINATIONS IN SOME SPICES SOLD IN SELECTED MARKETS IN KATSINA STATE, NIGERIA" has been carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Mustapha HARUNA _____ _____

Signature Date

CERTIFICATION

This thesis entitled "FUNGI AND AFLATOXIN CONTAMINATIONS IN SOME SPICES SOLD IN SELECTED MARKETS IN KATSINA STATE, NIGERIA" by Mustapha HARUNA, meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to the knowledge and literary presentation.

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DEDICATION

I dedicate this thesis to my Family.

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ABSTRACT

Dried, raw, powdered and processed samples of fourteen different spices were randomly collected from five markets in Katsina state, Nigeria. The markets were Yar'kutungu, Tsohuwar kasuwa and Katsina central market in Katsina metropolis (Sudan savanna), Funtua and Kafur markets (Northern guinea savanna). The spices were Clove, African nutmeg, Ashanti pepper, Candlewood, Ethiopian pepper, Pineapple of the bush, Ginger, Garlic, Chillies, Thyme, "Yaji", Curry and Dadawa which were selected based on availability and usage popularity. Agar plate method and Standard Dilution method were used in studying fungi. Samples were cultured on PDA supplemented with chloronphenicol and later sub-cultured and identified according to morphological and microscopic characteristics using standard identification keys. Seven different fungal genera and 12 species were isolated and identified as *Aspergillus flavus, Aspergillus parasiticus, Aspergillus fumigatus, Aspergillus versicolor, Aspergillus niger, Aspergillus terreus, Phoma glomerata, Rhizopus stolonifer, Penicillium citrinum, Nigrospora sphaerica, Rhizoctonia sp, and Mucor hiemalis. A. flavus and A. parasiticus* were most dominant. Aflatoxin Assay was carried out using ELISA (Enzyme-linked Immunosorbent Assay) technique. AgraQuant Total Aflatoxin Assay test kit and Mycosep immuno affinity columns were used for aflatoxin assay. Thirty three (47.1 %) out of seventy (70) samples analysed were contaminated by aflatoxin in the range of 0.1->20 µg/kg, some above EU and NAFDAC limits while 37 (52.9 %) samples tested negative for aflatoxin contamination. Spices from the five markets were contaminated by different fungi and 33 samples contained aflatoxin. Monitoring of aflatoxin contamination by regulatory agencies is recommended.

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CHAPTER ONE

1.0 INTRODUCTION

Mycotoxins are secondary metabolites produced by fungi which contaminate a large variety of food, with toxic effect on animals and humans (Ghali *et al.*, 2008). Many fungi produce mycotoxins, *Aspergillus, Penicillium and Fusarium* species are among the most common producers (Rustemeyer *et al.*, 2010). Many Plants used by man may be contaminated by these mycotoxins in two ways: fungi growing as pathogens on plants or growing saprophytically on stored plants (Glenn, 2007). These fungi are capable of growing on a great variety of food commodities and animal feed material when the conditions of temperature, relative humidity and product moisture are favourable (Lamanaka *et al.*, 2007; Rosi *et al.*, 2007). Human food can be contaminated with mycotoxins at various stages in the food chain (Shapira and Paster, 2004). It has been reported that 5-10 % of agricultural products in the world are spoiled by mould contamination to the extent that they cannot be consumed by humans or livestock (Topal, 1993). Food contaminated by mycotoxins, when consumed by humans or animals, may cause mycotoxicosis and poisoning, resulting in death (Richard *et al.*, 1993).

Nearly 400 types of mycotoxins have been discovered since early sixties, and are generally categorized into groups based on structural similarities (Bennet and Klich, 2003) and their major toxic effects. From the standpoint of health and trade, important mycotoxins are aflatoxins, ocharatoxins, deoxynivalenol, zearalenone, fumonisin, T-Z and T-2 like toxins (Adegoke, 2004). Among all mycotoxins, aflatoxins are a group of highly toxic secondary metabolic products named as aflatoxin B1 (AFBI), aflatoxin B2

(AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Tarin *et al*., 2004; Kamkar, 2005). Aflatoxins are produced by some *Aspergillus* spp namely *A. flavus*, and *A. parasiticus* during the growth on food and or feed. *A. flavus* produces only B aflatoxin, while the other two species produce both B and G aflatoxin groups (Creppy, 2002; Sweeney and Dobson, 1998). Aflatoxins M1, M2 were originally discovered in the milk of cows fed on moldy grains. These compounds are products of a conversion process of AFB1 in the animal liver. However, aflatoxin M1 is present in the fermentation broth of *A. parasiticus* (Bhat and Vashanti, 1999).

Aflatoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive to most animal species and humans (Sharma and Marquez, 2001). Aflatoxin B1 has the highest potency as a toxin and is classified as group 1 carcinogen by International Agency for Research on Cancer (IARC) (Anonymous, 1993). The order of toxicity is AFBI> AFGI > AFB2 > AFG2 (Chiavoro *et al*., 2001). Humans are exposed to aflatoxins via risky foods such as milk and dairy products, cereals, cacao, coffee, grapevine, dried fruit seeds and various spices (Brera *et al.,* 2002 and Colak *et al.,* 2006). Spices are aromatic or pungent fragrance vegetable substances used in minute quantities to enrich alter or mark the flavour of food (Bokhari, 2007). Spices are increasingly being used in a range of meals to enhance flavour (taste), aroma (smell) and create variety in kitchen (Riordon and Wilkinson, 2008). However, spices may be contaminated with aflatoxins at every stage of development like pre-harvest, during drying, storage and transport (Elshafie *et al*., 2002; Vasanthi and Bhat,1998). Previous studies have demonstrated that spices are contaminated with various microorganisms including toxigenic moulds (especially *Aspergillus* spp.) which have aflatoxin producing potential (Fazekas *et al.,* 2005).

1.1 Statement of Research Problem

Spices pose a serious threat to health of man because of the contaminants associated with it due to poor handling practice. Spices are usually dried on the ground in the open air in poor hygienic conditions that promote more growth of moulds and production of mycotoxins (Martins *et al.,* 2001). Spices are usually kept outside in metal or plastic containers, wooden boxes, sometimes on bare floor exposing them to dust and fungal spores contamination. Spices are often added to food without further processing or are eaten raw (Llewellyn *et al.,* 1992), and may result in serious health hazards.

1.2 Justification

A number of spices are cultivated while some are imported into Nigeria. A spice may have other uses, including medicinal, religious ritual, cosmetics and perfume production. In Nigeria, several spices are routinely used in homes for cooking. Spices are usually processed in poorly hygienic form which favours growth of moulds and subsequent mycotoxin production. Most of the researches on fungi and mycotoxin contamination have concentrated more on food and feeds as well as beverages. On a global scale, the contamination of spices with mycotoxin have been reported in many countries such as Hungary (Fazekas *et al*., 2005), Bahrain (Musaiger *et al*., 2008), and Morroco (Zinedine *et al.,* 2006). Regardless of the daily use of these spices in Nigerian homes little work has been carried out on mycobiota and aflatoxin contamination. In Nigeria the only few reports on spices contamination with mycotoxins were mostly from the Southern part of the country. For example Ezekiel *et al*., (2013), in Lagos studied aflatoxin contamination in three different types of spices (Ashanti pepper, Black pepper and African nutmeg)

using Thin Layer Chromatographic (TLC) technique. While Luka *et al*., (2013), reported mycotoxin contamination in three different types of condiments (Dadawa, Ogiri and Okpehe) obtained from Jos and Kaduna markets (Northern part of the country) using ELISA technique. These spices are consumed in large quantities either fresh or processed in Katsina State. Katsina state has two different climatic conditions with the Southern part being more humid than the Northern part. The state falls within Northern guinea savanna in the southern part and Sudan savanna in the northern part of the state. There is no report on fungi and aflatoxin contamination in spices and condiments in Katsina State. Hence the need to carryout this research.

1.3 Aim

To isolate and identify fungi associated with selected spices and to screen for aflatoxin contamination in selected markets in Katsina metropolis, Funtua and Kafur.

1.4 Objectives of the Study

1 To isolate and identify fungi associated with the spices samples.

2 To determine mycotoxin contamination of spices.

3 To correlate fungi and aflatoxin contaminations of the spices between the two ecological zones

1.5 Hypotheses

1 Spices from these markets do not harbour any fungi.

2 There is no mycotoxin contamination in spices and condiments.

3 There is no difference in fungi and aflatoxin contamination between the two ecologica zones.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Spices Definition

A spice is an aromatic or pungent fragrance vegetable substance used in minute quantities to enrich, alter or mask the flavour of food (Bokhari, 2007). Many spices have antimicrobial properties. This may explain why spices such as ginger, garlic and clove are more commonly used in warmer climates, which have more infectious disease, and why use of spices is especially prominent in meat, which is particularly susceptible to spoilage (Thomas *et al*., 2012). A spice may have other uses, including medicinal, religious ritual, cosmetics or perfume production.

2.2 History of Spices

The spice trade developed throughout South Asia and Middle East since 2000 BC with cinnamon and pepper, and in East Asia with herbs and pepper. The Egyptians used herbs for embalming and their demand for exotic herbs helped stimulate world trade. By 1000 BC, medical systems based upon herbs could be found in China, Korea and India. Early uses were connected with magic, medicine, religion, tradition, and preservation (Linda, 2007). Arab merchants facilitated the routes through the Middle East and India. This resulted in the Egyptian port city of Alexandria being the main trading center for spices. The most important discovery prior to the European spice trade were the monsoon winds (40 CE). Sailing from Eastern spice growers to Western European, consumers gradually replaced the land-locked spice routes once facilitated by the Middle East Arab caravans

(Scully, 1995). Given medieval medicine's main theory of humourism, spices and herbs were indispensable to balance "humours" in food (Linda, 2007), a daily basis for good health at a time of recurrent pandemics. Spices were all imported from plantations in Asia and Africa, which made them expensive. From the 8th until the 15th century, the Republic of Venice had the monopoly on spice trade with the Middle East, and along with it the neighboring Italian city-states. The trade made the region rich. It has been estimated that around 1,000 tons of pepper and 1,000 tons of the other common spices were imported into Western Europe each year during the Late Middle Ages. The value of these goods was the equivalent of a yearly supply of grain for 1.5 million people (Adamson, 2004).

2.3 Nutrient Composition of Spices

Because spices tend to have strong flavours and are used in small quantities, spices tend to add few calories to food, even though many spices, especially those made from seeds, contain high portions of fat, protein and carbohydrate by weight. Many spices, however, can contribute significant portions of micronutrients to the diet. For example, a teaspoon of paprika powder contains about 1133 IU of Vitamin A, which is over 20 % of the recommended daily allowance specified by the United States Food and Drug Administration (USDA, 2013). When used in larger quantity, spices can also contribute a substantial amount of minerals, including iron, magnesium, calcium, and many others, to the diet. Most spices have substantial antioxidant activity, owing primarily to phenolic compounds, especially flavonoids, which influence nutrition through many pathways, including affecting the absorption of other nutrients (Ninfali *et al*., 2007). These

antioxidants also can act as natural preservatives, preventing or slowing the spoilage of food, leading to a higher nutritional content in stored food.

2.4 World Spice Producers

Many countries cultivate spices, but the major producers of spices are listed in Table 2.1. Among the world top ten spice producing countries, India is ranked the highest producer while Srilanka ranks the least.

2.5 Aflatoxins

Aflatoxins are a group of mycotoxins produced by *Aspergillus* spp, including *A. flavus*, and *A. parasiticus*. A quarter of the world's food crops are estimated to be affected by mycotoxins, creating a large economic loss in the developed and developing countries (Kumar *et al.*, 2008). Other reports indicate even higher contamination rate of aflatoxin (Njobeh *et al*., 2009). Exposure to higher levels of aflatoxin contamination increases cancer incidence, including risk of hepato-cellular carcinoma and neural tube defects especially in 6- to 9-year-old girls (Umoh *et al*., 2011). One of the reason which makes aflatoxins one of the most challenging mycotoxin is the fact that it could be produced by the responsible fungi not only at pre-harvest time but also at post harvest stages including storage. Aflatoxin B1, B2, G1 and G2 are the most important members of the aflatoxin group, which chemically are coumarin derivatives with a fused dihydrofurofuran moiety. Presence of aflatoxin B1, B2, G1 and G2 may naturally occur in different ratios depending on different matrices.

Table 2.1: World Top Ten Spice Producing Countries

	Annual Production (metric tonnes)	
Country	**Year (2010)**	**Year (2011)**
India	1,474,900	1,525,000
Bangladesh	128,517	139,775
Turkey	107,000	113,783
China	90,000	95,890
Pakistan	53,647	53,620
Iran	18,028	21,307
Nepal	20,360	20,905
Colombia	16,998	19,378
Ethiopia	27,122	17,905
Sri lanka	8,293	8,437

Source: UN Food and Agriculture Organization (FAO, 2011).

Cereals notably corn, nuts such as peanuts, pistachio and Brazil nuts, oil seeds such as cottonseed, as well as copra, the dried meat of coconut, are some of the commodities with greater risk of aflatoxin contamination (Yassin *et al*, 2011). Because peanuts, cottonseed and copra constitute the most important source of edible oils, they are of particular interest (Idris *et al*., 2010). Other commodities such as cocoa beans, linseeds, melon seeds and sunflower seeds have been infrequently contaminated with mycotoxins with lower importance rate compared to other commodities (de Magalhaes *et al*., 2011).

2.5.1 Types of aflatoxins

Although 17 aflatoxins types have been isolated (WHO, 1979), only 4 of them are well known and studied extensively from toxicological point of view. Being intensely fluorescent in ultraviolet light the four are designated by letters B1, B2, G1 and G2 representing their blue and green fluorescence in UV light. Two other familiar aflatoxins are M1 and M2 which are found in the milk of animals previously exposed to aflatoxin B1 and B2. Of all the above-named aflatoxins, aflatoxin B1 is the most acutely toxic to various species (Anonymous, 1993). Toxigenic *A. flavus* isolates generally produce only aflatoxins B1 and B2, whereas *A. parasiticus* isolates generally produce aflatoxins B1, B2, G1 and G2 (Creppy, 2002). Other metabolites B2a, aflatoxicol, aflatoxicol H1 and aflatoxins P1 and Q1 have been identified (FDA, 1979). Aflatoxin M1 is a metabolite of aflatoxin B1 in humans and animals. Aflatoxin M2 is a metabolite of aflatoxin B1 in milk of cattle fed on contaminated foods. Although aflatoxins B1, B2 and G1 are common in the same food sample, AFB1 predominates (60-80 % of the total aflatoxin content).

Generally AFB2, AFG1 and AFG2 do not occur in the absence of AFB1. In most cases AFG1 is found in higher concentrations than AFB2 and AFG2 (Weidenborner, 2001).

2.5.2 Favorable conditions for aflatoxin biosynthesis

The formation of aflatoxins is influenced by physical, chemical and biological factors. The physical factors include temperature and moisture. The chemical factors include the composition of the air and the nature of the substrate. Biological factors are those associated with the host species (Hesseltine, 1983). Specific nutrients, such as minerals (especially zinc), vitamins, fatty acids, amino acids and energy source (preferably in the form of starch) are required for aflatoxins formation (Wyatt, 1991). The limiting temperatures for the production of aflatoxins by *A. flavus* and *A. parasiticus* are reported as 12 to 41° C, with optimum production occurring between 25 and 32° C (Lillehoj, 1983).

2.5.3 Structure of aflatoxin

In an independent study in early sixties, Asao *et al*. (1963) and Van Dorp *et al*. (1963) characterized the chemical and physical nature of the aflatoxins B1, B2, G1 and G2. Chemically, aflatoxins are difurocoumarolactones (difurocoumarin derivatives). Their structure consists of a bifuran ring fused to a coumarin nucleus with a pentenone ring (in B and M aflatoxins) or a six membered lactone ring in G aflatoxins.The structure of aflatoxins BI, B2, GI and G2 are shown in Figure 1.

The four compounds are separated by the color of their fluorescence under long wave ultraviolet illumination (B=blue, G= green) (Devero, 1999).

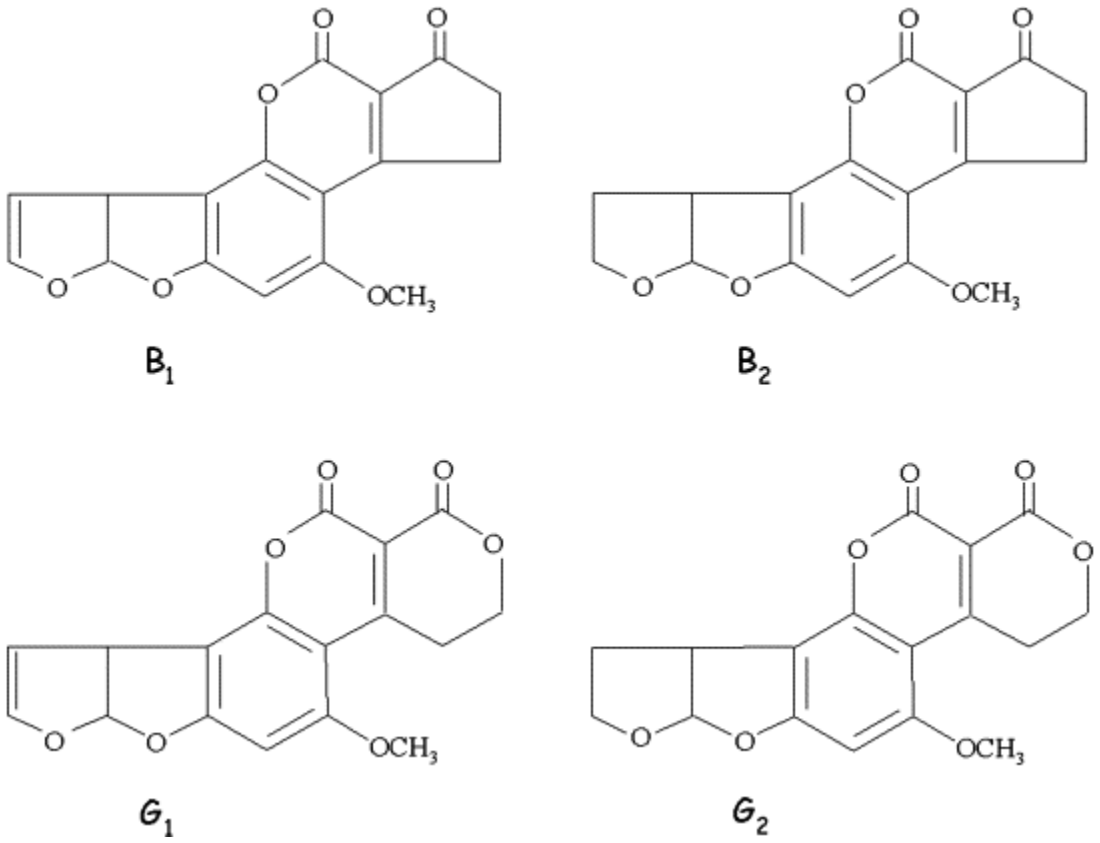


Figure 2.1: Structure of aflatoxins (B1, B2, G1 and G2)

Source: Adejumo and Adejoro (2014).

2.5.4 Physical properties of aflatoxins

These compounds were originally isolated by groups of investigators in England (Sargeant *et al.,* 1961). The molecular formula of aflatoxin B1 was established as C17H1206 and of aflatoxin G1 as C17H1207; aflatoxins B2 and G2 were found to be the dihydro derivatives of the parent compounds, C17H1406 and C17H1407 (Hartley *et al*., 1963). Some physical properties of the compounds are summarized in Table 2.2.

2.5.5 Natural occurrence of aflatoxin contamination in raw agricultural products

Natural occurrence of aflatoxins in raw agricultural products poses severe health and economic risks worldwide. The Food and Agriculture Organization (FAO**)** estimates that many basic foods could be contaminated by mycotoxin producing fungi, contributing to huge global losses of foodstuffs, about 1000 million metric tons each year (Bhat *et al*., 2010). The economic impact of feed contamination with mycotoxins include productivity reduction and organ damage (Upadhaya and Park, 2010). Mycotoxin contamination reports in animal feed indicate a variety of contamination levels (Monbaliu *et al*., 2010). Fungi which produce mycotoxin belong to *Aspergillus*, *Penicillium* and *Fusarium* species (Rustemeyer *et al*., 2010). *Aspergillus* and *penicillium* constitute a major part of the fermented feed microbiota (Roige *et al*., 2009). Animal feeds, such as hay and straw, might be contaminated during pre-harvest or drying stages (Bhat *et al*., 2010). Despite extensive research done during the last few decades, which helped authorities around the world to establish control measures, still aflatoxin contamination in food and agricultural commodities remains as one of the most challenging and serious food safety problem.

Table 2.2: Molecular Formula, Weight and Melting Point of Aflatoxins

Aflatoxin	MF	MW (g/mole)	MP (º C)
B_1	$C_{17}H_{12}O_6$	312	268-269
B_2	$C_{17}H_{14}O_6$	314	286-289
G_1	$C_{17}H_{12}O_7$	328	244-246
G_2	$C_{17}H_{14}O_7$	330	237-240
M_1	$C_{17}H_{12}O_7$	328	299
M_2	$C_{17}H_{14}O_7$	330	293

MF=Molecular formula, MW= Molecular weight and MP= Melting point

Source: www.icrisat.org/aflatoxin/aflatoxin.asp. Retrieved (08/ 12/ 2014).

2.5.6 Aflatoxin contamination in herbs and spices

Medicinal plants are various plants with medicinal properties, which were the core of traditional therapy for the most of human history. Although the toxic effect of some were known for centuries, only in the recent modern time, the safety of these plants from the contamination point of view come to light. One of the safety concerns in herbal medicine presently is the presence of mycotoxins, notably aflatoxins, as their use have been increasing in the recent years after a decline in their use for almost a century. It has been reported that spices and herbs that were used for the improvement of some forms of liver disorder might be contaminated with high concentrations of aflatoxins, with aflatoxin B1 at an alarming level of 2230 μg/kg (Moss, 1998).

Abdulkadar *et al*. (2004) in Qatar found aflatoxin B1 contamination in mixed spices powder in the range of 0.16–5.12 μg/kg, while chilli powder showed a higher range of 5.60–69.28 μg/kg. A Turkish study conducted from September 2008 to February 2009, detected aflatoxin B1contamination in 80% (48/60) of unpacked and packed ground red pepper samples within the range of 5-55.9 μg/kg (Set and Erkmen, 2010). Zinedine *et al*. (2006) in Morocco reported relatively low contamination levels in spice samples including paprika, ginger, cumin, and pepper. The highest level of aflatoxin was found in red paprika (9.68 μg/kg) (Zinedine *et al*., 2006). Close study of all mycotoxin rejected lots (211 reports of 432 at the time) from 2007 to 2011, based on online information available from RASFF, revealed that the highest aflatoxin levels were found in curry powder from Nigeria, whole nutmeg from Indonesia, dried paprika from Peru and suya pepper from Ghana, followed by paprika powder from UK (RASFF, 2011). In a study on

garlic samples, no aflatoxins were found at levels >0.1 μg/kg. However, aflatoxin levels between 4.2-13.5 μg/kg were detected in ginger (Patel *et al*., 1996). A detailed UK study of aflatoxin contamination in some spices including curry powder, pepper, cayenne pepper, chilli, paprika, ginger, cinnamon and coriander showed 95% contamination below 10 μg/kg of total aflatoxins, while only 9 out of the 157 retail samples had higher levels (Macdonald and Castle, 1996).

Contrary to the long history and the wide use of herbal medicines, there are only a few publications in regard to their mycotoxin contamination compared to the large number of publications on the contamination of cereals and oil seeds (Trucksess and Scott, 2008). The European Pharmacopeia has set limits for aflatoxin B1 and total aflatoxins at 2 and 4 μg/kg respectively, for some medicinal herbs (Pharmacopeia, 2007). Although in one study in South Africa, no aflatoxin contamination was found in some medicinal plants (Sewram *et al*., 2006), while others reported levels ranging from 2.90–32.18 μg/kg (Yang *et al*., 2005). Aflatoxins were only found in 1 out of 5 *Aerra lanata* medicinal plant samples from Sri Lanka at 500 μg/kg (Abeywickrama and Bean, 1991). In another survey in India, 60 % samples of medicinal plant seeds were contaminated with AFB1, ranging from 20 to 1180 μg/kg (Trucksess and Scott, 2008). In Thailand, five out of 28 herbal medicinal products were found to be contaminated with aflatoxins at 1.7–14.3 μg/kg (Tassaneeyakul *et al*., 2004). In Malaysia and Indonesia, 16 of the 23 commercial traditional herbal medicines (jamu and makjun) analyzed contained a low level of total aflatoxins (Ali *et al*., 2005). In a study by Hitokoto *et al*. (1978) aflatoxins were not detected in the 49 powdered herbal drugs. Ten percent of the tablets of *Cascara sagrada* dried bark were contaminated with aflatoxins in Argentina (Trucksess and Scott, 2008).

Trucksess and Scott (2008) found that 30 % of the ginseng products purchased in USA were contaminated with AFB1 at levels of about 0.1 μg/kg.

2.5.7 Mycotoxin regulation

Despite a global effort underway to harmonize mycotoxin and aflatoxin standard, there is still variation in regulation across countries. Each country sets it's acceptable limit and differs among foods and feeds. Maximum accepted levels of aflatoxins in foods and products for human consumption range from 0.5 ppb in milk to 20 ppb for processed foods (USAID, 2012). EU has set total aflatoxins acceptable limit for spices at 10 ppb and 5 ppb for aflatoxin B1 (EC, 2002). The Nigerian NAFDAC acceptable limits is 10 ppb for total aflatoxin and 5 ppb for aflatoxin B1. Acceptable limits for Animal feed set by US, EU, and NAFDAC are 100-300, 20 and 50 ppb respectively (CAC, 1995; EC, 2002; USAID, 2012).

2.5.8 Fate of aflatoxins during processing

Aflatoxins, like most of the mycotoxins, are stable compounds. Therefore, most of the processing steps during food production such as temperatures below 250 º C have little or no effect on their content, which may lead to contaminated finished products. Cooking experiments showed that aflatoxin levels in spiced sauces are not reduced by domestic cooking with either microwave or convetional gas oven heating. Menon and Xavier (2010) reported that there was no appreciable reduction of aflatoxins after microwave and gas cooking of ginger paste.

Effect of roasting on the aflatoxin content of pistachios have been recently investigated (Yazdanpana *et al*., 2005). At 200º C, most of the aflatoxins were destroyed (Hwang and Lee, 2006). Microwave field (500 MHz–10 GHz) exposure could also result in aflatoxin destruction. Both microwave power and exposure time play a major role in the extent of such destruction. Reports indicated that only 16 min exposure of contaminated peanuts to a microwave power level of 1.6 kW resulted in a loss of almost 95% in its aflatoxin content. However, there are some other processing steps such as alkaline cooking, nixtamalization (tortilla process), extrusion, roasting, flaking and modified processing methods that may reduce the aflatoxin content, but cannot eliminate the aflatoxin completely (Arzandeh and Jinap, 2011). Physical sorting is also another effective measure in the reduction of aflatoxins, as high as 40–80% (Bullerman and Bianchini, 2007). Marginal losses are considerable only if they are beyond the uncertainty of measurements at the given concentration. Some reports indicated a total destruction, of 1600 μg/kg of aflatoxin, in yellow dent contaminated corn by frying process (Magan and Olsen, 2004). Using aflatoxin degradation enzyme named myxobacteria aflatoxin degradation enzyme (MADE), obtained from the extracellular enzyme of *Myxococcus fulvus,* was proposed to be an effective decontamination material with wide temperature range, pH tolerance and reasonable cost (Ji *et al*., 2011).

Application of different microorganisms to degrade aflatoxin was started in 1960 with a positive demonstration of removing aflatoxin by *Flavobacterium aurantiacum* in milk, vegetable oil, corn, peanut, peanut butter and peanut milk.It has been shown that pH and temperature influenced the uptake of toxin by the cells. However, the bright orange pigmentation caused by *Flavobacterium aurantiacum* ,limits its application in food

(Smiley and Draughon, 2000). Other microorganisms also have aflatoxin degradation such as *Rhodococci species.*, *Lactobacillus rhamnosus and Enterococcus faecium* (Topcu *et al*., 2010).Genetically modified plants could have aflatoxin lowering potential and also other applications (Davison, 2010). Aqueous and organic extracts of plant materials such as *Tagetes minuta, Lippia javanica*, *Amaranthus spinosus* and *Vigna unguiculata* have been successfully used against *A. flavus* and *A. parasiticus* (Houssou *et al*., 2009).

Extrusion cooking is a technique that cooks the food product by heating under high pressure, while passing through continuous processing machinery, considerably reducing the food moisture content. Low transient time within the extruder can lead to limited aflatoxin loss despite high temperature and pressure (Linko *et al*., 1984). Zorlugenc *et al*. (2008) evaluated the effectiveness of the use of ozone and ozonated water on aflatoxin B1 content of dried figs. Treatment of spiked dried fig samples with aflatoxins showed higher degradation of AFB1 as ozonation time was increased in favour of the gaseous ozone compared with ozonated water (Zorlugenc *et al*., 2008). High temperature roasting of green coffee beans at 200º C for 12 min reported 79 % aflatoxin loss, which increased to 94 % as the exposure time increased to 15 minutes (Magan and Olsen, 2004). The aflatoxin reduction in coffee bean during roasting was also found to be dependent on the type and temperature of roasting with moderate reductions of approximately 42 to 56 % (Bullerman and Bianchini, 2007). Gamma radiation was also reported to decrease the total aflatoxins and aflatoxin B1 levels gradually, with increase in gamma radiation dose from 0 to 10 kGy (Gupta *et al*., 2009). However, in another study a 24-43 % reduction in aflatoxin contamination was observed with irradiation at 60 kGY (Jalili *et al*., 2010).

Gaseous ozone was effectively used against aflatoxin B1 at 13.8 mg/L (Zorlugenc *et al*., 2008).

2.5.9 Health hazards associated with aflatoxin

2.5.9.1 *Aflatoxicosis*

The poisoning as a result of consumption of substances or foods contaminated with aflatoxin is called aflatoxicosis. The disease is characterized by high fever, high colored urine, vomiting, and edema of feet, Jaundice, rapidly developing ascitis, portal hypertension and a high mortality rate. The disease is associated to the very poor, who are forced by economic circumstances to consume badly molded corn containing aflatoxins between 6.25 -15.6 ppm, an average daily intake per person of 2-6 mg of aflatoxins (Keeler and Tu, 1983).

Aflatoxins have been linked with many human diseases such as Hepatitis (Hendrickse, 1991), Cirrhosis (Lata, 2010), Hepatocellular carcinoma (HCC) (Liu and Wu, 2010), Reye's syndrome (Stora *et al*., 1983), Lung cancer (Oyelami *et al*., 1997) and Immuno-suppression and reproductive problems (Cusumano *et al*., 1996; Shuaib *et al*., 2010).

2.5.10 Methods of estimation of aflatoxin concentration

2.5.10.1 *Immunological methods*

Immunological methods are based on the affinities of the monoclonal or polyclonal antibodies for aflatoxins. Due to the advancement in biotechnology, highly specific antibody-based tests are now commercially available for measuring aflatoxins in foods in

less than ten minutes. There are two major requirements for immunological methods. First requirement is high quality antibodies and second is methodology to use the antibodies for the estimation of aflatoxins. Being low molecular weight molecules, aflatoxins cannot stimulate the immune system for the production of antibodies. Such molecules of low molecular weight, which cannot evoke the immune system, are called haptens. Therefore, before immunization, aflatoxins must be conjugated to a carrier molecule which is a larger molecule like proteins. Bovine serum albumin (BSA) is most commonly used as a carrier protein and hapten is conjugated with it. The three types of immunochemical methods are: immunuaffinity column assay (ICA), radioimmunoassay (RIA), and enzyme-linked immunosorbent assay (ELISA). Immunoaffinity columns are mainly used for clean-up purposes and RIA has limited use in aflatoxins analysis. ELISA is most commonly used for the estimation of aflatoxins (AOAC, 2002).

2.5.10.2 *Enzyme-linked immunosorbent assay* (ELISA)

The enzyme-linked immunosorbent assay is the most widely used test to detect aflatoxins, due to its simplicity, sensitivity and adaptability. There are two types of enzyme-linked immunosorbent assay, which are direct competitive enzyme-linked immunosorbent assay and indirect competitive enzyme-linked immunosorbent assay. In direct competitive enzymelinked immunosorbent assay method, specific antibody is coated to a solid phase such as a microtiter plate, whereas in indirect competitive enzyme-linked immunosorbent assay method, toxin-protein conjugate is coated onto the microtiter plate. In aflatoxin analysis, direct competitive enzyme-linked immunosorbent assay is used. The enzyme-linked immunosorbent assay is detection and quantification of

an antigen (aflatoxin) in a sample by using an enzyme labeled toxin and antibodies specific to aflatoxin. The enzyme-linked immunosorbent assay is based on antigen-antibody reaction (Aycicek *et al.,* 2005). Antigen is that substance which can elicit production of antibodies when introduced into warm blooded animals. Whereas antibodies are glycoproteins which are produced as a result of an immune response, after introduction of antigens, leading to the production of a specific antigen-antibody complex. In the direct competitive enzyme-linked immunosorbent assay, specific antibodies for aflatoxin are coated on to the wells in the microtiter strip. The test samples or aflatoxin standards are added to the wells. After incubation and washing, enzyme conjugate (a conjugate of aflatoxin and bovine serum albumin is attached with an enzyme molecule (such as, horseradish peroxidase or penicillinase or alkaline phosphatase) is added to the wells. Free aflatoxin and aflatoxin enzyme conjugate compete for the aflatoxin antibody sites in the wells. Washing step removes any unbound enzyme conjugate. Then substrate/chromogen is added to the wells and incubated. The bound enzyme conjugate converts the colorless chromogen into a blue product. The stop solution is added which leads to color change from blue to yellow. Then measurement is made photometrically at 450 nm in an ELISA reader. The absorbance is inversely proportional to the aflatoxin concentration in the sample i.e., the lower the absorbance, the higher the aflatoxin concentration (Newsome, 1987).

Other analytical methods that are used for estimation of aflatoxin include: Thin-layer chromatography (TLC), High performance thin-layer chromatography (HP-TLC), High performance liquid chromatography (HPLC) and Liquid chromatography with mass spectrometric detection (AOAC, 2000).

2.5.12 Mycotoxin control and prevention strategies

Mycotoxin control programs will result in economic gains as well as health improvement It is now realized by many developing countries that reducing mycotoxin levels in foods will confer international trade advantages as well as offer long-term health benefit to the local population (Ajoy and Priyanka, 2010).

2.5.12.1 *Education and extension*

Since the problem posed to the health and economy by mycotoxins is only popular among researchers, and is not known to a larger percentage of the populace including even the educated ones. Therefore the National agency in each country such as the National Agency for Food and Drug Administration and Control in Nigeria should initiate a program to educate people. There should be regular programs on radio and televisions on mycotoxin hazards and discussion on the issue should also feature regularly on daily newspapers and magazines (WHO, 2006).

2.5.12.2 *Seminars and workshops*

Seminars and workshops provide avenues to exchange discoveries, and get information on work going on in other laboratories. The meeting enables scientists to assess the past and present work, and streamline the areas of future studies (WHO, 2006).

2.5.12.3 *Adoption of good agronomic practices*

Agronomic practices have been shown to have profound effect on mycotoxin contamination of crops in the field. Avantaggio *et al*. (2002) found that extremely high

fumonisin contamination levels were found in maize ears visibly damaged by insects, and recommended field control of insects to reduce fumonisin contamination.

Early harvesting has been advocated as a means of reducing the risk of aflatoxin contamination. Though, many farmers are aware of the need for early harvesting, however, labour constraints, unpredictable weather, the need for cash and the threat of thieves, rats and other animals often compel farmers to harvest at inappropriate time (Amyot, 1983).

Among the recommendation for solving mycotoxin problem, rapid drying of agricultural products to low moisture is often emphasized, because all scenarios leading to mycotoxin contamination relate to non-maintenance of stored products at safe moisture content. Most African farmers spread their harvests to dry under the sun, which often require longer time (Awuah and Ellis, 2002)

2.5.12.4 *Physical separation*

Sorting out of physically damaged and infected grains (known from colourations) from the apparently healthy ones is an efficient and feasible method of reducing mycotoxin contamination. This could be done manually or by using electronic sorter. Infection of seeds or grain often imparts colourations or changes some other physical characteristics. Hell *et al*. (2000) advocated the removal of insect damaged cobs at harvest to reduce aflatoxin contamination.

2.5.12.5 *Sanitation and the use of improved storage structures*

Clearing the remains of previous harvests and destroying infested crop residues are basic sanitary measures against storage deterioration. Cleaning of stores before loading in the new harvests was correlated to reduced aflatoxin levels (Hell *et al*., 2000).

Traditional storage structures used by farmers for on the farm storage include containers made of plant materials (woods, bamboo, thatch) or mud placed on raised platforms and covered with thatch or metal roofing sheet. Essentially the stores are constructed to prevent insect and rodent attack and to prevent moisture from getting into the harvest. The adoption of high yielding varieties (mostly with poor storability) by farmers has made the traditional storage systems to become inadequate (Ajoy and Priyanka, 2010).

2.5.12.6 *Smoking*

Smoking is also an efficient method of protecting agricultural products against infestation by fungi. Daramola (1986) reported that the efficacy of smoking in protecting against insect infestation was found to be effective However, the problem with smoking is that if not carefully applied, it may discolour the product and change the taste.

2.5.12.7 *Use of plant products, synthetic pesticides and resistant varieties*

Some traditionally useful plants have been shown to exhibit fungitoxic properties. Awuah, (1996) reported that the following plants *Occimum gratissimum, Cymbopogon citratus, Xylopia aethiopica, Monodora myristica, Syzigium aromaticum, Cinnamum*

verum and *Piper nigrum* are effective in inhibiting formation of nonsorbic acid, a precursor in aflatoxin synthesis pathway.

When used in the right quantity, pesticides (insecticides or fungicides) can result in highly economic gains (Giga and Biscoe, 1989). Seed fumigation with ethylene oxide and methyl formate was found to significantly reduce the incidence of fungi including toxigenic species on stored groundnuts and melon seeds (Bankole, 1996). Kavita and Reddy (2000) reported that sodium chloride (2.5, 5.0 and 10.0 %), propionic acid (1.0, 2.5 and 5.0 %), acetic acid (1.0, 2.5 and 5.0 %) (aflatoxin inhibitors) inhibited aflatoxin B1 production in *A. flavus* inoculated groundnuts and maize kept in gunny bags. However, all treatments except sodium chloride have adverse effect on seeds germination and viability. The poor education background of the farmers often leads to misuse of pesticides.

Differences exist in the storability of different crop varieties and therefore it is preferable for farmers to grow crop varieties that have long durability in store. In most cases, this is not practicable, as most of the high yielding varieties increasingly being introduced to farmers are more susceptible to storage deterioration than the traditional varieties (Marin *et al*., 2009). Scientists at USDA have identified two maize lines that are resistant to *A. flavus* and *F.moniliforme* infection (Hamilton, 2000).

2.5.12.14 *Biological control*

Biological control can be achieved by introducing atoxigenic *A. flavus* strain to soil of developing crop. Researchers at the International Institute of Tropical Agriculture (IITA),

Cotonou, Benin, have found atoxigenic strain of *A. flavus* that is applied in the maize field in a formulation known as Aflasafe. This atoxigenic *A. flavus* strain when applied can displace harmful strains that produce large amounts of toxins (IITA, 2003).

2.5.13 Aflatoxin producing fungi

Aflatoxins are produced by some *Aspergillus* spp. which are *A. flavus* and *A. parasiticus*. However, *A. flavus* is the major producer (Bradburn *et al*., 1993).

2.5.13.1 *Aspergillus flavus*

Aspergillus flavus is a saprophytic and pathogenic fungus with a cosmopolitan distribution (Ramirez-Camejo *et al*., 2012). It is best known for its colonisation of cereal and legume grains. Post-harvest rot typically develops during harvest, storage, and/or transit. In addition to causing pre-harvest and post-harvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins, which when consumed are toxic to human and livestock (Agrios, 2005). *A. flavus* is also an opportunistic human and animal pathogen causing aspergillosis in immunocompromised individuals (Amaike and Nancy, 2011). *A. flavus* grows and thrives in hot and humid climates (Hedayati *et al*., 2007). *A. flavus* has a minimum and maximum growth temperatures of 12° C (54°F) and 48°C (118° F) respectively with 37° C (98.6° F) being the optimum growth temperature. With these temperatures in mind; *A. flavus* grows rapidly at 30-55° C, slows down at 12-15° C and it almost ceases growth at 5-8° C (Agrios, 2005). *A. flavus* growth occurs at different percentage levels of moisture for different crops. For starchy cereals, growth will occur at 13-13.2 % humidity. For Soybeans, growth will occur at 11.5-11.8 % humidity. For other crops, growth will occur

at 14 % humidity. (Agrios, 2005). With this data, it is apparent that *A. flavus* growth is prevalent in tropical countries (Amaike and Nancy, 2011).

Aspergillus flavus belongs to the kingdom fungi, phylum ascomycota, class eurotiomycetes, order eurotiales and family trichocomaceae.

Life cycle

A. flavus overwinters in the soil and will appear as propagules on decaying matter, either as mycelium or as sclerotia. Sclerotia germinate to produce additional hyphae and asexual spores call conidia. These conidia are said to be the primary inoculum for *A. flavus*. The propagules in the soil, which are now conidia, are dispersed via wind and insects (such as Stink bugs or Lygus). The conidia can land on and infect either cereals or legumes grains. The spores enter the corn through the silks and thus infect the kernel. Conidiophores and conidia are produced in the spring from sclerotial surfaces. There is a secondary inoculum for *A. flavus*, which is conidia on leaf parts and leaves. *A. flavus* grows on leaves after damage by leaf-feeding insects. Insects are said to be a source of inoculum and promote inoculum production (Hedayati *et al*., 2007).

2.5.13.2 *Aspergillus parasiticus*

Aspergillus parasiticus is also a mould known to produce aflatoxins (a potent liver carcinogen) (Horn *et al.,* 2009) although strains of it that do not produce this carcinogen exist. It is closely related to but separable to (based on DNA sequencing and Amplified fragment length polymorphism fingerprinting) *Aspergillus flavus* (Horn *et al.*, 2009). *A.*

parasiticus produces aflatoxins B1, B2, G1 and G2, unlike *A. flavus* which only produces B toxin (Anamika and Farid, 2000).

Aspergillus parasiticus belongs to the kingdom fungi, phylum ascomycota, class eurotiomycetes, order eurotiales and family trichocomaceae.

Life Cycle

A. parasiticus is disseminated via wind-borne spores but also has been shown to be transmitted through moist soil when in contact with damaged seeds (Horn *et al*., 2009). Although sexual reproduction has been observed, *Aspergillus parasiticus* normally reproduces in the asexual state. Spores are produced on crops, crop residue on the soil surface, and stored grains. Fungal spores travel to other hosts through the air and can overwinter on plant residue or as sclerotia in the soil (Gupta, 2012). In the asexual state, conidial heads are dark green and predominately unbranched. Conidia are distinctly roughened, globose to subglobose and are borne on stalks, which are commonly covered in small spines (Horn *et al.,* 2009). In the sexual state, the fungus is heterothallic

(i.e., requiring compatible male and female reproductive structures from different individuals). However, sclerotia are typically formed in individuals of a single mating type and not from sexual mating. The sclerotium is not only an overwintering structure but may also provide genetic recombination by forming ascospores (sexual spores) once sexually compatible individuals have mated. Ascospores have fine tuberculation (small, rounded swellings) and an equatorial line. Many details of the sexual life cycle remain unknown (Horn *et al*., 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Five markets from Katsina state were selected as study areas which comprised of Katsina central (12º 58□ 17.57□ □ N and 7º 36□ 0.42□ □ E), Tsohuwar kasuwa (12º 59□ 41.87□ □ N and 7º 35□ 28.33□ □ E) and Yar'kutungu (13º 0□ 15.85□ □ N and 7º 36□ 19.15□ □ E) markets in Katsina metropolis (Katsina Local Government Area). The other two markets were Funtua central market (11º 31□ 48.48□ □ N and 7º 18□ 49.30□ □ E) in Funtua town (Funtua Local Government Area) and Kafur market (1º 38□ 37.00□ □ N and 7º 41□ 15.00□ □ E) in Kafur town (Kafur Local Government Area). Katsina state lies on latitude 12º 30□ 0□ □N and longitude 80º 0□ 0□ □ E. Katsina local government lies between 13º 1□ 30□ □ N and 7º38□ 0□ □ E, Funtua local government lies between 11º 32□ N and 7º19□ E, while Kafur local government lies on latitude 11º 38□ 47□ □ and longitude 7º 41□ 41□ □ .

Katsina central market is a modern market with road network throughout the market. It consists of modern shops, stalls and it serves as a multi-purpose market where commodities such as food stuff, spices and many other agricultural produce, textile materials, kitchen wares, mobile phones, cosmetics and many other commodities are being sold. It is a daily market.

Tsohuwar Kasuwa was before a very big and famous market during tran-sahara trade alongside Kasuwar Kurmi in Kano, but now reduced to road side market due to

construction of housing and property. Commodities sold include: Spices , freshmeat, vegetables, poultry, imported rice and other food stuff, livestock. Pharmaceutical chemists are also abundant. It is both a daily and weekly market.

Yar'kutungu market is a local market where majority of the stalls in the market are made from millet stalk and tree branches. There are no asphalted road network as a result the market atmosphere is always dusty. Commodities sold include: Spices, groundnut cake (kulikuli) ,boiled leafy vegetables (e.g Moringa), garri, grains, dried moringa (zogale), textile material and many other traditional commodities . It is a weekly market, two market days a week (Tuesday and Friday).

Funtua central market consists of shops and stalls. Commodities sold include spices fertilizer, food stuff, textile, automotive spare parts e.t.c. Shops are located too close to each other and it is a daily market.

Kafur market is a local market consisting of stalls made from either millet stalk or tree branches.Some market people spread their commodities by the roadside (Kafur-Malumfashi road) for sale including those who sell spices. Market day is once in a week (Sunday). Local governments in which study areas are located are shown in Figure 3.1.

3.2 Sample Collection

A total of two hundred and ten dried, raw (not powdered), powdered and industrially processed samples representing different types of spices were randomly collected in new polythene bags from the five markets. These spices and condiments were chosen based

on their availability and popularity of usage. For each spice three (3) replicates were obtained and mixed to prepare one composite sample (Farid *et al*., 2013).

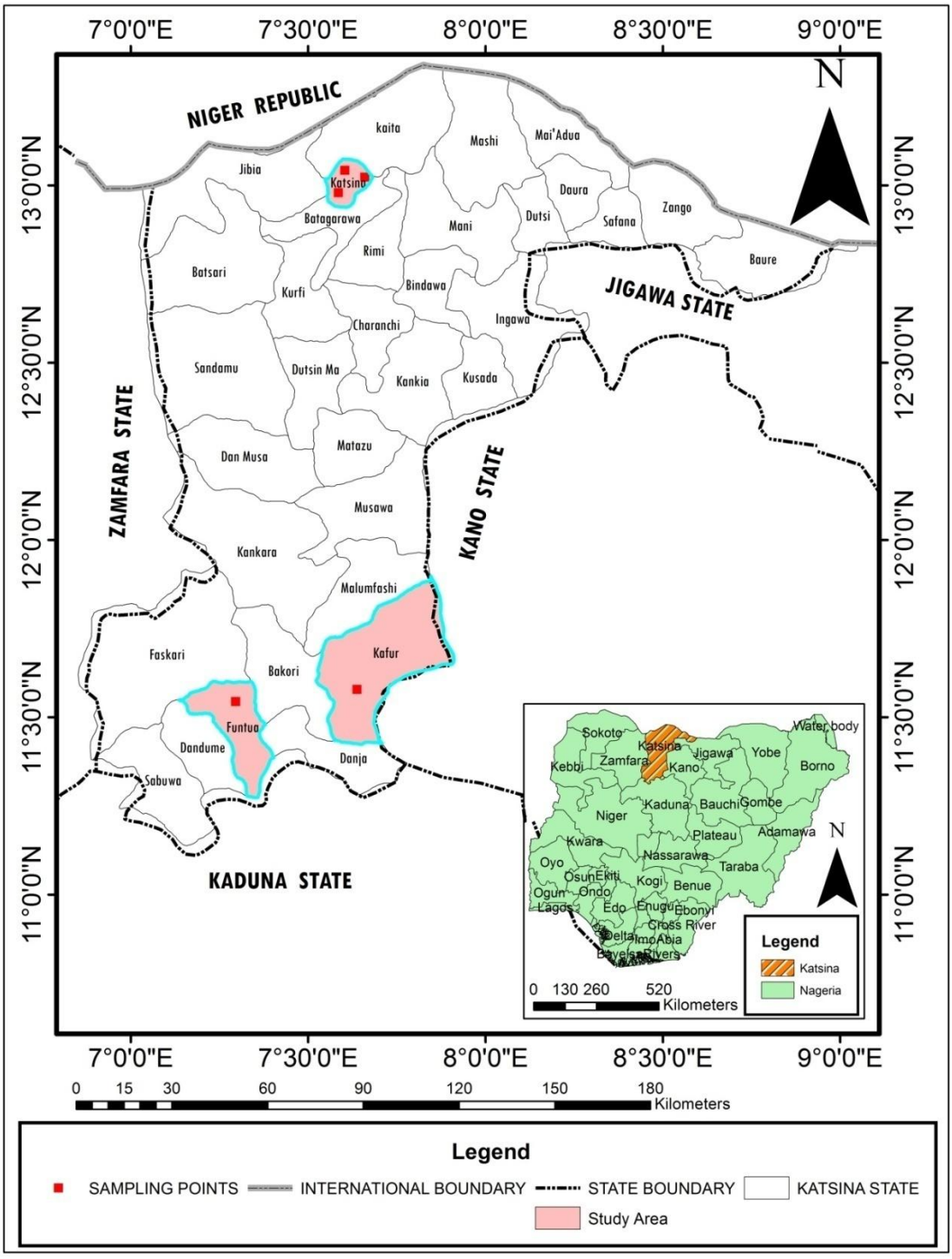


Fig 3.1 Study Areas of Katsina State

Source: Department of Geography, A. B. U. Zaria (2015).

Samples were collected in May 2014. Details of the spices used are presented in Table 3.1 and Plate I.

3.3 Isolation of Fungi

3.3.1 Media preparation

Potato Dextrose Agar (PDA) was used throughout the study. Thirty nine (39.0 g) grams of PDA was suspended in 1000 ml of distilled water. PDA was supplemented with Chlorenphenicol (500 g / L) to prevent bacterial contamination. The mixture was stirred till the PDA was completely dissolved. It was then autoclaved at 121º C for 15 minutes. According to the manufacturer's description, the media was composed of Potatoes infusion (200 g/ L), Dextrose (20.00 g/ L) and Agar (15.00 gram/ litre).

3.3.2 Isolation of fungi by direct plating method

The method described by Jha (1995) was used for raw spices which included: Clove, African nutmeg, Ashanti pepper, Candlewood, Ethiopian pepper, Pineapple of the bush, Ginger, Garlic, Chillies, Kajiji and Dadawa. Each raw sample was surface sterilized in 0.1 % sodium hypochloride for two minutes and was washed three times with sterile distilled water. The disinfected samples were transferred with sterile forceps into petri dishes containing autoclaved sterilized Potato Dextrose Agar (15 ml / plate), at the rate of (2-4) pieces per plate depending on the size of the particles, larger samples were cut into

smaller pieces. PDA was supplemented with 500 mg/ l chloromphenicol to restrict bacterial growth. Three replicates were made and plates were incubated at room temperature for 7 days.

Scientific Name	Common Name	Local Name (Hausa)	Part of plant Used
Eugenia aromatica (L.)	Clove	Karamfani	Bud
Monodora myristica (Gaertan)	African nutmeg	Gyadar miya	Seeds
Piper guineense (Schumach)	Ashanti pepper	Masoro	Seeds

Fagara zanthoxyloides (Lam.)	Candlewood	Fasakwari	Stem
Xylopia aethiopica (Dunal)	Ethiopian pepper	Kimba	Fruit
Thonningia sanguinea (Vahl.)	Pineapple of the bush	Kulla	Root
Zingiber officinale (Roscoe)	Ginger	Citta mai yatsu	Rhizome
Allium sativum (L.)	Garlic	Tafarnuwa	Bulb
Capsicum frutescence (L.)	Chillies	Barkono	Fruit
Cyperus tonkinensis (Hooper)	-	Kajiji	Fruit
Thyme vulgaris (L)	Thyme	Thyme	Leaves
-	-	Yaji	-
-	Locust bean	Dadawa	-
-	Curry powder	Curry	-

Table 3.1: Spices Studied with their Scientific, Common, Local Name and Part of Plant Used.



a



b



c



d



e



f

Plate I: Pictures of Spices studied. a clove, b African nutmeg, c Ashanti pepper, d candlewood, e Ethiopian pepper and f pineapple of the bush.



g



h



i



j



k



l



m



n

g ginger, h garlic, i chillies, j kajiji, k thyme, l curry, m dadawa and n yaji

3.3.3. Isolation of fungi by standard dilution plate method

This method was used to study fungi in powdered samples (Yaji, Curry and thyme). One gram of each composite samples (fine powder) was transferred into 250 ml screw-capped medical bottle containing 9 ml of sterile distilled water and was mechanically homogenized using a mechanical shaker at constant speed for 15 minutes. The sample water suspension was allowed to stand for 10 minutes. Five fold serial dilution (1:5) was prepared and 1ml portions of suitable dilutions of the resulting samples suspension was used to inoculate petri dishes each containing 15 ml Potato Dextrose Agar (PDA). Three replicates were made and inoculated at room temperature for 7 days (Aziz *et al*., 1998).

3.4 Fungal Identification and their Occurrence Percentage

Fungi colonies and isolates were identified according to morphological (colony colour, surface texture) and microscopic characteristics (Nature of spore, conidiophores, sporangiophore, and vessicles) using identification keys by Davise (2002), Klich (2002), Robert *et al.* (2004).

Percentage of fungal occurrences were calculated using the formula:

$$\% = \frac{\text{Total number of individual fungal occurrence}}{\text{Total number of fungal occurrence}} \times 100$$

(Gonzalez *et al*., 1995).

3.5 Determination of Aflatoxin Contamination in Spices Using ELISA Technique

Elisa (AqraQuant Total Aflatoxin Assay 1/20) test kit was used for aflatoxin determination in the samples.This technique was used by Colak *et al*. (2006).

3.5.1 Sample extraction

Spices and condiments were individually ground using laboratory mill (Romer). Sample extraction was performed according to the manufacturer's recommendation (Aqra Quant Total Aflatoxin Assay 1/20) test kit sourced from Romer Labs Singapore Pte Ltd. Twenty five ml of acetonitrile /water (84/16) was added to 5 g of each ground spice and the solution was extracted by shaking for 30 minutes using orbital shaker. The sample was allowed to settle and the top layer of extract was filtered through a whatman no 1 filter paper and the filtrate was collected for clean up.

3.5.2 Sample clean up

Sample Cleanup was performed with MycoSep 226 aflazon according to the manufacture recommendation. Four ml of extract was transferred into a glass tube, 4 ml extract was prepared. MycoSep column was placed firmly into the top portion of the glass tube and pushed it through until 0.5 ml of purified extract was removed. This 0.5 ml of the purified extract was transfered into a 2 ml vial and evaporated to dryness. The residue was reconstituted with 0.5 ml of 70/30 methanol/water and used for Elisa testing.

3.5.3 Aflatoxin determination

Aflatoxin analysis of the spices was carried out at NAFDAC Laboratory Kaduna.

According to AqraQuant Total aflatoxin Assay 1/20 test kit manual, 200 µL of conjugate was dispensed into each green-bordered Dilution well and 100 µL of each of aflatoxin standard (0, 1.0, 2.0, 4.0, 10.0 and 20.0 µg/kg) and sample was added into the appropriate Dilution well. Each well was carefully mixed by pipetting it up and down three times and 100 µL of the contents from each Dilution well was immediately transferred into a corresponding Antibody Coated Microwell, it was then incubated at room temperature for 15 minutes. The contents of the microwell strips were emptied followed by washing each microwell with distilled water. The washing was repeated 5 times. Microwell strips were then tapped using absorbent paper towels to expel as much residual water as possible after the fifth wash, and a dry towel was used to dry the microwells bottom. Into each microwell, 100 µL of the substrate was added and incubated at room temperature for five minutes and blue color developed, then 100 µL of stop solution was added into each microwell strip. This changed the colour of the content from blue to yellow. The strips were read with microwell reader using an absorbance filter of 450 nm.

The microwell reader read the absorbance of both the aflatoxin standards and samples. Since the amount of aflatoxin in each standard was known, the amount of aflatoxin in the unknown (samples) was measured by interpolation from this standard that is by comparing the absorbance of standards and that of samples.

3.6 Statistical Analysis

Data collected were subjected to ANOVA analysis using Statistical Package for Social Sciences (SPSS) version 21.0. Means were separated using Duncan Multiple Range Test (DMRT) at 5 % level of significance ($P = < 0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 Fungal Identification and their Occurrence Percentage

A total of 12 different fungi species were isolated from different spices from the selected markets and identified according to their cultural and microscopic characteristics observed using identification manuals by Davise (2002), Klich (2002), Robert *et al.* (2004) (Table 4.1).Their distribution among the markets was variable. Spices obtained from Funtua central market contained more fungi (250) and was followed by Kafur (182),Yar'kutungu (81), Tsohuwar kasuwa (59) and Katsina central (56) markets. There was non- significant differences ($P > 0.05$) in the number of fungi among the markets.These results are shown in Table 4.2 and Plates II-XIII.

Percentage occurrence of each isolated fungi from spices obtained from the five markets was determined (Table 4.1). Percentage occurrence was considered high at the value of ≥ 12 %, moderate at the range of 5-11 % and low at < 5 %. There was highly significant differences ($P \leq 0.01$) in the number of colonies among the fungi with *Aspergillus flavus* having the highest mean colony of 57.20 which is non significant from *A. parasiticus* (49.00) (Table 4.2).

4.1.1 Percentage occurrence of fungi in samples from tsohuwar kasuwa

In this study, fifty nine isolates representing 7 species of 4 genera were isolated from 14 spices obtained from tsohuwar kasuwa. *Aspergillus* species were the most dominant fungi

Table 4.1: Observed Characteristic of Fungi Isolated Adapted from Davise (2002), Klich (2002), Robert *et al*. (2004)

Fungi	Macroscopic	Microscopic
A. flavus (Link)	Colonies were olive to lime green with a cream reverse.Texture was woolly.	Conidiophores was coarsely roughened, vesicles globose
A. parasiticus (Speare)	Deep dark green colonies. Reverse dull yellow	Vessicles were sphaerical
A. fumigatus (Fresenius)	Colonies were smoky grey-green with a slight yellow reverse. Texture was woolly.	Conidiophore was smooth-walled with a dome-shaped vesicle.
A. niger (Vantiegham)	Colonies were initially white, quickly becoming black with conidial production. Reverse was pale yellow.	Conidiophore was smooth, vesicle globose
A. terreus (Thom)	Colonies were cinnamon in colour. Reverse was yellow. Colonies texture was finely granular.	Conidiophore was smooth-walled and hyaline, globose vesicles.
A. versicolor (Vuillemin)	Colonies were pinkish-green. Reverse was reddish	Conidiophore was hyaline, smooth-walled. Vesicles were small.
Mucor hiemalis (Wehmer)	White initially and became greyish brown in time. From the reverse, it was white.	Rhizoid and stolon were absent. Sporangiophores were short, erect, taper towards their apices.
Nigrospora sphaerica	Colony was white and wooly, then became grey with black areas and turned to black from both front and reverse.	Conidiophore had a single conidium at the apex. Conidium was black, slightly flattened horizontally.
Penicillium citrinum (Thom)	Blue-green colony , appearing leathery. Reverse yellow	Conidia produced in columns and globose.
Rhizopus stolonifer (Ehrenb)	Colony was white initially and turned grey to yellowish brown in time. The reverse was white to pale. The texture was typically cotton-candy	Sporangiophore was unbranched. Rhizoidand stolons present
Phoma glomerata	Dull green colony.	Large round pycnidium. Conidia one-celled and ovoid .
Rhizoctonia sp	Colony was brown in colour	The hyphae branch at right angles with constrictions at branch points.

Table 4.2: Percentage Occurrence of Fungal Isolates of Five Markets

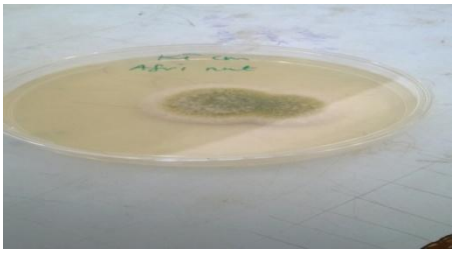
Fungal Isolates	Market					Total	Mean Number of Colonies±SE
	Tsohuwar Kasuwa	Katsina Central Market	Yar'kutungu Market	Funtua Central Market	Kafur Market		
Aspergillus flavus	21 (35.59)	15 (26.79)	16 (19.75)	126 (50.40)	108 (59.34)	**286 (45.54)**	57.20±24.60a
Aspergillus parasiticus	22 (37.29)	20 (35.71)	26 (32.10)	112 (44.80)	65 (35.71)	**245 (39.01)**	49.00±17.78a
Apsergillus niger	0 (0.00)	4 (7.14)	0 (0.00)	0 (0.00)	1 (0.55)	**5 (0.80)**	1.00±0.77b
Aspergillus terreus	0 (0.00)	0 (0.00)	7 (8.64)	0 (0.00)	0 (0.00)	**7 (1.11)**	1.40±1.40b
Aspergillus versicolor	2 (3.39)	3 (5.36)	7 (8.64)	0 (0.00)	0 (0.00)	**12 (1.91)**	2.40±1.29b
Aspergillus fumigatus	3 (5.08)	3 (5.36)	1 (1.23)	3 (1.20)	3 (1.65)	**13 (2.07)**	2.60±0.40b
Mucor hiemalis	0 (0.00)	1 (1.79)	0 (0.00)	1 (0.40)	1 (0.55)	**3 (0.48)**	0.60±0.24b
Phoma glomerata	0 (0.00)	2 (3.57)	2 (2.47)	0 (0.00)	0 (0.00)	**4 (0.64)**	0.80±0.49b
Rhizopus stolonifer	7 (11.86)	5 (8.93)	7 (8.64)	3 (1.20)	3 (1.65)	**25 (3.98)**	5.00±0.89b
Rhizoctonia sp.	1 (1.69)	0 (0.00)	8 (9.88)	2 (0.80)	0 (0.00)	**11 (1.75)**	2.20±1.50b
Penicillium citrinum	0 (0.00)	2 (3.57)	5 (6.17)	0 (0.00)	1 (0.55)	**8 (1.27)**	1.60±0.93b
Nigrospora sphaerica	3 (5.08)	1 (1.79)	2 (2.47)	3 (1.20)	0 (0.00)	**9 (1.43)**	1.80±0.58b
Total	**59 (9.39)**	**56 (8.92)**	**81 (12.90)**	**250 (39.81)**	**182 (28.98)**	**628**	**10.47±3.39**
	4.92±2.31a	4.67±1.81a	6.75±2.19a	20.83±13.27a	15.17±9.98a	**10.47±3.39**	

	P value
Fungi Isolat**es**	0.000**
Markets	0.471ns

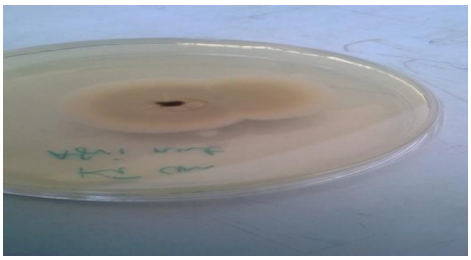
ns= non significant

Means with the same alphabets either on row or column are not significant (P > 0.05).

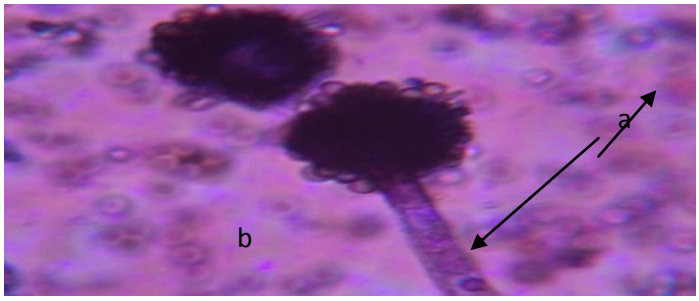
.



A: Colony is olive to lime green

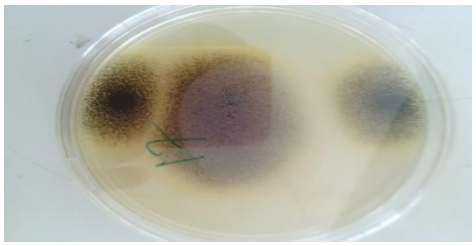


B: Cream in colour

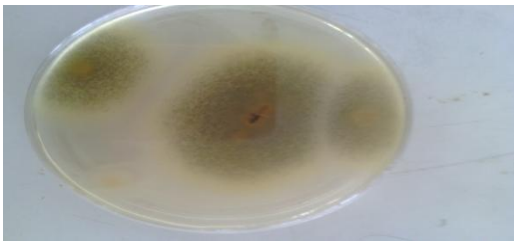


C: a Conidiophore. b Vessicle. Mag x10

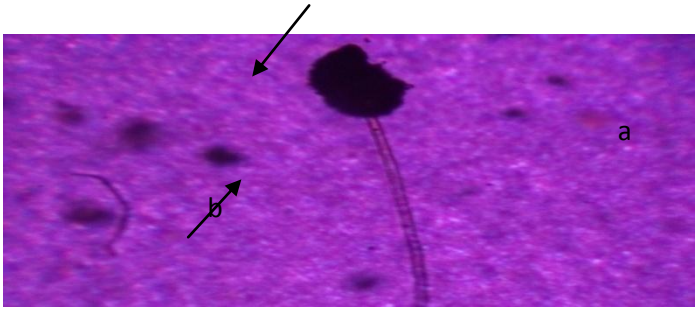
Plate II: Pure culture of *Aspergillus flavus* Link 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.



A: Blue-green

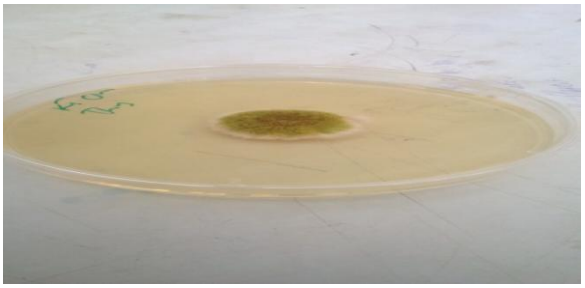


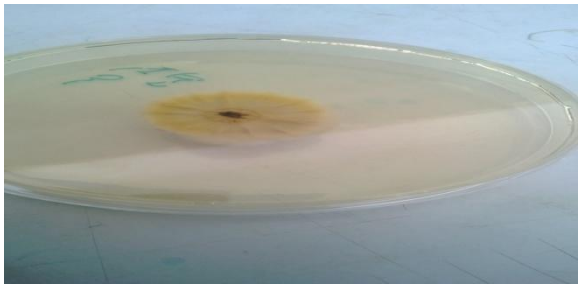
B: Dull yellow to dull green



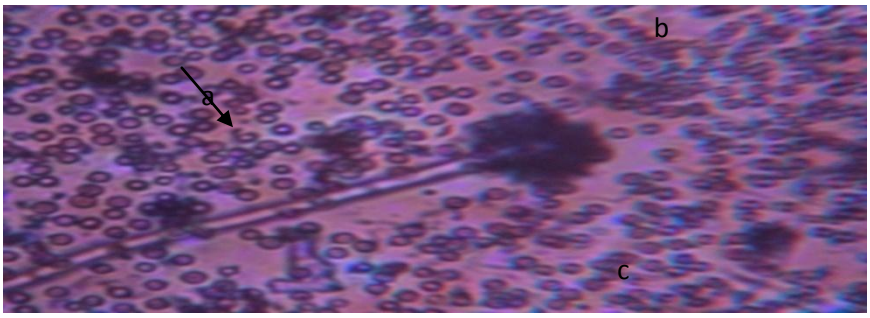
C: a Conidiophore. b Vessicle. Mag x10

Plate III: Pure culture of *Aspergillus parasiticus* Thom 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.





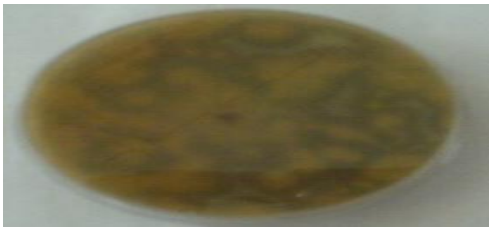
A: Smoky grey-green colony B: Yellow in colour



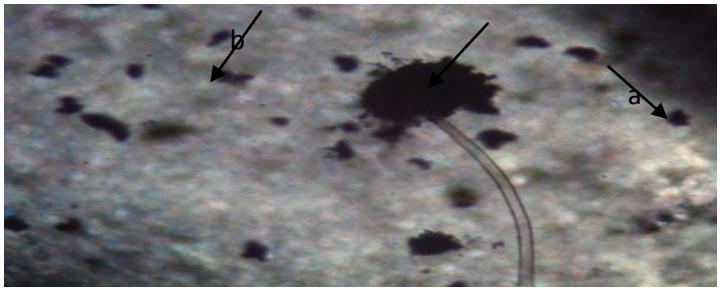
C: a Conidiophore. b Vessicle. c Conidia. Mag x10

Plate IV: Pure culture of *Aspergillus fumigatus* Fresenius 'A' Upper surface 'B' Reverse side 'C' Microscopic feature



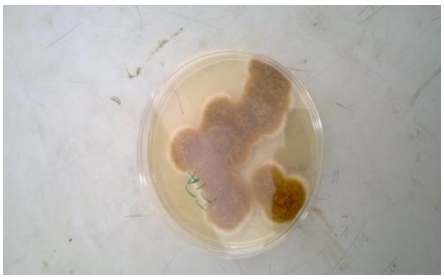


A: Colony black B: Pale yellow



C: a Conidiophore. b Vessicle. Mag x10

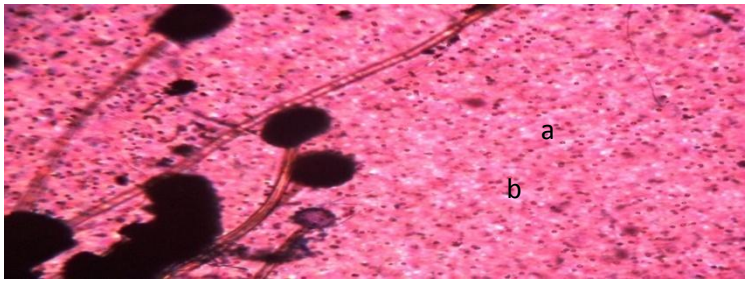
Plate V: Pure culture of *Aspergillus niger* Vantiegham 'A' Upper surface 'B'Reverse side 'C' Microscopic feature.



A:Colony cinnamon in colour

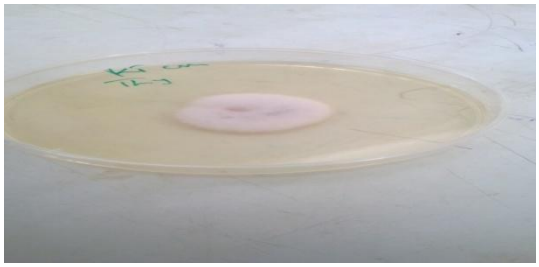


B: Yellow colour

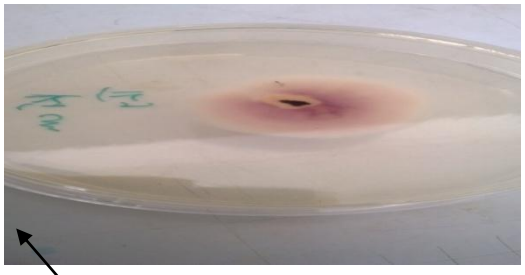


C: a Conidiphore. b Vessicle. Mag x10

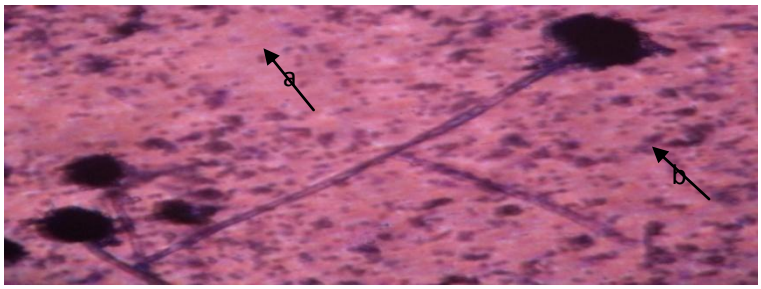
Plate VI : Pure culture of *Aspergillus terreus* Thom 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.



A: Pinkish green

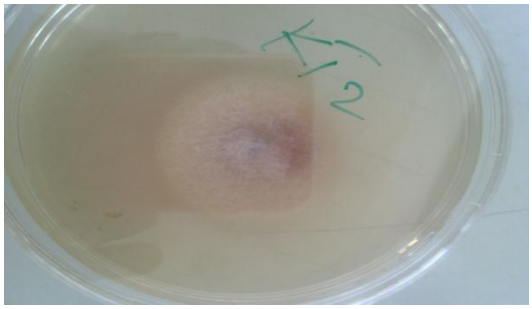


B: Pinkish in colour



C: a Conidiophore. b Vessicle. Mag x10

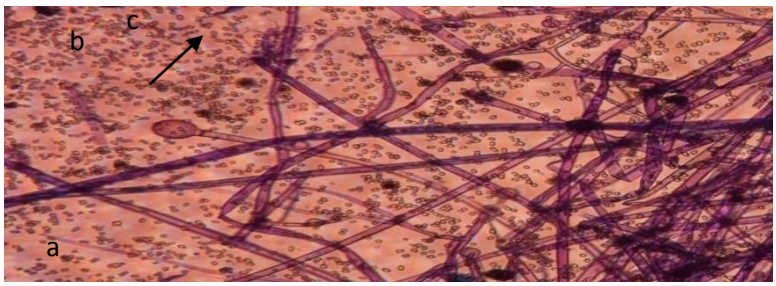
Plate VII: Pure culture of *Aspergillus versicolor* Vuillemin 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.



A: White turn to greyish brown

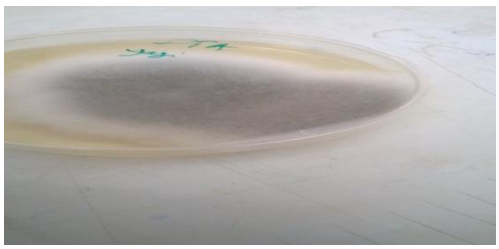


B: White in colour

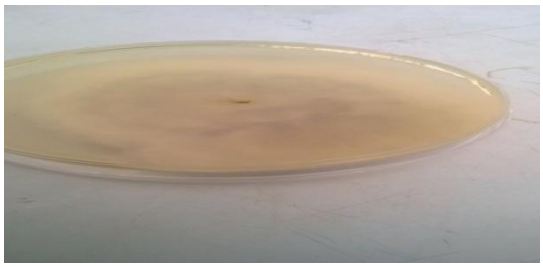


C: a= Sporangiophore, b= Empty sporangium, c= sporangiospores. Mag x10

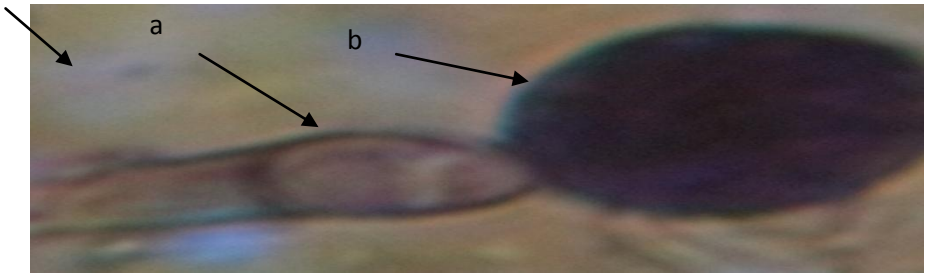
Plate VIII: Pure culture of *Mucor hiemalis* Wehmer 'A' Upper surface 'A' Reverse side 'C' Microscopic feature.



A: Initially white turn to grey with black areas

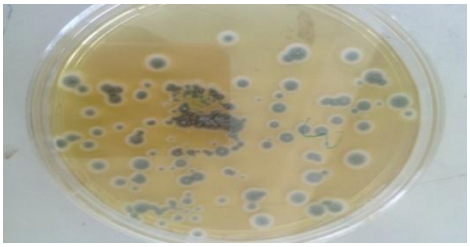


B: Grey eventually turns to black

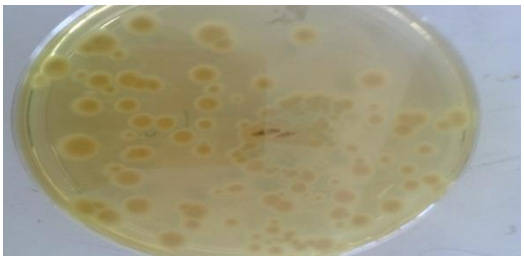


C: a= Conidiophore. b= Conidium. Mag x40

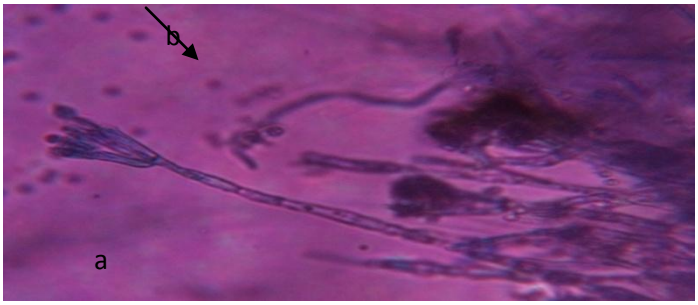
Plate IX : Pure culture of *Nigrospora sphaerica* Saccardo 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.



A: Blue-green colony

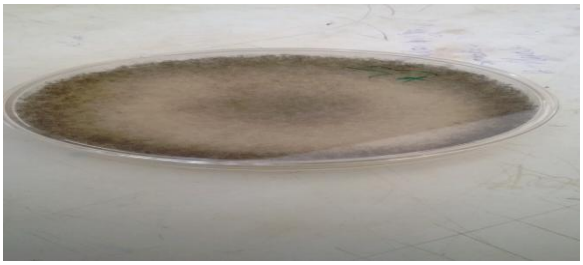


B: Yellow- orange

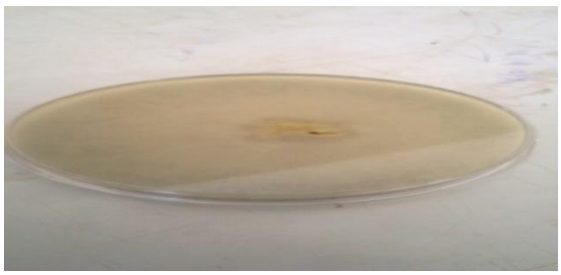


C: a Conidiophore. b Chain of conidia. Mag x10

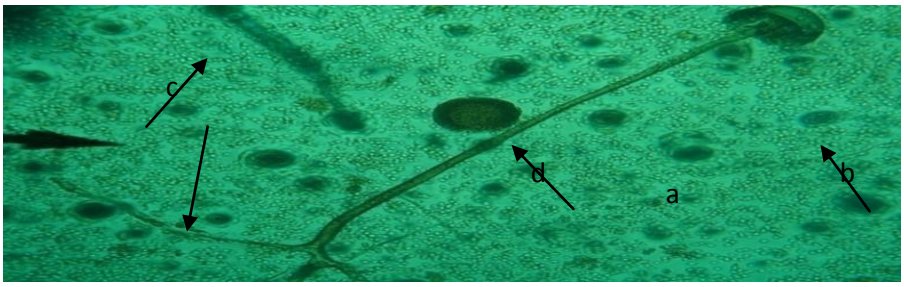
Plate X: Pure culture of *Penicillium citrinum* Thom 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.



A: Colony white turns grey to yellowish

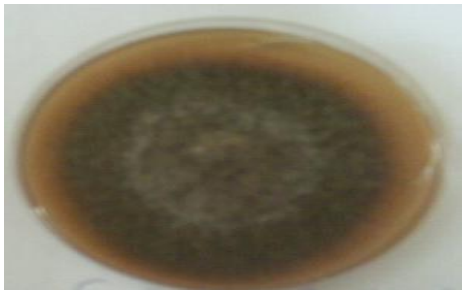


B: White to pale

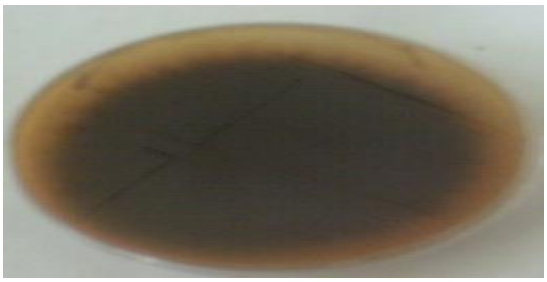


C: a= Sporangiophore. b= Sporangium. c = Stolon. d= Rhizoid. Mag x10

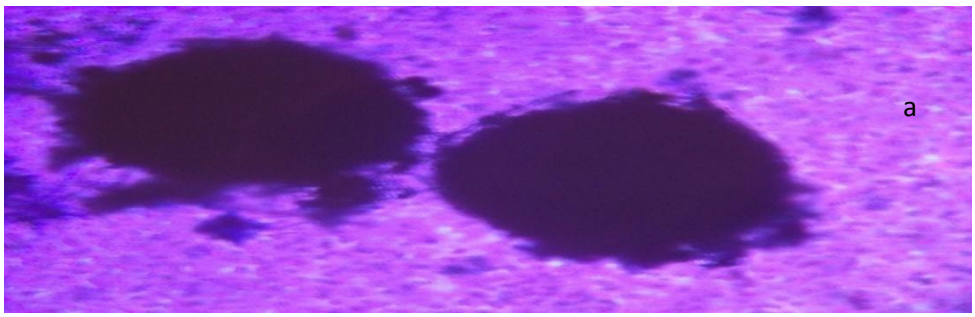
Plate XI: Pure culture of *Rhizopus stolonifer* Ehrenb 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.



A: Dull green

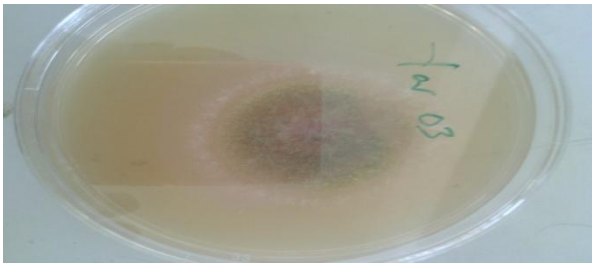


B: Dull green



C: a= Dark round pycnidium. Mag x40

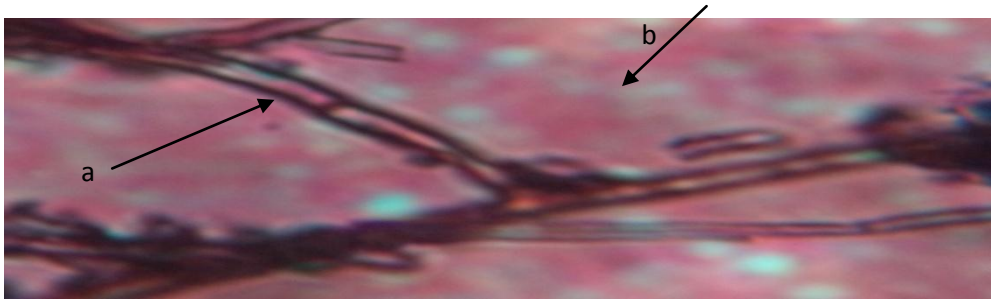
Plate XII: Pure culture of *Phoma glomerata* Corda 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.



A : Brown in colour



B: Green-brown



C: a= Hyphae. b= Hyphae constriction at right angle. Mag x40

Plate XIII : Pure culture of *Rhizoctonia* sp (de Candolle) 'A' Upper surface 'B' Reverse side 'C' Microscopic feature.

isolated. Among the *Aspergillus* spp., *A. parasiticus* and *A. flavus* had the highest occurrence and were found to contaminate all the samples except garlic, ginger, Ashanti pepper and chilli powder. *Rhizopus stolonifer, Nigrospora sphaerica* and *A. fumigatus* had moderate level of occurrence, while the least occurring species were, *A. versicolor*, *Rhizoctonia* sp. respectively (Table 4.2).

4.1.2 Percentage occurrence of fungi in samples from Katsina central market

Fifty six fungal isolates representing 10 species of 6 genera were isolated from samples obtained from Katsina central market. *Aspergillus* was the most common genus, represented by 5 species and 43 isolates. Highest occurring fungi were *A. parasiticus* and *A flavus* respectively and were found to contaminate (8 and 9) samples respectively. Moderate level of occurrence was recorded in *Rhizopus stolonifer*, *A. niger*, *A. versicolor* and *A. fumigatus*. The least occurring fungi were *Phoma glomerata*, *Penicillium citrinum*, *Mucor hiemalis* and *Nigrospora sphaerica* (Table 4.2).

4.1.3 Percentage occurrence of fungi in samples from Yar'kutungu market

A total of 81 isolates belonging to 6 genera of 10 species were isolated from samples obtained from Yarkutungu Market. However, of these 79 isolates the most highly occurring species were *A. parasiticus*, *A. flavus* and contaminated almost all the samples. Moderately occurring fungi were *Rhizoctonia* sp., *A. terreus*, *A. versicolor*, *Rhizopus stolonifer* and *Penicillium citrinum*. Least occurred fungi were *Nigrospora sphaerica*, *Phoma glomerata* and *A. fumigates* (Table 4.2).

4.1.4 Percentage occurrence of fungi in samples from Funtua central market

Two hundred and fifty isolates representing 7 species of 5 genera were isolated from 14 spices and condiments samples obtained from Funtua central market. *Aspergillus* was the most common genus represented by 241 isolates . Among *Aspergillus* spp, *A. flavus* and *A. parasiticus* had the highest level of occurrence and contaminated all the samples except candlewood which was not contaminated with *A. flavus*. The least level of occurrence was recorded in *A. fumigatus, Rhizopus stolonifer, Nigrospora sphaerica, Rhizoctonia* sp. *and Mucor hiemalis* respectively (Table 4.2).

4.1.5 Percentage occurrence of fungi in samples from Kafur maarket

A total of 182 isolates representing 4 genera consisting of 7 species were isolated from samples obtained from Kafur market. *Aspegillus* was the most frequently occurring genus. Among the *Aspergillus* species, *A. flavus* and *A. parasiticus* had the highest level of occurrence and contaminated all the samples. The least level of occurrence was recorded in *A. fumigatus, Rhizopus stolonifer*, *A. niger*, *Mucor hiemalis* and *Penicillium citrinum* respectively(Table 4.2).

4.3 Percentage Contamination of Spices by Fungi

Spices from all the five markets were contaminated by various fungi species. Percentage contamination was considered high at ≥ 8, moderate 5-7 and low < 5 %.

Statistical analysis of the fungal contamination of spices from the five markets revealed that there was highly significant differences ($P \leq 0.01$) in fungi contamination of spices

among the markets with Funtua central market having the highest mean colony of 17.86 which is non- significant from Kafur market (13.00). There was non- significant differences ($p > 0.05$) in fungi contamination among the spices. These results are shown in Table 4.3.

4.3.1 Percentage contamination of spices by fungi from Tsohuwar kasuwa

Highest contaminated samples with fungi from Tsohuwar kasuwa were African nutmeg (15.25 %), Pineapple of the bush (13.56 %), Clove (11.86 %) and Chillies (10.17 %). Moderately contaminated samples were Dadawa, Curry, Thyme, Kajiji, and Ethiopian pepper (6.78 %) followed by Candlewood (5.08 %). Lowest contamination was recorded in Ginger and Ashanti pepper (3.39 %) followed by Garlic and yaji (1.69 %) (Table 4.3).

4.3.2 Percentage contamination of spices by fungi from Katsina central market

Spices that had highest contamination with fungi from Katsina central market were clove (16.07 %), thyme and kajiji (12.50), Ethiopian pepper (10.71 %), African nutmeg and yaji (8.93 %). Moderately contaminated samples were garlic, ginger and dadawa (5.36). Lowest contaminated samples were Chillies, Pineapple of the bush and Candlewood (3.57 %) followed by curry and Ashanti pepper (1.79 %) (Table 4.3).

4.3.3 Percentage contamination of spices by fungi from Yar'kutungu market

Highest contaminated samples with fungi from Yar'kutungu market were curry (14.8 %), followed by kajiji and pineapple of the bush (9.88 %) and chillies (8.64 %).

Table 4.3 : Percentage Contamination of Spices with Fungi for the Five Markets

Spices	Market					Total (%)	Mean Number of Colonies±S.E
	Tsohuwar Kasuwa (%)	Katsina Central Market (%)	Yar'kutungu Market (%)	Funtua Central Market (%)	Kafur Market (%)		
Chillies	6 (10.17)	2 (3.57)	7 (8.64)	25 (10.00)	33 (18.13)	**73 (11.62)**	14.60±6.07a
Garlic	1 (1.69)	3 (5.36)	2 (2.47)	23 (9.20)	22 (12.09)	**51 (8.12)**	10.20±5.03a
Ginger	2 (3.39)	3 (5.36)	6 (7.41)	5 (2.00)	3 (1.65)	**19 (3.03)**	3.80±0.73a
Dadawa	4 (6.78)	3 (5.36)	5 (6.17)	6 (2.40)	3 (1.65)	**21 (3.34)**	4.20±0.58a
Curry	4 (6.78)	1 (1.79)	12 (14.81)	18 (7.20)	11 (6.04)	**46 (7.32)**	9.20±3.02a
Thyme	4 (6.78)	7 (12.50)	6 (7.41)	15 (6.00)	10 (5.49)	**42 (6.69)**	8.40±1.91a
African nutmeg	9 (15.25)	5 (8.93)	6 (7.41)	20 (8.00)	19 (10.44)	**59 (9.39)**	11.80±3.22a
Ashanti pepper	2 (3.39)	1 (1.79)	4 (4.94)	6 (2.40)	5 (2.75)	**18 (2.87)**	3.60±0.93a
Clove	7 (11.86)	9 (16.07)	5 (6.17)	19 (7.60)	18 (9.89)	**58 (9.24)**	11.60±2.89a
Kajiji	4 (6.78)	7 (12.50)	8 (9.88)	21 (8.40)	14 (7.69)	**54 (8.60)**	10.80±3.02a
Candle wood	3 (5.08)	2 (3.57)	4 (4.94)	5 (2.00)	3 (1.65)	**17 (2.71)**	3.40±0.51a
Yaji	1 (1.69)	5 (8.93)	4 (4.94)	14 (5.60)	10 (5.49)	**34 (5.41)**	6.80±2.31a
Pineapple of the bush	8 (13.56)	2 (3.57)	8 (9.88)	47 (18.80)	13 (7.14)	**78 (12.42)**	15.60±8.04a
Ethiopian pepper	4 (6.78)	6 (10.71)	4 (4.94)	26 (10.40)	18 (9.89)	**58 (9.24)**	11.6±4.45a
Total	**59 (9.39)**	**56 (8.92)**	**81 (12.90)**	**250 (39.81)**	**182 (28.98)**	**628**	**8.97±1.02**
Mean Number of Colonies±S.E	4.21±0.67b	4.00±0.67b	5.79±0.66b	17.86±3.01a	13.00±2.29a	**8.97±1.02**	
	P value						
Spices	0.304ns						
Markets	0.000**						

Means with the same alphabets either on row or column are not significant (P > 0.05).

Moderate contamination was recorded in ginger, thyme and African nutmeg (7.41 %), followed by dadawa and clove (6.17 %). Lowest contaminated samples were yaji, Ashanti pepper, candlewood and Ethiopian pepper (4.94 %) followed by garlic (2.47 %) (Table 4.3).

4.3.4 Percentage contamination of spices by fungi from Funtua central market

Spices that had highest contamination with fungi from Funtua central market were pineapple of the bush (18.0 %), Ethiopian pepper (10.40 %), chillies (10.00 %), garlic (9.20 %), kajiji (8.40 %) and African nutmeg (8.00 %). Moderately contaminated samples were clove (7.60 %), curry (7.20 %), thyme (6.00 %) and yaji (5.60 %). Lowest contamination was recorded in Ashanti pepper and dadawa (2.40 %), followed by ginger and candlewood (2.00 %) (Table 4.3).

4.3.5 Percentage contamination of spices by fungi from Kafur market

Highest contaminated samples with fungi from kafur market were chillies (18.13 %), followed by garlic (12.09 %), African nutmeg (10.44 %), clove and Ethiopian pepper (9.89 %). Moderately contaminated samples were kajiji (7.69 %), pineapple of the bush (7.14 %), curry (6.04 %), thyme and yaji (5.49 %). Lowest contamination was recorded in Ashanti pepper (2.75 %), followed by ginger, dadawa and candlewood (1.65 %) (Table 4.3).

4.4 Aflatoxin Contents of Spices

Aflatoxin contamination of spices from the five markets were determined. Aflatoxin concentration was considered high at ≥ 9 µg/kg, moderate level at 5-9 µg/kg and low at < 5 µg/kg.

4.4.1 Spices from Tsohuwar kasuwa market with associated fungi and aflatoxin content

Fungi species isolated from each spice obtained from Tsohuwar kasuwa were compared with aflatoxin content of the sample (Table 4.4). Any sample that was contaminated with *A. flavus and A. parasiticus* contained aflatoxin. Aflatoxin was not detected in samples that were contaminated by either *Rhizopus stolonifer, Nigrospora sphaerica*, or *A. versicolor*. Ten out of 14 spices samples (71.4%) obtained from this market contained total aflatoxins ranging from 0.1->20µg/kg. Contaminated samples were pineapple of the bush and Clove with the highest level (>20 µg/kg) followed by African nutmeg (11.42 µg/kg). Moderate level was in Ethiopian pepper (7.3 µg/kg). The lowest aflatoxin concentration was recorded on chillies (2.7 µg/kg), thyme (0.6 µg/kg), curry and candlewood (0.1 µg/kg) followed by kajiji and dadawa (0.03 µg/kg) . Aflatoxin was not detected in garlic, ginger, chilli powder and Ashanti pepper.

4.4.2 Spices from Katsina central market with associated fungi and aflatoxin content

Fungi species isolated from each spice obtained from Katsina central market were compared with aflatoxin content of the sample (Table 4.5). Spices and condiments that were contaminated with either *A. flavus, A. parasiticus* or both contained aflatoxin except chillies, garlic and dadawa. Aflatoxin was not detected in spices and condiments that

Table 4.4 : Spices from Tsohuwar Kasuwa Market with Associated Fungi and Aflatoxin Content

Spice	Fungi isolated	Total aflatoxin conc. (µg/kg)	Absorbance (OD) 450nm
Chillies	*A. flavus, A. parasiticus, Rhizopus stolonifer*	2.7	2.123
Garlic	*A. versicolor*	N.D	2.853
Ginger	*Rhizopus stolonifer, Nigrospora sphaerica*	N.D	3.00
Dadawa	*A. flavus, A. parasiticus, Rhizopus stolonifer*	0.3	2.639
Curry	*A. flavus, A. parasiticus, Rhizopus stolonifer*	0.1	2.675
Thyme	*A. flavus, A. parasiticus, A. fumigatus*	0.6	2.567
African nutmeg	*A. flavus, A. parasiticus, A. fumigatus Nigrospora sphaerica*	11.4	1.072
Ashanti pepper	*Rhizopus stolonifer*	N.D	2.686
Clove	*A. flavus, A. parasiticus*	>20	0.560
Kajiji	*A. flavus, A. parasiticus, Nigrospora sphaerica*	0.3	2.628
Candlewood	*A. flavus, A. parasiticus, A. versicolor*	0.1	2.663
Yaji	*Rhizopus stolonifer*	N.D	3.00
Pineapple of the bush	*A. flavus, A. parasiticus, Rhizoctonia sp*	>20	0.390
Ethiopian pepper	*A. flavus, A. parasiticus, A. fumigatus*	7.3	1.428
S.E		1.97	

N.D= Not Detected, Maximum limit set by EU/NAFDAC= 10µg/kg

Table 4.5 : Spices from Katsina Central Market with Associated Fungi and Aflatoxin Content

Spices	Fungi isolated	Total aflatoxin conc. (µg/kg)	Absorbance (OD) 450nm
Chillies	*A. flavus, A. niger*	N.D	3.00
Garlic	*A. Parasiticus, A. niger*	N.D	2.997
Ginger	*A. flavus, A. versicolor*	11.6	1.052
Dadawa	*A. flavus, A. parasiticus*	N.D	2.875
Curry	*Rhizopus stolonifer*	N.D	2.698
Thyme	*A. flavus, A. parasiticus, A. fumigatus, Rhizopus stolonifer*	2.6	2.139
African nutmeg	*A. flavus, A. parasiticus, Nigrospora sphaerica*	9.9	1.179
Ashanti pepper	*Rhizopus stolonifer*	N.D	3.00
Clove	*A. flavus, A. parasiticus , A. niger*	>20	0.495
Kajiji	*A. flavus, A. parasiticus, Mucor hiemalis, Rhizopus stolonifer*	0.8	2.521
Candlewood	*A. flavus, Penicillium citrinum*	0.7	2.553
Yaji	*A. flavus, A. parasiticus, A. versicolor A. fumigatus, Rhizopus stolonifer*	8.1	1.345
Pineapple of the bush	*Phoma glomerata*	N.D	3.00
Ethiopian pepper	*A. parasiticus, Penicillium citrinum*	7.3	1.429
S.E		1.64	

N.D= Not Detected, Maximum limit set by EU/NAFDAC= 10µg/kg

were contaminated with either *Phoma glomerata* or *Rhizopus stolonifer*. Eight out of 14 spices samples (57.1%) obtained contained total aflatoxins in the range of 0.7->20µg/kg. Highest level of aflatoxin contamination was recorded in clove (>20 µg/kg), followed by ginger (11.6 µg/kg) and African nutmeg (9.9 µg/kg). Moderate level of contamination was recorded in yaji (8.1 µg/kg) and Ethiopian pepper (7.3 µg/kg). The lowest aflatoxin contamination was recorded in thyme (2.6 µg/kg) followed by kajiji (0.8 µg/kg) and candlewood (0.7 µg/kg). Aflatoxin was not detected in garlic, pineapple of the bush, dadawa, chillies, curry and Ashanti pepper.

4.4.3 Spices from Yar'kutungu market with associated fungi and aflatoxin content

Fungi species isolated from each spice obtained from Yar'kutungu market were compared with aflatoxin content of the sample (Table 4.6). All spices and condiments were contaminated with either *A. flavus, A. parasiticus* or both which are aflatoxin producing fungi. However, all the samples contained aflatoxin except curry and candlewood. Eleven out of 14 spices and condiments samples (78.6%) obtained from Yar'kutungu market were contaminated with total aflatoxins ranging between 0.1-20µg/kg. High aflatoxin contamination was recorded in pineapple of the bush (>20 µg/kg) followed by chilli powder (15.5 µg/kg), clove (12.4 µg/kg), and African nutmeg (11.6 µg/kg). Moderate level of contamination was recorded in Ethiopian pepper (7.4 µg/kg) and ginger (7.3 µg/kg). The lowest aflatoxin contamination was recorded in chillies (3.8 µg/kg) followed by dadawa (3.3 µg/kg), thyme (1.9 µg/kg), kajiji (0.6 µg/kg) and Ashanti pepper (0.1 µg/kg). Aflatoxin was not detected in garlic, candlewood and curry.

Table 4.6 :Spices from Yar'kutungu Market with Associated Fungi and Aflatoxin Content

Spice	Fungi Isolated	Total aflatoxin conc. (µg/kg)	Absorbance (OD) 450nm
Chillies	*A. flavus, A. parasiticus, Rhizopus stolonifer, Rhizoctonia* sp., *Penicillium citrinum*	3.8	1.919
Garlic	*A. parasiticus, A. versicolor*	N.D	3.00
Ginger	*A. flavus, A. parasiticus, A. versicolor, Nigrospora sphaerica*	7.3	1.416
Dadawa	*A. parasiticus, Rhizopus stolonifer*	3.3	2.020
Curry	*A. flavus, A. parasiticus, A. terreus, A. versicolor, Rhizopus stolonifer, Rhizoctonia* sp.	N.D	3.00
Thyme	*A. parasiticus, A. fumigatus, Rhizoctonia* sp.	1.9	2.285
African nutmeg	*A. flavus, A. parasiticus, A. versicolor, Rhizopus stolonifer, Penicillium citrinum*	11.6	1.053
Ashanti pepper	*A. flavus, A. terreus, Phoma glomerata*	0.1	2.675
Clove	*A. flavus, A. parasiticus*	12.4	1.002
Kajiji	*A. parasiticus, A. versicolor, Rhizopus stolonifer, Rhizoctonia* sp., *Penicillium citrinum*	0.6	2.570
Candlewood	*A. flavus, A. terreus, A. versicolor, Rhizoctonia* sp.	N.D	3.00
Yaji	*A. flavus, Rhizopus stolonifer, Penicillium citrinum*	15.5	0.839
Pineapple of the bush	*A. flavus, A. parasiticus, Rhizoctonia* sp.	>20	0.571
Ethiopian pepper	*A. flavus, A. parasiticus*	7.4	1.421
S.E		1.76	

N.D = Not Detected, Maximum limit set by EU/NAFDAC= 10µg/kg

4.4.4 Spices from Funtua central market with associated fungi and aflatoxin content

All spices from Funtua central market were contaminated with *A. flavus* and /or *A. Parasiticus* which are aflatoxin producing fungi. However, only chillies, garlic, African nutmeg, Ashanti pepper and Ethiopian pepper contained aflatoxin (Table 4.7). Four out of 14 spices (28.6%) were contaminated with total aflatoxins ranging between 3.3-17.2µg/kg. Contaminated samples with highest level were African nutmeg (17.2 µg/kg) and chillies (16.6 µg/kg). Moderate level was recorded in Ethiopian pepper (5.7 µg/kg) and the lowest level was recorded in garlic (3.3 µg/kg). Aflatoxin was not detected in pineapple of the bush, thyme, dadawa, ginger, chilli powder, kajiji, candlewood, curry, clove and Ashanti pepper.

4.4.5 Spices from Kafur market with associated fungi and aflatoxin content

Aflatoxin producing fungi namely *A. flavus and A. parasiticus* contaminated all the samples obtained from Kafur market (Table 4.8). However, aflatoxin was detected in only chillies and African nutmeg. Two out of 14 spices samples (14.3%) were contaminated with total aflatoxins in the range of 3.7-12.4µg/kg. Aflatoxin contamination was recorded in African nutmeg and chillies only. African nutmeg had the highest level of contamination of (12.4 µg/kg) while chillies had the lowest level of aflatoxin contamination (3.7 µg/kg) Aflatoxin was not detected in garlic, pineapple of the bush, thyme, dadawa, ginger, chilli powder, kajiji, candlewood, curry, clove, Ashanti pepper and Ethiopian pepper.

Table 4.7 : Spices from Funtua Central Market with Associated Fungi and Aflatoxin Content

Spices	Fungi Isolated	Total aflatoxin conc. (µg/kg)	Absorbance (OD) 450nm
Chillies	*A. flavus, A. parasiticus, Rhizoctonia* sp., *Rhizopus stolonifer*	16.6	0.193
Garlic	*A. flavus, A. parasiticus,*	3.3	0.508
Ginger	*A. flavus, A. parasiticus, Mucor hiemalis*	N.D	0.874
Dadawa	*A. flavus, A. parasiticus, Rhizopus stolonifer*	N.D	0.806
Curry	*A. flavus, A. parasiticus,*	N.D	0.923
Thyme	*A. flavus, A. parasiticus,*	N.D	0.906
African nutmeg	*A. flavus, A. parasiticus*	17.2	0.178
Ashanti pepper	*A. flavus, A. parasiticus*	N.D	0.940
Clove	*A. flavus, A. parasiticus*	N.D	0.815
Kajiji	*A. flavus, A. parasiticus*	N.D	0.667
Candlewood	*A. parasiticus, Nigrospora sphaerica*	N.D	0.726
Yaji	*A. flavus, A. parasiticus, Rhizopus stolonifer*	N.D	0.809
Pineapple of the bush	*A. flavus, A. parasiticus*	N.D	1.014
Ethiopian pepper	*A. flavus, A. parasiticus, A. fumigatus*	5.7	0.450
S.E		1.63	

N.D = Not Detected, Maximum limit set by EU/NAFDAC= 10µg/kg

Table 4.8 : : Spices from Kafur Market with Associated Fungi and Aflatoxin Content

Spice	Fungi Isolated	Total aflatoxin conc. (µg/kg)	Absorbance (OD) 450nm
Chillies	*A. flavus, A. parasiticus, A. fumigatus*	3.7	0.500
Garlic	*A. flavus, A. parasiticus, A. niger*	N.D	0.760
Ginger	*A. flavus, A. parasiticus, Mucor hiemalis*	N.D	0.924
Dadawa	*A. flavus, A. parasiticus*	N.D	0.991
Curry	*A. flavus, A. parasiticus, Rhizopus stolonifer*	N.D	0.840
Thyme	*A. flavus, A. parasiticus, A. fumigatus*	N.D	0.871
African nutmeg	*A. flavus, A. parasiticus*	12.4	0.292
Ashanti pepper	*A. flavus, A. parasiticus, Rhizopus stolonifer*	N.D	0.933
Clove	*A. flavus, A. parasiticus,*	N.D	1.053
Kajiji	*A. flavus, A. parasiticus*	N.D	0.783
Candlewood	*A. flavus, A. parasiticus, Penicillium citrinum*	N.D	0.853
Yaji	*A. flavus, A. parasiticus*	N.D	0.675
Pineapple of the bush	*A. flavus, A. parasiticus, A. fumigatus*	N.D	1.059
Ethiopian pepper	*A. flavus, A. parasiticus, Rhizopus stolonifer*	N.D	0.872
S.E		0.90	

N.D = Not Detected, Maximum limit set by EU/NAFDAC= 10µg/kg

Aflatoxin concentration of each spice obtained from five markets was compared (Fig. 4.1 and 4.2). Among the garlic samples from five markets, only garlic from Funtua central market contained aflatoxin. Pineapple of the bush from Tsohuwar kasuwa and Yar'kutungu market contained aflatoxin, Thyme from Tsohuwar kasuwa, Katsina central market, Yar'kutungu market contained aflatoxin. Only two samples of dadawa from Tsohuwar kasuwa and Yar'kutungu market were contaminated with aflatoxin. Aflatoxin was only detected in ginger samples from Katsina central and Yar'kutungu markets. African nutmeg samples from all the five markets were contaminated with aflatoxin. . Only chilli powder from Katsina central and Yar'kutungu markets were contaminated with aflatoxin. Kajiji from Tsohuwar kasuwa, Katsina central and Yar'kutungu markets were contaminated with aflatoxin. Aflatoxin was detected in candlewood samples from Tsohuwar kasuwa and Katsina central markets. Aflatoxin was detected in chillies samples from all the markets except Katsina central market. Aflatoxin was detected in only curry samples from Tsohuwar kasuwa. Only clove sample from Tsohuwar kasuwa contained aflatoxin. Aflatoxin was detected in only Ashanti pepper sample from Yar'kutungu market. Aflatoxin was detected in Ethiopian pepper samples from all the market except Kafur market.

Highly significant differences ($P \leq 0.01$) was observed in aflatoxin concentrations among the spices. The highest aflatoxin concentration was in African nutmeg (12.500) which is not significant from clove (10.480), pineapple of the bush (8.000), Ethiopian pepper (5.540) and chillies (5.360). Meanwhile, the least aflatoxin concentration was in curry and Ashanti pepper (0.020). There was non-significant differences ($P > 0.05$) in aflatoxin concentrations among the markets.

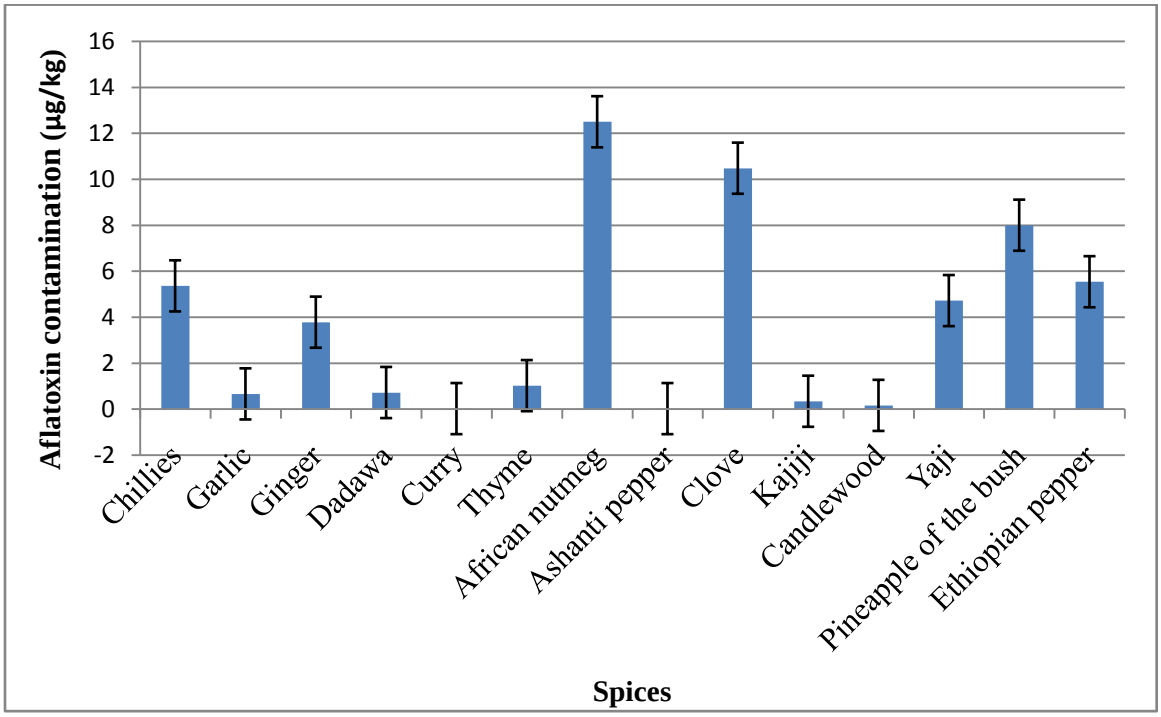


Fig. 4.1 : Mean Aflatoxin Contamination of Spices

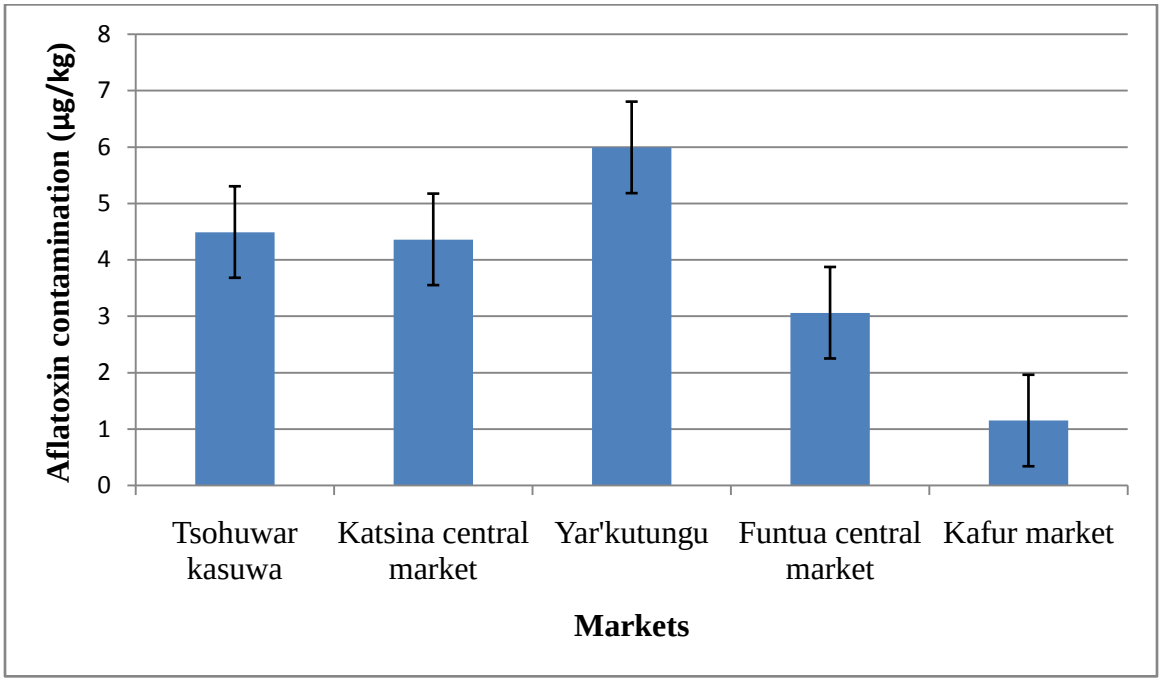


Fig. 4.2 Mean Aflatoxin Contamination in Different Markets

Aflatoxin absorbance of each spice was statistically determined. It was observed that there was non significant differences ($P > 0.05$) in aflatoxin absorbance among the spices while highly significant differences was observed among the markets. The highest absorbance was in spices from Tsohuwar kasuwa and Katsina central market (2.16) which is not significant from Yar kutungu market (1.91). Least mean absorbance was recorded in spices from Kafur (1.55) followed by Funtua (0.70) markets (Table 4.9).

Table 4.9: Comparison of Aflatoxin Absorbance of Spices Obtained from Five Markets in Katsina State

Spices	Absorbance (OD) 450nm					Mean±S.E
	Tsohuwar Kasuwa	Katsina Central Market	Yar'kutungu Market	Funtua Central Market	Kafur Market	
Chillies	2.123	3.000	1.919	0.193	0.500	**1.547±0.52a**
Garlic	2.853	2.997	3.000	0.508	0.760	**2.024±0.57a**
Ginger	3.000	1.052	1.416	0.874	0.924	**1.453±0.40a**
Dadawa	2.639	2.875	2.020	0.806	0.991	**1.866±0.42a**
Curry	2.675	2.698	3.000	0.923	0.840	**2.027±0.47a**
Thyme	2.567	2.139	2.285	0.906	0.871	**1.754±0.36a**
African nutmeg	1.072	1.179	1.053	0.178	0.292	**0.755±0.21a**
Ashanti pepper	2.686	3.000	2.675	0.940	0.933	**2.047±0.46a**
Clove	0.560	0.495	1.002	0.815	1.053	**0.785±0.11a**
Kajiji	2.628	2.521	2.570	0.667	0.783	**1.834±0.45a**
Candle wood	2.663	2.553	3.000	0.726	0.853	**1.959±0.48a**
Yaji	3.000	1.345	0.839	0.809	0.675	**1.334±0.43a**
Pineapple of the bush	0.390	3.000	0.571	1.014	1.059	**1.207±0.47a**
Ethiopian pepper	1.428	1.429	1.421	0.450	0.872	**1.120±0.20a**
Mean±S.E	**2.16±0.24a**	**2.16±0.24a**	**1.91±0.23a**	**0.70±0.06b**	**1.55±0.11b**	**1.551±0.11**

	P value
Spices	0.319ns
Markets	0.000**

Means with the same alphabets either on row or column are not significant (P> 0.05)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Isolation and Identification of Fungi

Spices have been reported to be heavily contaminated by many storage fungi including *Aspergillu*s, *Penicillium*, *Rhizopus*, *Eurotium*, *Cladosporium*, *Trichoderma*, *Mucor* and *Stachybotrys* (Hashem and Alamri, 2010). Spices from Funtua central (250) and Kafur (182) markets contained more fungi than those obtained from Tsohuwar kasuwa (59), Katsina central (56) and Yar'kutungu market (81) in Katsina metropolis. This might be due to the difference in ecological zone. Funtua and Kafur towns are more humid (Northern guinea savanna) than Katsina town (Sudan savanna). Warm and humid climatic conditions favour the growth of fungi (Huho, 2011).

5.2 Percentage Occurrence of Fungi Species

Percentage occurrences of different fungi species revealed that *Aspergillus* had the highest frequency of occurrence in all spices obtained from all the markets. The reason for their high incidence might be due to their cosmopolitan nature and they are known to be obligate saprophytes so can survive in the environment with a wide range of temperatures varying from 18-32º C (Shehu and Bello, 2011). This result is in agreement with the result obtained by Sumanth *et al*. (2010) in India who isolated fungal genera from tested spices (ammi, caraway, cardamom, coriander and cumin), and found that the most common fungi isolated were *Aspergillus* spp. Another research conducted by

Bokhari (2007) in Saudi Arabia revealed that *Aspergillus* was the most common genus isolated from samples of spices (clove, anise, black pepper, red pepper, black cumin, peppermint, cardamom, ginger and marjoram) tested. Among the *Aspergillus* species encountered, *A. flavus* and *A. parasiticus* were most prevalent. This might be due to their abilty to survive in a wide range of varying environmental factors (temperature and humidity) as compared to other species isolated. The temperature ranges for *A. flavus* is (12-48º C), *A. parasiticus* (17-42º C), *A. fumigatus* (12-50º C), *A. niger* (25-30º C), *A. versicolor* (22- 26º C) and *A. terreus* (35-40º C). There is also variation in the water activity,*A. flavus* (0.50- 0.99), *A. parasiticus* (0.82), *A. fumigatus* (0.82), *A. niger* (0.85-0.93), *A. versicolor* (0.75-0.81) and *A. terreus* (0.78) (Shehu and Bello, 2011). Hedayati *et al*. (2007) reported that *A. flavus* can grow and thrive in both hot and humid conditions. Incidence of *A. flavus* in all the samples from all the five markets ranged from 17.7-59.9 % while *A. parasiticus* ranged from 32.9-43.3 %. This result is in agreement with the result obtained by Hashem and Alamri, (2010) who reported that among the species of *Aspergillus* previously reported to contaminate spices globally, *A. flavus*, *A. parasiticus* and *A. tamarii* were prevalent with *A. flavus* occurring more frequently. Moderate level of incidence was found in *Rhizopus stolonifer* (6.6 %). In a similar work conducted by Hashem and Alamri (2010) in Saudi Arabia, moderate occurrences were reported for *Aspergillus*, *Rhizopus* and *Penicillium* species. The least level of incidence ranging between 0.56 -3.6 % was recorded for *Nigrospora sphaerica, A. fumigatus, A. niger, A. versicolor, A. terreus, Rhizoctonia sp, Muccor hiemalis, Penicillium citrinum* and *Phoma glomerata*. Several researchers reported moderate or low frequency of occurrence of *A. fumigatus*, *A. terreus* and *A. versicolor* in different spices (Hashem and Alamri, 2010;

Amal, 2011). Hashem and Alamri considered highest fungi occurrence at > 40 %, moderate 25-40 % and low < 25, while Amal considered highest value at ≥ 19 %, moderate 9-8 % and low < 9 %.

5.3 Percentage Contamination of Spices by Fungi

The fungi contamination of spices in this study showed that chillies were contaminated with *A. flavus, A. parasiticus, Rhizopus stolonifer*, *Rhizoctonia sp*, *Penicillium citrinum, A. niger and A. fumigatus.* Chillies are mostly spread on the ground or by the roadside for drying by the farmers, this enhances contamination by the fungi. Sometimes small quantity of water is sprinkled on chillies during packaging in order to reduce the spicy smell of it. This little moisture on the fruit may enchance mould growth during storage. In a similar report by El-kady *et al*. (1995) in Egypt, Freire *et al.* (2000) in Brazil and Ekhuemelo and Ekefan (2013) in Nigeria isolated *A. flavus, A. fumigatus, A. niger, A. parasiticus, Rhizoctonia* sp., *Rhizopus* sp. and *Penicillium* sp from chillies. Similarly Fungi isolated from garlic in this study were *A. flavus, A. parasiticus, A. niger and A. versicolor* which also in agreement with those isolated by Ghangoankar (2013) and Olalekan *et al*. (2003) who isolated similar fungi. Contamination of garlic with fungi might have occurred during drying or storage. Garlic is not completely dried at storage and this will enhance mould growth. Ginger samples were contaminated with fungal species of *A. flavus, A. parasiticus, A. versicolor, Nigrospora sphaerica, Rhizopus stolonifer and Mucor hiemalis*. Contamination of ginger by fungi might have occurred during drying. Independent works in India by Geeta and Reddy (1990) and Madhyastha and Bhat (1984) isolated *A. flavus and A. parasiticus* from ginger samples respectively.

Bokhari (2007) isolated *Rhizopus stolonifer and Mucor hiemalis* in ginger samples. Gnonlonfin *et al*. (2013) isolated *Nigrospora sphaerica* from ginger samples.

Fungi isolated from Clove obtained from all the markets were *A. flavus, A. parasiticus, A. niger*. This agreed with the result of Farid *et al*. (2013) who isolated *A. flavus* and *A. niger* from clove while Olalekan *et al*. (2003) isolated *A. parasiticus* in clove samples. Fungi species isolated from Ashanti pepper were *A. flavus, A. parasiticus, Rhizopus stolonifer, A. terreus* and *Phoma glomerata*. Similar works by Ezekiel *et al*.. (2013) in Lagos Nigeria reported contamination of Ashanti pepper with *A. flavus* and *Rhizopus* sp. while Oranusi *et al*. (2013) also in Nigeria isolated *A. parasiticus* from samples of Ashanti pepper. Chilli powder samples were contaminated with *A. flavus, A. parasiticus, Rhizopus stolonifer, A. versicolor, A. fumigatus* and *Penicillium citrinum*. . Chilli powder is produced using traditional processing method. Contamination of this product by fungi might have resulted during poor sanitary processing stages it passed through, or from the chilli pods from which the powder was produced. Rajasinghe *et al*. (2009) isolated *A. flavus, Rhizopus* sp. and *Penicillium* sp. from chilli powder while Erdogan, (2004) isolated *A. versicolor*. Fungi species isolated from curry samples were *A. flavus, A. parasiticus, A. terreus, A. versicolor, Rhizopus stolonifer* and *Rhizoctonia* sp. Similar work done on curry by Rajasinghe *et al*. (2009) in India reported contamination of curry with *A. flavus* and *Rhizopus* sp. In this study thyme samples were found to be contaminated with *A. flavus, A. parasiticus, A. fumigatus, Rhizoctonia* sp. and *Rhizopus stolonifer*. Olalekan *et al*. (2003) in Nigeria isolated *A. flavus* and *A. parasiticus* from thyme sample while Hashem and Alamri (2010) in Saudi Arabia isolated *Rhizopus stolonifer*. Thyme and curry powder might have become contaminated with fungi during

processing. Poor handling during processing could lead to contamination of products by fungi. Fungi isolated from African nutmeg were *A. flavus, A. parasiticus, A. versicolor, Rhizopus stolonifer, Penicillium citrinum, Nigrospora sphaerica and A. fumigatus*. High contamination of African nutmeg with fungi might be due to the nature of the fruit. The spice has two thick woody pericaps surrounding the mesh-like fragrant pulp. The dried pulp has perforations which may be an avenue for entry of fungal spores and a store for these spores during drying or storage. Similar work done by Ezekiel *et al*. (2013) in Lagos Nigeria reported *A. flavus, Rhizopus* sp. and *Penicillium* sp. from African nutmeg. Fungi isolated from Ethiopian pepper samples in this study were *A. flavus, A. parasiticus, Rhizopus stolonifer, A. fumigatus and Penicillium citrinum*. Oranusi *et al*. (2013) isolated *Rhizopus* sp. and *Penicillium* sp. from samples of Ethiopian pepper while Omafuvbe and Kolawole (2005) in Nigeria isolated *A. fumigatus*. Kajiji samples were found associated with *A. flavus, A. parasiticus, Nigrospora sphaerica, Mucor, Rhizopus stolonifer, A. versicolor, Rhizoctonia* sp. *and Penicillium citrinum.* Fungi isolated from Pineapple of the bush were *A. flavus, A. parasiticus, Rhizoctonia* sp., *Phoma glomerata* and *A. fumigatus*. High contamination of kajiji, Ethiopian pepper and pineapple of the bush by fungi might have occurred during transportation. Dadawa samples were associated with *A. flavus, A. parasiticus* and *Rhizopus stolonifer*. Dadawa is a fermented condiment produced locally due to lack of modern technology. Contamination of this product by fungi has been reported by Luka *et al.* (2013) from Jos Nigeria. Fungi isolated from candlewood were *A. flavus, A. terreus, A. versicolor, Rhizoctonia* sp., *Penicillium citrinum, A. parasiticus* and *Nigrospora sphaerica*. These were isolated in low frequency and might be due to to low water activity associated with it. It has been reported that the

microbial contamination of a products is dependent on the environment it passed through and to the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce (Oranusi *et al*., 2013), as well as drying.

5.5 Aflatoxin Contents of Spices

Out of seventy spices samples analysed for total aflatoxins during this study, thirty three (47.1 %) were contaminated by aflatoxins of varied concentrations ranging from 0.1- >20 µg/kg, while 37 (52.9 %) samples tested negative to aflatoxins contamination.

African nutmeg from all the markets except Katsina central markets contained aflatoxin above maximum acceptable limit (10 µg/kg) set by EU and NAFDAC (CAC, 1995;E.C, 2002.). There was little variation in aflatoxin concentration among the markets. Highest concentration were recorded in samples from Funtua central market and Kafur market respectively. This might be due to higher humidity as compared to other three markets in Katsina metropolis. Mean aflatoxin concentration of African nutmeg was 12.5 µg/kg. The high contamination of African nutmeg by aflatoxin might be due to the nature of it's matrice which is highly rich with nutrient content that promotes fungal growth and subsequent aflatoxin production. In a similar work by Ezekiel *et al*. (2013) in Lagos Nigeria reported aflatoxin B1 concentration of African nutmeg at 20 µg/kg. Maximum acceptable limit of (10 µg/kg) for total aflatoxin in spices set by EU and NAFDAC was used in comparison with aflatoxin concentration of spices and condiments in this work. Only garlic from Funtua central market contained aflatoxin (3.3 µg/kg) which is within the acceptable limit. This might be as a result of high humidity and long term storage of garlic. Concentration of aflatoxin in garlic in this study was slighty higher than the

concentration reported by William *et al*. (2014) of 2 µg/kg in garlic obtained from Nyahururu retail market in Kenya. Low aflatoxin contamination of garlic in this work might be attributed to presence of antimicrobial component (Allicin) known to inhibit microbial growth. Aflatoxin was only detected in Pineapple of the bush obtained from Tsohuwar kasuwa and Yar'kutungu market at concentration above maximum acceptable limit. This might be due to the unhygienic nature of the markets that promoted contamination.

Only thyme from Tsohuwar kasuwa, Katsina central market and Yar'kutungu markets contained aflatoxin concentration (0.6, 2.6 and 1.9µg/kg) respectively which were within acceptable limit. Low aflatoxin concentration of thyme might be due to the presence of thyme essential oil (thymol) that inhibits mould growth as well as industrial processing. Aflatoxin was only detected in dadawa obtained from Tsohuwar kasuwa and Yar'kutungu market at concentration of 0.3 and 3.3 µg/kg respectively which are within acceptable limit. Detection of aflatoxin in dadawa from these two markets might be due to unhygienic conditions of the markets. This is similar to the result of Luka *et al*. (2013) who reported aflatoxin contamination in Dadawa from Jos at the range of 0.21-3.45 µg/kg. Ginger was found to be contaminated with aflatoxin in samples obtained from Katsina central market and Yar'kutungu market with aflatoxin concentration of 11.6 and 7.3 µg/kg respectively. Aflatoxin concentration in ginger from Katsina central market was above maximum acceptable limit. This might be associated to long period of ginger storage in the shops, spices may be contaminated with aflatoxins in the field, during drying, storage and transport (Elshafie *et al*., 2002). Ginger from Yar'kutungu market contained aflatoxin concentration within acceptable limit and contamination might be due

to the nature of handling the product in the market. In a similar report by Patel *et al*. (1996) who reported aflatoxin levels in ginger between 4.2-13.5 µg/kg. Aflatoxin was only detected in chilli powder obtained from Katsina central market and Yar'kutungu market (8.1 and 15.5 µg/kg) respectively. Only chilli powder from Yar'kutungu market contained aflatoxin concentration above maximum acceptable limit. In a similar work conducted by Klieber (2000) who reported aflatoxin contamination in chilli powder in Australia at the range of 5-10 µg/kg. Only Kajiji obtained from Tsohuwar kasuwa, Katsina central market and Yar'kutungu markets contained aflatoxin at concentrations of 0.3, 0.8 and 0.6 µg/kg respectively. All the aflatoxin concentrations are within the acceptable limit. Kajiji from Katsina central market had the highest aflatoxin concentration which might be due to long period of storage. It was followed by sample from Yar'kutungu market which might be due to dusty nature of the market. Aflatoxin was only detected in Candlewood obtained from Tsohuwar kasuwa and Katsina central market at concentrations of (0.1 and 0.7 µg/kg). These were within the aflatoxin acceptable limit. Chillies from all the markets except Katsina central market contained aflatoxin. However, Funtua central market had the highest aflatoxin concentrations (16.6 µg/kg) which is above aflatoxin acceptable limit. This might be due to high humidity during storage. Other three markets Tsohuwar kasuwa, Yar'kutungu and Kafur markets had aflatoxin levels (2.7, 3.8 and 3.7 µg/kg) respectively which are all within aflatoxin acceptable limit. In a work conducted by Food Safety Authority of Ireland (2004) reported aflatoxin contamination in Chillies ranging from 2- 9.7 µg/kg. Another study conducted by Hell *et al*. (2009) reported aflatoxin contamination in dried Chillies in West Africa at 3.2 µg/kg. Aflatoxin was only detected in Curry obtained from Tsohuwar

kasuwa at concentration of 0.1 µg/kg which is within aflatoxin acceptable limit. This agreed with the result of Menon and Xavier (2010) who reported that 8 samples of Curry powder contained aflatoxins at < 1 µg/kg. Clove obtained from Tsohuwar kasuwa, Katsina central market and Yar'kutungu market had aflatoxin concentration of (> 20, >20 and 12.4 µg/kg) respectively which are above aflatoxin acceptable limit. In a similar work conducted by William *et al*. (2014) reported mean aflatoxin contamination in Clove obtained from Nyahururu retail market in Kenya at the level of 7 µg/kg. Aflatoxin was only detected in Ashanti pepper obtained from Yar'kutungu market at concentration of 0.1 µg/kg which is within aflatoxin acceptable limit. Low aflatoxin concentration in Ashanti pepper might be due to the presence of piperine. Piperine has been reported to inhibit aflatoxin biosynthesis in *Aspergillus parasiticus* (Madhyastha and Bhat, 1984). Ethiopian pepper from all the markets except Kafur market contained aflatoxin concentrations which are all within aflatoxin acceptable limit. Aflatoxin concentration of Ethiopian pepper from the four markets namely Tsohuwar kasuwa, Katsina central market, Yar'kutungu and Funtua central market were 7.3, 7.3, 7.4 and 5.7 µg/kg respectively.

Aflatoxin was detected in most spices obtained from three markets in Katsina metropolis compared to those samples obtained from Funtua and Kafur markets. However, interms of aflatoxigenic fungi spices and condiments from Funtua and Kafur markets had more number of isolates than those samples from three markets in Katsina metropolis. This might be due to the inability of the aflatoxigenic fungi to produce aflatoxins in the samples. Elshafie *et al*., (2002) in the Sultanate of Oman did not detect any aflatoxins in 15 selected samples out of 105 spices samples including cloves and ginger, but found

45% of their 20 *Aspergillus flavus* isolates to be aflatoxigenic. It has been established that spices may support fungal growth (e.g *A. flavus*) but inhibit the production of aflatoxins than in cereals (MacDonald and Castle,1996). Therefore it is suggested that not all *A. flavus* strains are aflatoxigenic on this kind of matrix (Elshafie *et al*., 2002). Bokhari (2007) also reported that some spices inhibit aflatoxin biosynthesis but not the growth of the toxigenic fungi.

Yar'kutungu market had the highest number of contaminated samples(11) out of 14 samples. It also had the highest mean concentration of aflatoxin (5.99 µg/kg) than the other four markets. This was due to the nature of the markets. The market ground is neither cemented nor asphalted as such market atmosphere is always dusty due to movement of people. People selling spices and condiments mostly spread different kinds of mat directly on the bare ground on which spices and condiments are placed. This provide avenue for fungi contamination of spices and condiments including mycotoxigenic species.

Tsohuwar kasuwa recorded ten (10) contaminated samples with mean aflatoxin concentration of 4.49 µg/kg. This is a roadside market and people selling spices and condiments place them on mats spread directly on the ground which is cemented. Contamination of the samples might have resulted from poor hygienic condition of the road/roadside.

Eight samples collected from Katsina Central market contained aflatoxin with mean aflatoxin concentration of (4.36 µg/kg). It is a modern market and spices and condiments are kept inside the shop or shop verandah in metal, plastic or wooden containers.

Containers are placed directly on tables not ground. Aflatoxin contamination of sample might have occurred as a result of long period of storage.

Funtua central market recorded aflatoxin contamination of 4 samples with mean aflatoxin concentration of (3.06 µg/kg). Market consists of shops and spices sold are kept in metal, plastic or wooden containers placed directly on tables. Unlike spices and condiments sold in Katsina central market that are stored for a long time, people selling spices and condiments in Funtua central market do not keep huge quantity of these commodities in the shops. Therefore chances of contamination of spices and condiments during storage is reduced.

Two (2) out of 14 samples of spices collected from Kafur market contained aflatoxin with mean concentration of (1.15 µg/kg). The market is a weekly market, people selling spices and condiments come from different places along with their commodities. People selling spices and condiments stay by the roadside. The difference in mean aflatoxin concentration of this market and that of Tsohuwar kasuwa which is also a roadside market might result from difference in hygienic conditions of the markets. Tshohuwar kasuwa is more unhygienic than Kafur market because it is located in the ancient city with high human activities which contributes to the poor sanitation of the market. While there is no high human activities in Kafur market apart from market day which is once in a week.

Although aflatoxin concentrations detected in majority of spices and condiments are within acceptable limit but continuos accumulation of this toxin over time will have different types of health effects on humans.

CHAPTER SIX

6.0 SUMMARY CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Fungi and aflatoxin contaminations of 14 different types of spices obtained from 5 markets (Tsohuwar kasuwa, Katsina central, Yar'kutungu, Funtua and Kafur markets) were determined. These were clove, African nutmeg, Ashanti pepper, candlewood, Ethiopian pepper, pineapple of the bush, ginger, garlic, chillies, kajiji, thyme, yaji, dadawa and curry. The spices were contaminated with 12 different fungal species, including mycotoxigenic fungi. These were *Aspergillus flavus, A. parasiticus, A. fumigatus, A. versicolor, A. niger, A. terreus, Rhizopus stolonifer, Penicillium citrinum, Phoma glomerata, Nigrospora sphaerica, Rhizoctonia* sp. *and Mucor hiemalis. Aspergillus* was the predominantly occurring genus with *A. flavus* and *A. parasiticus* being most frequently occurring species with percentage occurrences of 45.54 and 39.01 % respectively. The least occurring fungus was *Mucor hiemalis* (0.48 %). Pineapple of the bush had the highest fungal contamination (12.42 %) followed by chillies (11.62 %),.while candlewood was the least contaminated (2.71 %). Higher fungal isolates were recorded in samples obtained from Funtua (250) and Kafur (182) markets (Northern guinea savanna) compared with the other 3 markets in Katsina metropolis (Sudan savanna). Aflatoxin Assay was carried out using ELISA (Enzyme-linked Immunosorbent Assay) technique. AgraQuant Total Aflatoxin Assay test kit and Mycosep immuno affinity columns were used for aflatoxin assay. Thirty three (47.1 %) out of seventy (70) samples analysed were contaminated by aflatoxin in the range of 0.1->20µg/kg, while 37

(52.9 %) samples tested negative for aflatoxin contamination. African nutmeg had the highest aflatoxin contamination (12.500 µg/kg), followed by clove (10.480 µg/kg). The least contaminated samples were curry and Ashanti pepper (0.020 µg/kg). Yar'kutungu market had the highest aflatoxin contamination of samples with mean aflatoxin concentration of 5.99 µg/kg, followed by Tsohuwar kasuwa (4.49 µg/kg) while Kafur market had the least aflatoxin contamination (1.15 µg/kg).

6.2 Conclusions

1 Twelve different species of fungi including aflatoxigenic fungi were isolated from spices sold in five markets in Katsina State. Seven fungal genera were isolated from the spices and Aspergillus was the most dominant isolated genus. Among *Aspergillus* species *A. flavus* and *A. parasiticus* were the most frequently occurring. Pineapple of the bush had the highest fungal contamination (12.42 %) followed by chillies (11.62 %), while candlewood was the least contaminated (2.71 %).

2 Aflatoxin contamination was recorded in thirty three (47.1%) out of seventy sample with chillies (Funtua central), ginger (Katsina central), African nutmeg (all the markets except Katsina central), clove (all the markets except Funtua and Kafur) pineapple of the bush (Tsohuwar kasuwa and Yar'kutungu) and yaji (Yar'kutungu) Markets having aflatoxin concentrations above maximum permissible limits (10µg/kg) set by EU and NAFDAC.

3 Spices from Funtua central and Kafur markets had the highest fungi contamination compared to spices from Tsohuwar kasuwa, Katsina central and Yar'kutungu markets, but less contaminated by aflatoxin.

6.3 Recommendations

1. Some spices have high aflatoxin concentration than recommended limits, regulatory agencies for e.g. NAFDAC should ensure regular monitoring of aflatoxin contamination in spices.

2. Further work on different ways by which fungi and aflatoxin contaminate spices is required.

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APPENDICES

Appendix I : Percentage Occurrence of Fungal Isolates in Samples Obtained from Tsohuwar Kasuwa

Samples	*A. flavus*	*A. parasiticus*	*A. fumigatus*	*A. versicolor*	*Rhizoctonia* sp.	*Rhizopus stolonifer*	*Nigrospora sphaerica*	
Chillies	2	3	-	-	-	1	-	
Garlic	-	-	-	1	-	-	-	
Ginger	-	-	-	-	-	1	1	
Dadawa	1	2	-	-	-	1	-	
Curry	1	2	-	-	-	1	-	
Thyme	1	2	1	-	-	-	-	
African nutmeg	4	3	1	-	-	-	1	
Ashanti pepper	-	-	-	-	-	2	-	
Clove	4	3	-	-	-	-	-	
Kajiji	1	2	-	-	-	-	1	
Candle wood	1	1	-	1	-	-	-	
Yaji	-	-	-	-	-	1	-	
Pineapple of the bush	4	3	-	-	1	-	-	
Ethiopian pepper	2	1	1	-	-	-	-	
Total	21	22	3	2	1	7	3	59
% occurrence	35.6	37.3	5.1	3.4	1.6	11.9	5.1	

Appendix II : Percentage Occurrence of Fungal Isolates in Samples Obtained from Katsina Central Market

Samples	*A. flavus*	*A. parasiticus*	*A. niger*	*A. versicolor*	*A. fumigatus*	*Mucor hiemalis*	*Phoma glomerata*	*Rhizopus stolonifer*	*Penicilliu m citrinum*	*Nigrospor a sphaerica*	
Chillies	1	-	1	-	-	-	-	-	-	-	
Garlic	-	1	2	-	-	-	-	-	-	-	
Ginger	1	-	-	2	-	-	-	-	-	-	
Dadawa	1	2	-	-	-	-	-	-	-	-	
Curry	-	-	-	-	-	-	-	1	-	-	
Thyme	1	3	-	-	2	-	-	1	-	-	
African Nutmeg	3	1	-	-	-	-	-	-	-	1	
Ashanti Pepper	-	-	-	-	-	-	-	1	-	-	
Clove	5	3	1	-	-	-	-	-	-	-	
Kajiji	1	4	-	-	-	1	-	1	-	-	
Candle wood	1	-	-	-	-		-	-	1	-	
Yaji	1	1	-	1	1	-	-	1	-	-	
Pineapple of the bush	-	-	-	-	-	-	2	-	-	-	
Ethiopian pepper	-	5	-	-	-	-	-	-	1	-	
Total	15	20	4	3	3	1	2	5	2	1	56
% occurrence	26.8	35.7	7.1	5.4	5.4	1.8	3.6	8.9	3.6	1.8	

Appendix III : Percentage Occurrence of Fungal Isolates in Samples Obtained from Yar'kutungu Market

Samples	*A. flavus*	*A. parasiticus*	*A. terreus*	*A. versicolor*	*A. fumigatus*	*Phoma glomerata*	*Rhizopus stolonifer*	*Rhizoctonia sp*	*Penicilliu m citrinum*	*Nigrospor a sphaerica*	
Chillies	1	3	-	-	-	-	1	1	1	-	
Garlic	-	1	-	1	-	-	-	-	-	-	
Ginger	2	1	-	1	-	-	-	-	-	2	
Dadawa	-	3	-	-	-	-	2	-	-	-	
Curry	2	1	5	2	-	-	1	1	-	-	
Thyme	-	4	-	-	1	-	-	1	-	-	
African nutmeg	1	2	-	1	-	-	1	-	1	-	
Ashanti pepper	1	-	1	-	-	2	-	-	-	-	
Clove	3	2	-	-	-	-	-	-	-	-	
Kajiji	-	3	-	1	-	-	1	1	2	-	
Candle wood	1	-	1	1	-	-	-	1	-	-	
Yaji	2	-	-	-	-	-	1	-	1	-	
Pineapple of the bush	1	4	-	-	-	-	-	3	-	-	
Ethiopian pepper	2	2	-	-	-	-	-	-	-	-	
Total	16	26	7	7	1	2	7	8	5	2	81
% occurrence	19.8	32.1	8.6	8.6	1.2	2.5	8.6	9.9	6.2	2.5	

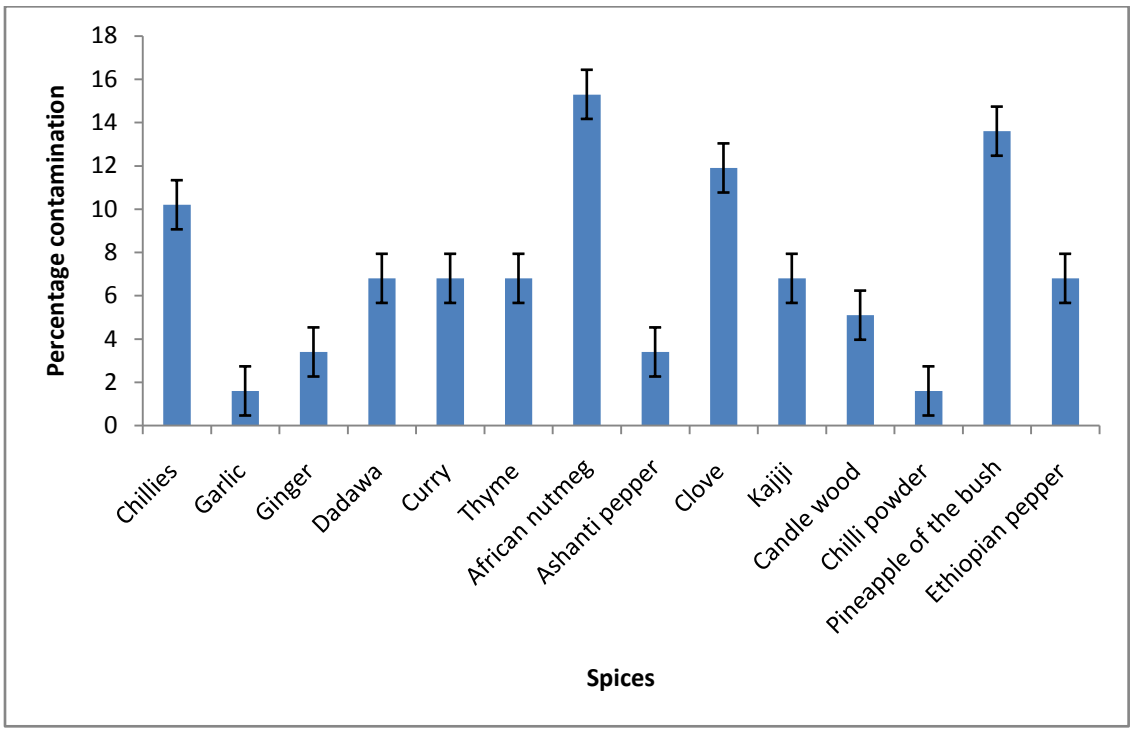
Appendix IV : Percentage Occurrence of Fungal Isolates in Samples Obtained from Funtua Central Market

Samples	*A. flavus*	*A. parasiticus*	*A. fumigatus*	*Mucor hiemalis*	*Rhizoctonia sp*	*Rhizopus stolonifer*	*Nigrospora Sphaerica*	
Chillies	6	16	-	-	2	1	-	
Garlic	20	3	-	-	-		-	
Ginger	2	2	-	1	-	-	-	
Dadawa	3	2	-	-	-	1	-	
Curry	15	3	-	-	-	-	-	
Thyme	12	3	-	-	-	-	-	
African nutmeg	18	2	-	-	-	-	-	
Ashanti pepper	5	1	-	-	-	-	-	
Clove	14	5	-	-	-	-	-	
Kajiji	6	15	-	-	-	-	-	
Candlewood	-	2	-	-	-	-	3	
Yaji	3	10	-	-	-	1	-	
Pineapple of the bush	2	45	-	-	-	-	-	
Ethopian pepper	20	3	3	-	-	-	-	
Total	126	112	3	1	2	3	3	250
% occurrence	50.4	44.8	1.2	0.4	0.8	1.2	1.2	

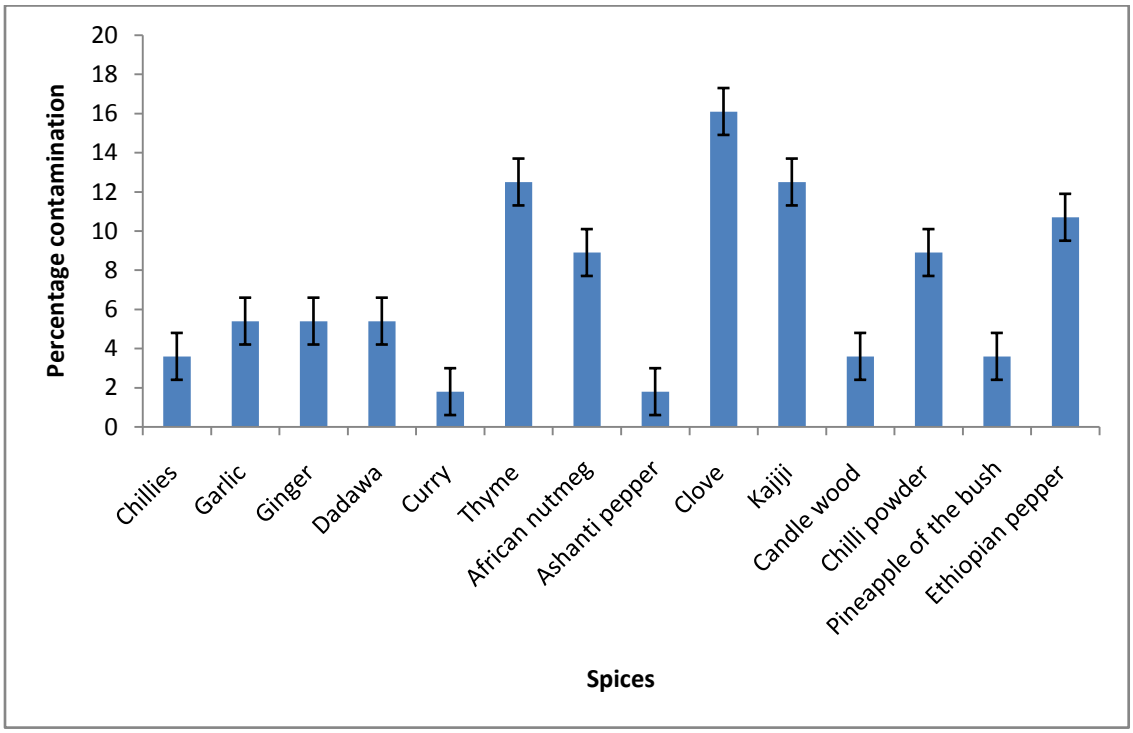
Appendix V : Percentage Occurrence of Fungal Isolates in Samples Obtained from Kafur Market

Samples	*A. flavus*	*A. parasiticus*	*A. fumigatus*	*A. niger*	*Rhizopus stolonifer*	*Mucor hiemalis*	*Penicillium citrinum*	
Chillies	20	12	1	-	-	-	-	
Garlic	15	6	-	1	-	-	-	
Ginger	1	1	-	-	-	1	-	
Dadawa	2	1	-	-	-	-	-	
Curry	3	7	-	-	1	-	-	
Thyme	6	3	1	-	-	-	-	
African nutmeg	14	5	-	-	-	-	-	
Ashanti pepper	3	1	-	-	1	-	-	
Clove	10	8	-	-	-	-	-	
Kajiji	9	5	-	-	-	-	-	
Candlewood	1	1	-	-	-		1	
Yaji	4	6	-	-	-	-	-	
Pineapple of the bush	9	3	1	-	-	-	-	
Ethopian pepper	11	6	-	-	1	-	-	
Total	108	65	3	1	3	1	1	182
% occurrence	59.3	35.7	1.6	0.5	1.6	0.5	0.5	

Appendix VI: Fungi Contamination of Spices from 'a' Tsohuwar Kasuwa and 'b' Katsina Central Markets

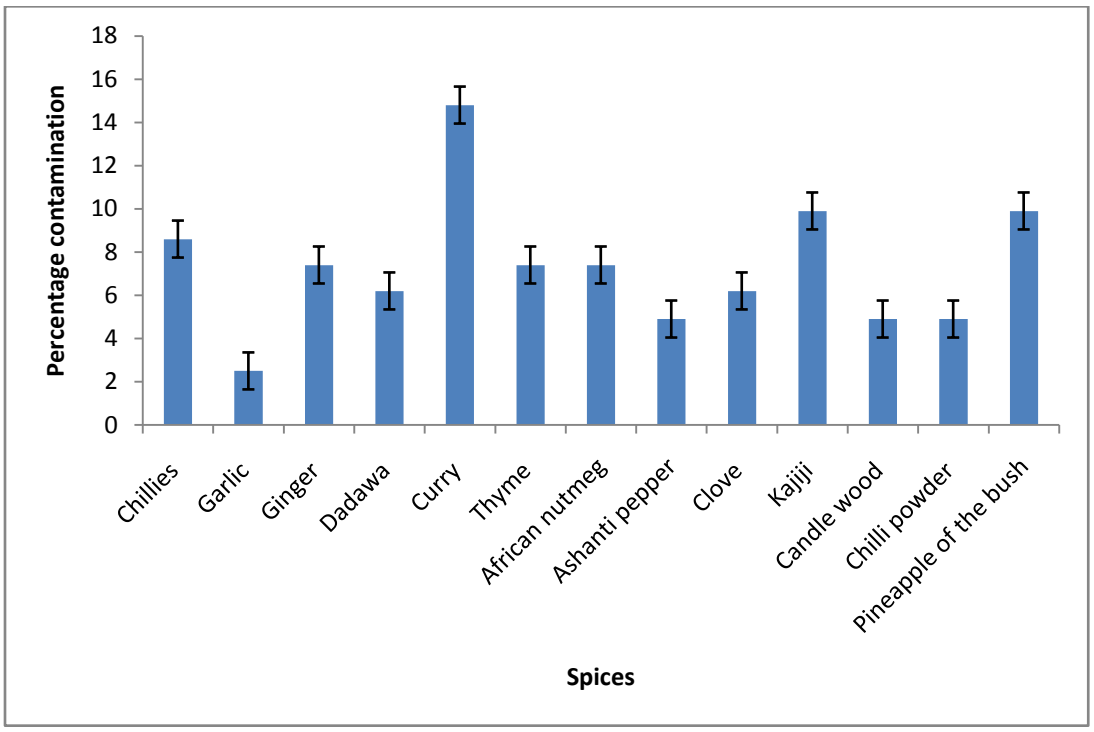


a

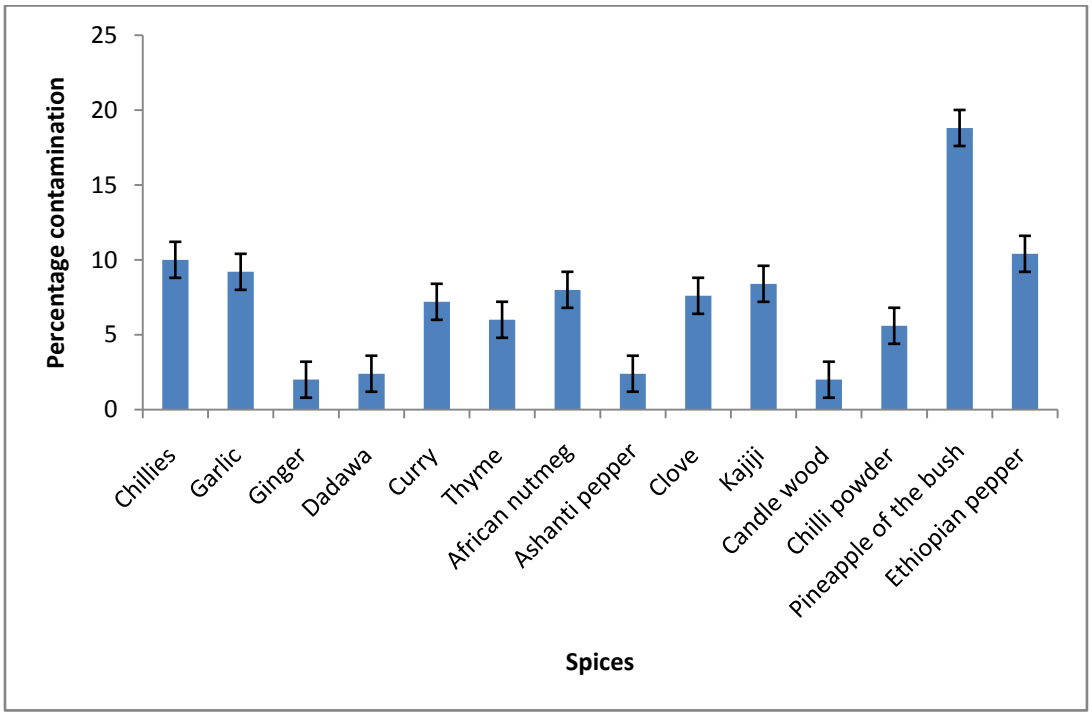


b

Appendix VII: Fungi Contamination of Spices from 'a' Yar'kutungu and 'b' Funtua Central Markets.



a



B

Appendix VIII: Fungi Contamination of Spices from Kafur Market.

