THE ISOLATION AND IDENTIFICATION OF AFLATOXIN PRODUCING MOLD IN COMMONLY CONSUMED FOOD SAMPLES IN OYE EKITI AND THE AFLATOXIN CONCETRATION IN THEM.

BY

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DEDICATION

This project work is dedicated to the glory of almighty God the giver of wisdom knowledge and understanding. Also to my parents pastor and Mrs Aladelokun.

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"You'll never walk alone" (YNWA).

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ABSTRACT

Aflatoxins are naturally occurring mycotoxins which are common occurrence in stored produce in this part of the country. The aim of the study is to isolate and identify aflatoxin producing molds from selected food samples sold in Oye-Ekiti central market and to provide information on the levels of aflatoxins in order to ascertain the safety level of the foods. The isolation and identification of molds were carried out using their morphological characteristics as the basic diagnostic method to determine the molds producing aflatoxin in the following raw food samples (maize, yam, beans and onion). The food samples were cultured on Sabouraud Dextrose Agar (SDA). Aflatoxin levels were determined using Enzyme Linked Immunosorbent Assay (ELISA). The highest aflatoxin level was found in maize.

Chapter one

1.0 INTRODUCTION

Oye Ekiti is a town situated in the northern part of Ekiti state south-west Nigeria with the market found in the center of the town. Its market that sells mostly consumable or perishable food crops such as yam, beans, rice, okro, onion pepper, tomato, water melon, maize. Maize, onion, beans, yam are food commodities which are greatly consumed in the Oye-Ekiti community, they become infected, spoils due to poor storage facilities in the locality. Food crops like onion, yam, and beans, maize spoil easily due to low relative humidity and its storage in moisture environments. Shelf life of this food products have been a problem which seemed not to have a solution due to the food crops becoming infected with fungi infections such as rot disease, black mold by *Aspergillus* species which not only cause threadlike growth of mold but also cause food poisoning by producing toxigenic substances called aflatoxin which is harmful to consumers health.

1.1 Onion

The onion (*Allium cepa.*) (Latin 'cepa' = onion), also known as the bulb onion or common onion, is a vegetable and is the most widely cultivated species of the genus Allium. The onion is most frequently a biennial or a perennial plant, but is usually treated as an annual and harvested in its first growing season. The onion plant has a fan of hollow, bluish-green leaves and the bulb at the base of the plant begins to swell when a certain day-length is reached. In the autumn the foliage dies down and the outer layers of the bulb become dry and brittle. The crop is harvested and dried and the onions are ready for use or storage. It's consumed everywhere in Nigeria and sold in virtually in all localities in Nigeria, it's consumed in virtually every home during cooking in

Oye Ekiti. The crop is prone to attack by a number of pests and diseases, particularly the onion fly, the onion eelworm and various mold that cause rotting. Black mold (*Aspergillus niger*)

1.2 Maize

Maize, English-speaking countries as corn, is a large grain plant domesticated by indigenous peoples in Mesoamerica in prehistoric times, worldwide production of maize is 785 million tons, with the largest producer, the United States, producing 42%. Africa produces 6.5% and the largest African producer is Nigeria with nearly 8 million tons. It's produced in Wet rainforest, covering most of Eastern States of Nigeria and the South-Western part, Derived Savanna, fringing the forests and forming the transition to the southern Guinea Savanna, Southern Guinea Savanna. It is cultivated everywhere in Nigeria during the raining season and it takes about ninety days for it to reach maturity.

The leafy stalk produces ears which contain the grain, which are seeds called kernels. Maize kernels are often used in cooking as a starch. The six major types of maize are dent, flint, pod, popcorn, flour, and sweet (Ranere *et al.,* 2009). They are been known to be infected by various plant storage pest which reduces their economic value and their consumption, examples are mold infection from Aspergillus species e.g *A. funmigatus, A. niger, A. flavus,* which infects the stored grain due to its storage in environment with moist atmospheric condition. . (National Science Foundation 2014)

1.3 Yam

Yam is the common name for some plant species in the genus Dioscorea (family Dioscoreaceae) that form edible tubers. These are perennial herbaceous vines cultivated for the consumption of their starchy tubers in Africa, Asia, Latin America, the Caribbean and Oceania. There are many

cultivars of yam. Although some varieties of sweet potato (Ipomoea batatas) are also called yam in parts of the United States and Canada, sweet potato is not part of the family Dioscoreaceae but belongs in the unrelated morning glory family Convolvulaceae. Yams are monocots, related to lilies and grasses. Native to Africa and Asia. There are over 600 varieties of yams and 95 percent of these crops are grown in Africa with Nigeria producing 1/3 of it been the largest producer of yam in Africa.

Yam spoilage usually occurs during the storage period due to the atmospheric condition of the storage environment. Storing yam at low temperature reduces the respiration rates. However, temperatures below 12 °C (53.6 °F) cause damage through chilling, causing a breakdown of internal tissues, increasing water loss and yam's susceptibility to decay. The symptoms of chilling injury are not always obvious when the tubers are still in cold storage. The injury becomes noticeable as soon as the tubers are restored to ambient temperatures. Sprouting rapidly increases a tuber's respiration rates, and accelerates the rate at which its food value decreases. Yam spoilage are caused by mold mostly of the aspergillus species which grow on it when stored in humid environment to form white to green like-mold on it.

1.4 Cowpea

Bean is a seed in a pod of certain leguminous plants of the family Fabaceae, originally of *Vicia faba,* an Old World species called broad bean or fava bean. The mature seeds of the principal beans used for food, except soybeans, are rather similar in composition, although they differ widely in eating quality. Rich in protein and providing moderate amounts of iron and vitamins B_1 and B_2, beans are used worldwide for cooking in either fresh or dried form. The term bean usually excludes crops used mainly for oil extraction (such as soy-beans and pea-nuts), as well as those used exclusively for sowing purposes (such as clover and alfalfa). Leguminous crops

harvested green for food, such as snap peas, snow peas, and so on, are not considered beans, and are classified as vegetable crops.

Beans has been concerns due to its spoilage from various means such as bean weevils a pest that feeds on the cotyledon in the bean seed and action of fungi group specifically the mold group aspergillus species which grow on them when stored in environment that favors the growth of the fungi which cause spoilage of the beans with white cotton-like growth on it which confirms the growth of the mold (Leong *et al.*, 2008).

1.5 MOLDS AND FUNGI

Fungi are eukaryotic organisms that, like algae, have rigid cell walls and may be either unicellular or multicellular. Some may be microscopic in size, while others form much larger structures, such as mushrooms and bracket fungi that grow in soil or on damp logs. Unlike algae, fungi do not contain chlorophyll and thus cannot carry out photosynthesis. Fungi do not ingest food but must absorb dissolved nutrients from the environment. Of the fungi classified as microorganisms, those that are multicellular and produce filamentous, microscopic structures are frequently called molds, whereas yeasts are unicellular fungi.

In molds cells are cylindrical in shape and are attached end to end to form threadlike filaments (hyphae) that may bear spores. Individually, hyphae are microscopic in size. However, when large numbers of hyphae accumulate for example, on a slice of bread or fruit jelly they form a fuzzy mass called a mycelium that is visible to the naked eye.

The procedure of identification involves grouping different isolated organisms within a specific categorization format. The process of identifying a mycelial fungus entails the study of its macro as well as micro morphology (cell structure and formation) depending on the growth. The identification of molds or mycelial is basically done in consistent with the organism's macro and micro morphology (form and structure) (Wasowicz 2006).

The species of *Aspergillus* is of immense economic value. Many mold, such as those similar to the genera *Aspergillus, Penicillium* and *Cladosporium*, are notable mold that work to spoil foods. On the other hand, the mold species known as *Aspergillus fumigatus* is liable for causing the disease aspergillosis in humans. However *Aspergillus flavus* as well as associated species of mold are responsible for the production of aflatoxin, aflatoxin belongs to the group of most potent naturally occurring carcinogens discovered thus far. Aflatoxin are toxic and among the most carcinogenic substances known. After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M_1, they have found mostly in cereals, legumes, tubers, vegetables.

A. flavus and *A. parasiticus, A. fumigatus* are weedy molds that grow on a large number of substrates, in particular under high moisture conditions. Aflatoxins have been isolated from all major cereal crops, and from sources as diverse as peanut butter and cannabis. The staple commodities regularly contaminated with aflatoxins include cassava, chillies, corn, cotton seed, millet, peanuts, rice, sorghum, sunflower seeds, tree nuts, wheat, and a variety of spices intended for human or animal consumption.

1.6 AFLATOXIN

Aflatoxins are naturally occurring mycotoxins that are Chemical structure of aflatoxin B_1 produced by *Aspergillus flavus* and *Aspergillus parasiticus*, species of fungi. The name, aflatoxin, was created around 1960 after the discovery that the source of "Turkey 'X' disease" was *Aspergillus flavus* toxins. Aflatoxins are toxic and among the most carcinogenic substances known. After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or hydroxylated to become the less harmful aflatoxin M_1. The cause of death was identified as aflatoxin, found in moldy groundnut meal imported from Brazil. Since then, sporadic incidences of aflatoxicosis have occurred in many species around the world. Almost all livestock animals are sensitive to the toxicity of aflatoxins, but a great variability exists in that sensitivity (Ehrlich *et al.*, 2010)

When processed, aflatoxins get into the general food supply where they have been found in both pet and human foods, as well as in feed-stocks for agricultural animals. Aflatoxin transformation products are sometimes found in eggs, milk products, and meat when animals are fed contaminated grains.

There are four major aflatoxins of toxicological importance: aflatoxin B_1 (afB_1), aflatoxin B_2 (afB_2), aflatoxin G_1 (afG_1), and aflatoxin G_2 (afG_2). These four aflatoxins were named according to their fluorescent properties under long wavelength ultraviolet light on thin-layer chromatography (TlC) plates. Aflatoxins B_1 and B_2 fluoresce blue, whereas aflatoxins G_1 and G_2 fluoresce green. The subscript numbers 1 and 2 indicate major and minor compounds, respectively. AfB_1 and afB_2 are hydroxylated and excreted in the milk as afM_1 and afM_2, which are of a lower toxicity than the parental aflatoxin. There are more than fourteen metabolites of the four major aflatoxins that have been chemically characterized. Certain environmental

factors and insects usually damage corn, small grains, peanuts, cottonseed, etc., and thereby promote the growth of mold and the production of aflatoxins.

Aflatoxins are mainly produced by *Aspergillus flavus, A. parasiticus*, and *A. nomius*. Other mold known to produce aflatoxins are *A. bombycis, A. fumigatus, A. ochraceus*, and *A. pseudotamarii*. Among all these fungi, *A. flavus* has been most extensively studied for the production of aflatoxins. The production of aflatoxins is associated with spore production by species of *Aspergillus* (Calvo *et al.* 2002). Strains of *A. flavus* can vary in aflatoxin capability from nontoxic to highly toxigenic and are more likely to produce more afB_1 than aflatoxin afG_1. *A. flavus* and other species can also produce cyclopiazonic acid (Cpa). Strains of *A. parasiticus* generally have less variation in toxigenicity and generally produce afB_1 and varying amounts of afB_2, afG_1, and afG_2. The aflatoxin profile produced by *A. nomius* is considered to be similar to *A. parasiticus*, and like *A. parasiticus, A. nomius* does not produce CPA. Recent studies have shown that *A. nomius* is more important as a producer of aflatoxins than previously suspected (Johnson *et al.*, 2008). aflatoxigenic strains of *Aspergillus* can also produce sterigmatocystin.

1.6.1 Aflatoxin toxicity

Aflatoxins of toxicological concern in feedstuffs are afB_1, afB2, afG_1, and afG2 on a functional basis, analyses for aflatoxins generally are the sum of the concentrations of these four toxins (Coppock *et al,* 2012). Among all aflatoxins, afB_1 occurs with the greatest frequency in feedstuffs and is found to be the most toxic in all, the order of toxicity is $afB_1 > afG_1 > afB_2 > afG_2$. The lD50 of aflatoxin B_1 in ducklings, rabbits, dogs, and guinea pigs is about 1 mg/kg. This value is about 10 mg/kg in monkeys, cattle, pigs, rats and hamsters. Mice and sheep are less sensitive to the toxicity of aflatoxin B_1, as the lD50 values are 63 and 500 mg/ksg, respectively.

Toxicity due to aflatoxins, under natural conditions, is usually sub-acute or chronic, depending on the level of exposure. (Coppock*et al.* 2012). Occasionally, acute cases are also seen. In general, affected animals show reduced growth rate, weight loss, immune suppression, icterus, hemorrhagic enteritis, reduced performance, and ultimately death. The major target for the toxicity of aflatoxins is the liver. afB_1 is known to cause hepatocellular carcinomas (HCC) in many animal species and in humans (Mahoney *et al.* 2014).

CHAPTER TWO

2.0 LITERATURE REVIEW

Aflatoxin are secondary metabolites produced by some strains of Aspergillus. Aflatoxin B1 is the most toxic of the aflatoxin. It causes a variety of adverse effects in different domestic animals. Effects on chickens include liver damage, impaired productivity and reproductive efficiency, decreased egg production in hens, inferior egg shell quality, and carcass quality and most importantly from human perspective, increase susceptibility to diseases such as direct outbreak of human disease e.g. aflatoxicosis in Kenya, 265 people died usually through liver cirrhosis, Child stunting and underweight, and Immune suppression (Turner *et al.,*. 2003).

Aflatoxins were reported in the autopsy of kidney specimens of 58% of children with kwashiorkor at Obafemi Awolowo University Teaching Hospital, Ile-Ife (Oyelami *et al.*1998). In a similar study by (Onyemelukwe *et al,* 2012), aflatoxins were found in both the sera and the blood of children with kwashiorkor.

Aflatoxin contamination of various food stuffs and agricultural commodities is a major problem in the tropics and sub-tropics where climatic conditions, agricultural and storage practices favour the growth and aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*, the main aflatoxin producers. Aflatoxins are being consumed daily by the populace especially in developing and under-developed economies since most food stuffs are transported from the farm to the market without proper inspection of produce by the regulatory agencies and this puts everyone at risk of the dangers of aflatoxins.

Various researchers have reported high levels of aflatoxins (5000 µg/Kg) in groundnuts and maize (Kumar *et al.*2008), 15 µg/Kg of G1in Brazil nuts (Oslen *et al.* 2008), 600µg/kg in shea –

45 µg /Kg in same paste (Feng-Qin-Li *et al.* 2009), 45 µg/Kg in same paste (Feng-Qin-Li *et al.*2009),97.5 µg/Kg in red pepper (Marin *et al.* 2008).

Yam, beans, maize, onion commonly consumed food samples in Nigeria which has recently been found to be contaminated with toxigenic fungi. Recent research have increased awareness of chemical and natural contaminations in food, at the same time, consumers concern about food safety has also grown at international level.

Research has shown that strains of mold known as the *Aspergillus* species have been identified to be the major producers of aflatoxin in the food samples, Secondary metabolism is mainly a characteristic of filamentous mold in which aflatoxin is been produced. The diversity and complexity of secondary metabolites is astounding, and species of Aspergillus are rich in genes for secondary metabolism (Nierman *et al.*2005, Kobayashi *et al.,* 2007, Rokas *et al.*2007).

In a study conducted between September 1966 to June 1967 in Uganda which 480 different food samples were analyzed for aflatoxin, it was found that 29% contained between 1 –100 ppb, eight percent contained between 100-1000 ppb while four percent had more than 1000 ppb aflatoxin. Aflatoxins occurred most frequently in beans, followed by maize and sorghum, whereas millet and cassava were contaminated least frequently (Alpert *et al.*1967)

The aflatoxin problem has been reported to be more serious in tropical and subtropical regions of the world where climatic conditions of temperature and relative humidity favour the growth of *Aspergillus species*

Storage systems of produce have also been found to encourage aflatoxin contamination. Adequate storage facilities are not available especially at farm level. It has been reported that the majority of farmers and traders in store maize using woven polypropylene bags, which do not

protect the grains against aflatoxin contamination (Kaaya *et al.*2001). Grains stored or heaped on the floor (unshelled) and those stored under the verandah had 100% aflatoxin contamination (Kaaya *et al.* 2001).

At the retail markets, produce is not properly protected from environmental influence during storage. Most of the produce is not properly packaged, always exposed, making it susceptible to infection by mycotoxigenic molds. Maize flour, pounded/ milled groundnuts and shelled kernels are some of the produce suspected to be highly contaminated by aflatoxins due to their form.

Moisture content and grain physical condition are major factors in molds and mycotoxin contamination of grains. Despite slow drying processes and inadequate storage methods, the moisture content and insect damage of maize and groundnuts stored for three to seven months at farm level have been found to be low, within recommended levels. Average moisture content has been reported to be eight to 11% for maize (Nakamya 2004). These grain conditions have been described as major factors in the low aflatoxin levels observed in on-farm produce compared to produce in the markets in which the majority of grains were found to have moisture content above 14% and Maize stored by traders for six to seven months was reported to have mean aflatoxin levels implying that these grains were not suitable for local nor export markets.

In Nigeria, maize and cassava products are dietary staples and aflatoxin contamination in higher or lower levels in these and other staple products has been reported. The aflatoxin-producing mold which are strains of *Aspergillus species* one of the predominant mold on stored maize (Owolade *et al.* 2001); it has also been observed on freshly harvested maize (Bankole, 1994). An analysis of several food items from Nigerian markets by (Emerole *et al.*1982) showed mean levels of aflatoxins in yam (0.40 ppm), red pepper (0.70 ppm), millet (1.4 ppm), corn (1.20 ppm), black-eyed bean (0.5 ppm), rice (0.4 ppm), and groundnut (1.7 ppm). (Oyelami *et*

*al.*1996) found that 12 of the 48 maize-based gruels used as weaning foods were contaminated with aflatoxin.

The morphological characteristics that are more upstanding in Aspergillus are globose or column heads with the color. Phyalids grow in one or two series frequently from the same strain. Mold species lack a sexual reproductive stage, they do not reproduce by meiosis, and it is common the presence of brown reddish, or black sclerotia can be globose or vertically elongated (Diener *et al.*1986).

This mold contaminate seeds in the field, where they can grow as saprophytes in crop debris on the soil and in warehouses. A source of primary contamination can be sclerotia formed in the grains and in healthy maize. During dry seasons the plants are more susceptible to insect invasions that carry spores, beginning the development of the mold (Eaton and Groopman 1993).

Aflatoxin can remain longer time after the producing mold die, therefore, grains can have dangerous levels of aflatoxin although they have not a moldy appearance. The mold reduce their viability, nutritional and sanitary qualities to seeds and grains.

The principal factor for aflatoxin production are: the aflatoxigenic mold, the substrate environmental condition and the substrate humidity (an environmental relative humidity of 85% corresponds to a wetness content of 16.5-18% of the grain), temperature, and oxygen level of the storage container and time of storage.

The aflatoxin production in the substrate can happen in the field and in the storage conditions between 20 and 40˚C with a 10-20% of humidity and 70-90% of relative humidity in the air. These mold grows at temperatures of 8-55 ˚C and 25-35 ˚C for aflatoxin production as it can be

formed in storage conditions and in the cereals, legumes and vegetables which are cultivated in the field before harvest. (Sweeney *et al.*1999).

Aspergillus species synthesized aflatoxin by their metabolism.

Aflatoxins are produced at the end of the exponential phase or at the beginning of the stationary phase of the mold growth. The development of the fungus is favored if the grains are damaged by insects or rodents. Aflatoxin production reaches its maximum production rate at the fifth day, that is, when mold comes up to the stationary growth phase. Its aflatoxin production diminishes from the sixth to the eight days. After 24hours, *Aspergillus* reaches its optimal conditions of temperature (27-30 ˚C) and relative humidity (85%) to produce aflatoxin, its rate of production increases as far as 12 days that is when it has its highest production, later this rate lowers progressively (Wylie *et al.* 2004).

OBJECTIVE OF STUDY

Aflatoxins are being consumed daily by the populace especially in developing and under-developed economies since most food stuffs are transported from the farm to the market without proper inspection of produce by the regulatory agencies and this puts everyone at risk of the dangers of aflatoxins in Oye Ekiti a University town students purchase their raw food stuffs from the central market. This study is therefore targeted at getting information on the levels of aflatoxins in some selected produce sold at Oye-Ekiti market in order to know the safety level of the food samples commonly consumed in the locality of Oye-Ekiti and to identify the mold producing aflatoxin in the food samples.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Materials used:

- Stop watch: model 228 manufactured by GNC
- Whatman filter paper:
- Micro pipette: model P5000 manufactured by *Glasscolabs India*
- Test tube: 20ml
- Microscope: electron microscope manufactured by *Eastcolight*
- Petri dish: plastic model manufactured by medical-alexpo
- Electric blender: stationary electric blender manufactured by Hamilton electronics
- Food samples: maize(white)

  Yam (white)

  Beans (white kidney beans)

  Onion
- Methanol (70%):
- Conjugate: mycosep112 column
- Distilled water
- Lacto-phenol cotton blue stain (LPCB):
- Sabroid dextrose agar: TITAN BIOTECH LTD India
- Antibody containing well tube

3.2 METHODOLOGY

3.2.1 Sample collection

The food samples yam, beans, maize and onion were collected from Oye-Ekiti central market opposite the king's palace and collected in a clean container separately and taken to the laboratory.

3.2.2 Isolation and identification of mold producing aflatoxin

3.2.3 Serial dilution

1g of each of the sample was place into 9mls of sterile normal saline which is the stock solution

1mls of stock solution was placed into 9ml of sterile distilled water with the following sequence $10^{-1} \rightarrow 10^{-2} \rightarrow 10^{-3}$ in a sterile test tube. The process was repeated for each of the other samples.

3.2.4 Culturing

10^{-2} was taken of the three serial dilutions from each of the samples was taken and incubated in the Saboraud dextrose agar medium which is the media been used for culture in a sterile atmospheric condition.

The prepared cultured was then placed in the incubator at a temperature of 37°C for growth of the organism present in the food samples.

The organism that grew on the culture media plates were identified using morphological characteristics as the basic diagnostic means of identification

3.3.1 Cutting and grinding

The food samples were cut into four equal parts and little portions were taken from each of the four sections and grinded with a blender to little particulate matters after which it was collected in a clean container

3.3.2 Weighing

The four food samples were measured with an electric weighing balance and 5g was taken out of the food samples that was blended into a clean container and transferred into a test tube.

3.3.3 Extraction process

The extraction process was carried out under the following procedure;

- 25mls of 70% methanol was added to 5g of each sample in a test tube,
- The methanol was added to the food sample to extract the components in it.
- The extraction process took place for 135 minutes (2hrs 15 minutes)

3.3.4 ELISA aflatoxin assay

It was carried out under the following procedure

- 50µl was taken from each sample and placed in a mixing well with a micro pipette. The mixing well is a container were the extracts from the food samples are mixed together with the conjugate by rocking the container.

- 100µl of conjugate is added to the mixing well containing the sample, then it's mixed together. The conjugate (antigens) is added to the sample and are in proportion to enzymes in order to reduce uncombined enzymes or unlinked antibodies.

The conjugate possess not only catalytic activity of enzyme but also immunological competence of antibody

- 100µl of the mixture containing both the sample and the conjugate were transferred into an antibody coated well and timed for 15 minutes. This done so that the conjugate and also the substrate (food sample extract) can bind to the antibody present in the coated well.
- The wells are rinsed with water (running tap water), this is done to wash off the remaining antigens were not bonded by the antibody are been washed off.

- Stop solution was added to stop the reaction between the enzyme (antibodies) and the substrate (antigen). The stop solution used was 2 mol/L of H_2SO_4 in the ELISA plate.

- The color changed to blue which showed that the aflatoxin present is either afb1 or afb2.

- After which it's been placed in the ELISA read machine to read the aflatoxin concentration in the samples. This is done by placing the test tube containing the solution in the loading tray and the ELISA read machine determines the aflatoxin level in the samples through the amount of light each can absorb.

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

4.1 ISOLATION AND IDENTIFICATION OF FUNGAL GROWTH ON INCUBATED PLATE

Table 1.0 mold growth at 24 hours on the SDA media plate

Sample type	Microscopic morphological view at 24hours	Mold	Cfu
Maize	no detectable morphological change	Unidentified	(colony not formed)
Beans	no detectable morphological change	Unidentified	(colony not formed)
Yam	no detectable morphological change	Unidentified	(colony not formed)
Onion	no detectable morphological change	Unidentified	(colony not formed)

Table 2.0 mold growth at 48 hours on the SDA media plate

Sample type	Microscopic morphological view 48 hours	Mold	Cfu
Maize	Appeared to have a pale brown color, with the surface appearing to be spherical, the conidia has a glubose, conidia surface serrated to be finely roughened surface.	*Aspergilus flavus*	2.6×10^3
Beans	Appeared to have a brown grayish color with surface smooth walled and the conidia has a glubose shape and the conidia surface appears smooth	*Aspergilus fumigatus*	4×10^2
Yam	Appeared to have a brown grayish color	*Aspergilus fumigatus*	1.6×10^3

	with surface smooth walled and the conidia has a glubose shape and the conidia surface appears smooth		
Onion	Appeared to have a brown grayish color with surface smooth walled and the conidia has a glubose shape and the conidia surface appears smooth	*Aspergilus fumigatus*	2.8×10^3

Table 3.0 fungal growth after 72 hours on the SDA media plate

Sample type	morphological view after 72 hours	Mold	Cfu
Maize	Appeared to have a pale brown color, with the surface appearing to be spherical, the conidia has a glubose or ellipsoid shape, conidia surface serrated to be finely roughened surface.	*Aspergilus flavus*	(colony too many to be counted)
Beans	Appeared to have a brown grayish color with surface smooth walled and the conidia has a glubose shape and the conidia surface appears smooth	*Aspergilus fumigatus*	(colony too many to be counted)
Yam	Appeared to have a brown grayish color	*Aspergilus fumigatus*	(colony too many to be counted)

	with surface smooth walled and the conidia has a glubose shape and the conidia surface appears smooth		
Onion	Appeared to have a brown grayish color with surface smooth walled and the conidia has a glubose shape and the conidia surface appears smooth	*Aspergilus fumigatus*	(colony too many to be counted)

Key:CTC: Colony too numerous to count CFU: Colony forming unit CNF: Colony not formed

The result in table 1.0 showed that there was no colony formed on any of the samples therefore mold could not be identified. However at 48 hours of incubation which was table 2.0, it could be seen that colonies were formed with beans recording the highest followed by onion maize and the lowest yam. In beans yam and onion *Aspergillus fumigatus* were identified while in maize *Aspergillus flavus* were identified. The lowest in yam might be due to the water activity in it. Table 3.0 at 72 hours the colonies were already and the colony formed were too many to count

4.1.1 Microscopic view of morphological changes at 24 hours

After the plate was incubated, it was checked at 24 hours of incubation, no morphological changes were detected during the first 24 hours, spores were visible and appeared swollen, a large fraction of them were agglomerated, germination has started on each of the plates and the hyphae appeared non-branched were already formed on the plate as seen through microscopic view.

4.1.2 Microscopic view of morphological changes at 48 hours

After 48 hours of incubation germination was now completed and evident, the hyphae is branched with cross walls, branching was apparent and permanent aggregates were detected.

The plate containing the food sample A which is maize, The mold appeared to have a pale brown color, with the surface appearing to be spherical, the conidia has a glubose or ellipsoid shape and the conidia surface serrated to have a finely roughened surface. The obtained result for identification of *Aspergillus flavus* agrees with various author findings (Klich *et al.*2002, McClenny 2005 Okuda *et al.* 2000).

The microscopic examination of the mold growth on food samples B, C, D appeared to have a brown grayish color with surface smooth walled and the conidia has a glubose shape and the

conidia surface appears smooth as seen through microscopic examination. The obtained result for identification of *Aspergillus fumigatus* agrees with various author findings (Diba *et al.* 2007, Riddle 2005)

4.1.3Morphological changes at 72 hours

At 72 hours the fungal growth on the SDA media plate containing maize has turned gray with the peripheral still retaining the brown color, while the color on SDA plate containing other food samples (yam, beans and onion) appeared to be pale. After 72 hours of growth, the fungal had occupied the surface of the plate due to their increased rate of sporulation.

4.2 IDENTIFICATION OF FUNGI GROWTH ON THE SDA PLATES.

After 48hrs each plate was taken and a colony was picked from the plates.

Sample A microscopic view containing maize for.

Under the microscopic view, the mold appeared to be flat, serrated to cottony to wooly. Conidial wall ornamentation was used as the primary diagnostic character for the identification of *A. flavus* species (Okuda *et al.* 2000). Conidia of *A. flavus* have relatively thin walls which are finely to moderately roughen. Conidiogenous cells of *Aspergillus flavus* are phialides, in which the shape appears spherical to elliptical, this morphological identification was in accordance with various authors (Okuda *et al.* 2000, (Klich *et al.* 2002, McClenny 2005).

Microscopic view of the sample B, C and D

The other food samples (yam, beans, onion) microscopic view appeared that they are serrated to have the same characteristics; globular vesicles, conidiophores shaped, translucent semi

Table 4.0 shows that aflatoxin was present in some of the food samples with maize having the highest aflatoxin level 13ppb followed by beans, while onion and yam had no aflatoxin in them due to the level of wetness or their level of water activity this was in agreement with various authors (Fratamico, 2008, Mahoney *et al.* 2010).

Chapter five

5.0 Conclusion and Recommendation

The results of this study revealed that some agricultural commodities sold in our markets have higher levels of aflatoxin than the recommended standard level of SON for various food samples.

Therefore, there is need to create awareness by sensitizing the commodity traders and the populace on the dangers associated with the consumption of high levels of aflatoxin in agricultural commodities.

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