UNIVERSITY OF CAPE COAST

STUDIES OF *ASPERGILLUS FLAVUS* AND AFLATOXIN CONTAMINANTION OF GROUNDNUT *(Arachis hypogaea L*) FROM SIX MARKETS IN THE CENTRAL REGION, GHANA.

BY

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research work and that no part of it has been presented for another degree in this University or elsewhere.

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Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

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ABSTRACT

The study shows ***Aspergillus flavus*** **and aflatoxin contamination of groundnut (*Arachis hypogaea L*) from** groundnut samples collected from the six markets in the Central Region of Ghana namely: Swedru, Mankessim, Cape Coast, Fosu, Jukwa and Kasoa. All groundnut sellers interviewed were females and have no knowledge about aflatoxins. Thirty five percent 35% of the groundnut sellers had no formal education whilst 26.7% had basic primary education and 23.3% had Junior high school education.

The fungal organisms encountered on the groundnut samples from the six market centres were: *Aspergillus flavus, Aspergillus niger, Botryodiplodia theobromae, Macrophomina phaseolina, Penicillum* spp. and *Rhizopus* spp. Laboratory results indicate that on unamended PDA Fosu *A. flavus* isolate showed better growth (8.5 cm) than the rest (7.0 cm- 8.0cm). However, there was a strong radial growth inhibition of *A. flavus*, in the garlic amended PDA than the rest of the botanical extracts.

Whereas the HPLC analyses of the groundnut samples were all below the tolerable limit (20 ppb) of Ghana Bureau of Standards for consumption, Cape Coast recorded the highest of (9.1 ppb) and Jukwa recorded the lowest (1.8 ppb). The four types of aflatoxin detected in the groundnut samples from the six market centres were: B1, B2, G1 and G2.

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DEDICATION

To my lovely mother, Lagbeh S. Dorley and my late father Alhaji Sarliah B. Dorley, to my darling wife Mrs. Isatta S.S. Dorley and my children.

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LIST OF ACRONYMS

ANOVA	-	Analysis of Variance
BSFSD	-	Biotechnology Seed and Food Science Division's
C	-	Centigrade
CM	-	Centimeter
CRD	-	Completely Random Design
CRI	-	Crop Research Institute
CSIR	-	Council of Scientific Industrial Research
CV	-	Co efficient of Variance
DNA	-	deoxyribonucleic acid
e.g.	-	Example
EU	-	European Union
FAO	-	Food and Agriculture Organization
FDA	-	Food and Drug Administration
g	-	Gram (s)
ha	-	Hectare (s)
IACs	-	immune affinity columns
ISTA	-	International Seed Testing Association
kg	-	kilogram (s)
Lsd	-	Least Significant Difference
L	-	Liter
ml	-	milliter
mm	-	millimeter
Mg	-	Magnesium
MoFA	-	Ministry of Food Agriculture

Mol	-	molecular
Min	-	minu
Nm	-	nino meter (s)
NUV	-	Near Ultra Violet
HBV	-	Hepatitis B virus
HIV/AIDS	-	Human Immunodeficiecy Virus/ Acquired Immune Defficiency Syndrome
HPLC	-	High Performance Liquid Chromatography
HRS	-	Hours
H2O	-	Water
PA	-	Participatory Appraisal
PAT	-	Participatory Appraisal Technique
PDA	-	Potato Dextrose Agar
Ph	-	power of hydrogen
ppb	-	parts per billion
P	-	Probability
ppm	-	parts per million
RNA	-	ribonucleic acid
RA	-	Rapid Appraisal
Spp	-	Species
TFA	-	trifluoroacetic acid
t	-	Tonne (s)
UNBS	-	Uganda National Bureau of Standards
USA	-	United States of America
U V light	-	Ultra- Violet light

UK	-	United Kingdom
vv	-	volume/ volume
WHO	-	World Health Organization

CHAPTER ONE

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food and feed crop, which also serves as a component of crop rotation in many tropical countries (Pande, Saxena & Pandey, 2003; Upadhyaya, Reedy, Gowda & Singh, 2006). It is believed that groundnut was a cultivated annual of South America origin and domesticated in Brazil, Argentina, Paraguay, Peru and Bolivia (Tweneboah, 2000). The major groundnut producing countries in the world include India, China, America, the Gambia and Malaysia. Leading producing countries in Africa include Nigeria, Senegal, Niger and Sudan (Tweneboah, 2000). Developing countries account for 97% of the world's groundnut area and 94% of the total production (Food Agriculture Organization Statistics, 2010).Groundnut yield in this part of the world and particularly in Africa is lower than the world average due to prevailing abiotic and biotic factors (Pande *et al*., 2003; Upadhyaya *et al*., 2006; Caliskan, Arslan & Arioglu, 2008). The average yield of groundnut was 1.5 mt / ha and 4.8 mt / ha in 2010 and 2012, respectively (Food Agriculture Organization, 2012).

Groundnut is grown mainly in the northern part of Ghana, including Upper East, Upper West and Northern Regions. It is also grown in small quantities in small towns and villages in Brong Ahafo, Ashanti and Volta Regions. The production of groundnut is a source of employment and hence income to people in rural and urban areas, as well as those who sell in the

market centres (Tsigbey, Branddenburg & Clottey, 2004, Carlberg, 2008, Debrad & Saliyar, 2006, Waele & Swanevelder, 2001) thereby alleviating poverty. Groundnut is also a non-traditional export crop in Ghana, hence a source of foreign exchange. For instance in 2014 Ghana exported a volume of groundnut to the European Union amounting to 10.5 million Euros (Florkowski & Kolavalli, 2012).

Groundnuts play a vast role in food security in Ghana been a source of vegetable and protein (Izge, Mohammed & Goni, 2007). Groundnuts conspicuously exceed meat and eggs in carbohydrates, fats, proteins, vitamin B1, C, and niacin. They are also superior in terms of minerals such as calcium, phosphorous, magnesium and potassium, without cholesterol or excess saturated fatty acids (Roger, 2001). Groundnut is eaten fresh, roasted, boiled or grilled and in the preparation of soup (Waele & Swanevelder, 2001). It is used as butter, eaten alone, in sandwiches; into chocolate as well as in candies, pies and other products (World Book of Encyclopedia, 1990). It can thrive under hash environmental condition and plays important role in improving soil conditions by adding atmospheric nitrogen to the soil (Smart, Wicklow & Caldwell, 1990).

Problem Statement

In spite of the economic importance of groundnut there are great challenges associated with its production in Ghana. As a result, yields of groundnuts in the country are lower compared to the average for the developing countries (FAO, 2003; Nutsugah, Oboateng, Tsigbey & Brandenburg, 2007). The current average yield of groundnut in Ghana is 1.4

Mt ha^{-1} which is lower compared to the potential yield of 2.5 Mt ha^{-1} (Ministry Of Food Agriculture-Statistics Research Information Directorate, 2013), and far lower compared to the world average of 4.8 Mt ha^{-1} (FAO, 2012).

Notable among these challenges facing groundnut production in Ghana are fungal contaminants such as *Aspergillus flavus* and *A. parasiticus*. Which affect seed germination of groundnut by reducing the viability, resulting in poor production. Besides, infection of groundnut by these fungal organisms such as *A. flavus* and *A. parasiticus,* results in the production of mycotoxins such as aflatoxin which are toxic to both humans and animals (Kaaya, Eigal & Harris, 2006). Aflatoxin contamination of food commodities and its associated health risks have raised universal concern over the years. The presence of aflatoxins is therefore considered as one of the most important groundnut quality problems in many African countries including Ghana (Kaaya *et al*., 2006).

In addition, the moustiness and mouldy smell of the harvested produce of groundnut affect the market value and hence its profitability. Generally, mycotoxins are associated with fungi infection of groundnut which include loss of germination, moistness as well as mouldy smell (Sauer, Meronuck & Christensen, 1992; Frisvad, 1995), and aflatoxin contamination (McAlpin, Wicklow & Horn, 2002; Bankole & Adebanjo, 2003).

Furthermore, from 2009 to 2013, analytical results from EU control laboratories reported that aflatoxin levels exceeded European Union limits for groundnut and peanut butter from Ghana. This necessitated the EU to impose import controls for groundnut from Ghana (Ghana Export Promotion Authority, 2015).

It is therefore pertinent to develop an effective management strategy to reduce fungal infection of groundnut in order to improve viability of seed, market value of grains and also reduce the levels of aflatoxin contamination. Knowledge of factors affecting the quality of groundnuts at the various markets is an important prerequisite to the development of the effective strategy. Also information on types of fungal organisms infecting groundnuts and the associated types and levels of aflatoxin contamination of the groundnuts are also important in the development of such effective strategy (Sauer *et al*., 1992).

However, there is inadequate information on the factors affecting the quality of groundnuts at the various market centres. Chemical fungicides such as Thiram have been used to control fungal infection of groundnut (Okello, Briuma & Deom, 2010). The use of chemical pesticides, has both health and environmental hazards, and also very expensive, beyond the financial means of many farmers. Therefore, there is the need to identify sustainable and environmental friendly remedy to salvage this situation such as botanicals which can be found all over the communities at no cost, non-toxic to the environment and human.

Justification

When an effective strategy for managing fungal infection is developed, it will minimise fungal infection of groundnuts at the various markets. Further, by minimising fungal infection, mycotoxins, such as aflatoxins, which cause health risk to both humans and animals, will also be reduced. Furthermore, this could increase the volume of groundnut export to the foreign markets,

thereby improving the foreign exchange earning of the country and thus improving the economy of Ghana.

Objectives of the Study

Main objective

The main objective of the study was to determine the factors affecting the quality of groundnut, the types of fungal organisms and their management as well as identifying the types and levels of aflatoxins in groundnut from six major markets in the Central Region.

Specific objectives were as follows:

1. To determine sellers' perception of fungal contamination, their source of seeds, transportation and storage.
2. To identify seed-borne mycoflora of the groundnut samples from the six markets in the Central Region.
3. To determine the diversity of *Aspergillus flavus* isolates from the groundnut samples based on cultural and morphological characteristics.
4. To identify the efficacy of botanicals on the growth of *Aspergillus flavus in vitro*.
5. To determine the types and quantity of aflatoxins in the groundnut samples.

CHAPTER TWO

LITERATURE REVIEW

Quality of Groundnuts Seed

The increase in agricultural productivity greatly depends on the adequate availability of good and high quality seed. On the other hand, farmers' efforts to produce enough good quality seed to cover the whole nation are hindered by fungal and insects attack in the field, and storage. Hence damage caused by storage insect and fungi results in an increase in moisture content and temperature of the seed, loss of weight, vigour and viability of the seed and production of mycotoxins which are hazardous to both humans and animals (Akyaw & Danquah, 1997).

To ensure that seed is produced and stored with manageable or no insect and fungal damage, it should be harvested early at the full physiological maturity, the moisture content of the seed should be kept at the minimum level, and cracked seed removed before bagging. The storage environment as well as the warehouse should be adequately maintained, with best sanitation practices and the seed should be properly treated before bagging for storage (Akyaw & Danquah, 1997).

Fungal Infection of Grains and Contamination of Groundnuts

Several species of fungi infect agricultural crops both in the field and during storage. These include fungi of genera *Aspergillus, Fusarium,*

Penicillium, Alternaria, Cladosporium and *Nigrospora* species (Hocking, 1991), and have been mainly found associated with cereals, groundnuts and other crop species. In addition to reduction of yield in these crops, some of these moulds produce mycotoxins. Mycotoxins are toxic substances produced by fungi and can be classified according to their fungal origin, chemical structure and biological activity (Smith & Moss, 1985). Occurrence of these toxins in human foods is mainly as a result of direct contamination of the groundnut seed and other cereals (Scott, 1991).

Oilseeds especially groundnuts (peanuts), maize (corn), soybean, cottonseed, and copra are particularly favorable substrates for mycotoxin formation. Diseases in animals and human beings resulting from consumption of mycotoxins are called mycotoxicoses. Because of their serious effects, the incidence of moulds and levels of mycotoxins in foods and feeds should be frequently and routinely monitored. Over 200 mycotoxins have been reported but only those occurring naturally in foods are of significance in terms of food safety (Okello *et al*., 2010). These are produced mainly by species of *Aspergillus, Penicillium* and *Fusarium* (Table 1) (Okello *et al*., 2010).

Table 1: Common Mycotoxins found in some Foodstuffs

Mycotoxin	Main causal agent	Food Commonly contaminated
Aflatoxin	*Aspergillus flavus* and *A.parasiticus*	All grains, dried fruits
Fumonisin	*Fusarium verticillioides*	Maize
Zearalenone	*Fusarium graminearum*	Maize
Ochratoxin	*Aspergillus ochraceous*	Coffee, cocoa
Trichothecenes (T2,Toxinsanddeoxynivalenol)	*Fusarium spp*	Cereals(wheat, barley, maize, rice)
Patulin	*Penecillium digitatum*	Apples

Source: (adopted from Okello, 2010).

The Genus of *Aspergillus Flavus*

The genus *Aspergillus*, a member of the phylum Ascomycota, includes over 185 known species. Several species of *Aspergillus flavus* produce aflatoxins. These include *A. flavus* and *A. parasiticus*, as well as several less common taxa including *A. nomius, A. tamarii, A. pseudotamarii, A. minisclerotigenes* and *A. bombycis* (Klich & Pitt, 1988; Cotty, 1994). *Aspergillus* species classified outside of Flavi can also produce aflatoxins. For example, *Aspergillus ochraceoroseus* from *Ochraceorosei*, SCRR 1468, morphological resembling members of *Circumdati,* and the *ascomycete*

Emericella astellata, E. *venezuelensis* and (*Aspergillus nidulantes),* (Cary, Klich & Beltz, 2005) also produce *aflatoxin.* The group of *aflatoxin* producing species is more complex than previously thought.

The Life Cycle and Population Dynamics of *Aspergillus Flavus*

Aspergillus flavus are one of the most abundant and widely distributed soil-borne moulds and can be found anywhere on earth (Yu, Cleveland, Nierman & Bennett, 2005) *A. flavus* is a saprophytic fungus that is capable of surviving on many organic nutrient sources like plant debris, (tree leaves, decaying wood, animal fodder, cotton, compost piles, dead insects and animal carcasses), outdoor and indoor air environments, stored grains, and even on live humans and animals (Klich, 1998).

The life cycle in agricultural fields can be divided into two stages: (1) colonization of plant debris in soil and (2) invasion of seeds and grain in actively growing crop plants (Horn, 2007). Soil serves as a reservoir for primary inoculum of *A. flavus* and *A. parasiticus* (Horn, Green, Dorner & Powell, 1995; Payne & Brown, 1998). *A. parasiticus* appears to be more adapted to the soil environment, being prominent in peanuts, whereas *A. flavus* seems adapted to the aerial and foliar environment, being dominant in corn, cottonseed, and tree nuts (Diener, Sandeers, Paynes & Klich, 1987).

Under adverse conditions such as dry and poor nutrition, the mycelium congregates to form resistant structures called sclerotia (Yu *et al.,* 2005). Sclerotia are pigmented, compacted aggregates of hyphae, which resist unfavorable environmental conditions and are capable of remaining dormant for long periods (Wicklow & Shotwell, 1983; Cotty, 1988; Rollins &

Dickman, 1998). The fungus overwinters either as mycelium in plant debris and litter on the soil, on insects or as sclerotia in the soil (Dieneret *et al*., 1987). When growth conditions are favorable the sclerotia either germinate to produce additional hyphae or they produce conidia (asexual spores), which can be further dispersed in the soil and air (Bennett, Loeng, Kruger & Keyes, 1986; Cotty, 1988). The fungus mostly exists in the form of mycelium or asexual conidia spores.

Phases of Infection

Aflatoxin infection can be divided into two distinct phases with the infection of the developing crop in the first phase and increase infections after maturation in the second phase (Cotty, 2001). Both phases contribute to many infections event (Cotty & Jaime-garcia, 2007). Weather influences the two phases of contamination differently. During the first phase of contamination infections by *A. flavus* and *A. parasiticus* of susceptible crops are infected due to wounding of developing crops by birds, mammals, insects, mechanically or drought stress and elevated temperatures (Dowd & Groopman, 1998; Payne & Brown, 1998; Guo, Sobolev & Lynch, 2002). Its ability to attack seeds of both monocots and dicots, and to infect seeds produced both above and below the ground, demonstrates that this fungus has evolved a battery of mechanisms to breach the host's resistance (Yu *et al*., 2005). Conidia of plant, insect, and human derived strains of *A. flavus* rapidly colonize leaves, kernels, and insects injured during inoculation but do not affect uninjured plant or insect material (St. Leger, Screen & Shamps-pirzadeh, 2000).

The *Aspergillus Flavus* Species

Populations of *A. flavus* species are diverse and comprise individuals that differ greatly in phenotype, including characters such as conidial colour, sclerotium production, presence of diffusible pigments and growth rate (Raper & Fennell, 1965; Christensen, 1981; Horn, Greene, Sobolev & Dorner, 1996). On the basis of physiological and morphological criteria, *A. flavus* can be divided into two types of strains (Cotty, Probst & Jaime-Garcia, 1989). The S-type isolates of *A. flavus* produce numerous small sclerotia (average diameter <400 μm) and fewer conidia than other *A. flavus* isolates (Saito, Tsuruta, Siriacha, Kawasugi, Manabe & Buangsuwan, 1986; Cotty *et al*., 1989). The S strain was originally described as *A. flavus, Var parvisclerotigenus*, based on a type strain that produced on average much greater quantities of only B aflatoxins (Cotty *et al.,* 1989; Saito *et al.,* 1993). The L-type isolates of *A. flavus* produce larger and fewer sclerotia and is designated as "typical" isolates of *A. flavus* (Saito & Tsuruta, 1986).

Strains resembling the S-type but having different physiological criteria have been reported in different regions of the world. These strains can also produce aflatoxin G and were found in Argentina, Thailand, Australia and West Africa (Saito & Tsuruta, 1993; Geiser Pitt, & Taylor, 1998; Cotty & Cardwell, 1999; Fernandez Pinto, Patriarca, Locani & Vaamonde, 2001). Recent studies designated most of these isolates to the *A. minisclerotigenes* (Pildain, Frisvad, Vaamonde, Cabral, Vargga & Samson, 2008). The exact taxonomic affiliation of SBG commonly found in West Africa remains unclear.

Aflatoxins

Aflatoxins as toxic compounds produced by several species of aspergillus; a group of structurally related toxic secondary metabolites produced mainly by certain strains of *A. flavus* and *A. parasiticus. A. flavus,* in particular, is a common contaminant in agriculture (Bhatnagar, Cotty, & Cleveland, 2001; Bennett & Klich, 2003) and mostly found where certain grains are grown under stressful condition such as drought *A. bombysis, A. ochraceoroseus, A. nomius minisclerotigenes, A. pseudotamari, A.* and the strain SBG are also aflatoxin-producing species but occur less frequently (Goto & Wicklow, 1996; Cotty & Cardwell, 1999; Klich, Mullney, Daly & Cary, 2000; Peterson & Horn, 2001; Pildain *et al*., 2008). The four major aflatoxins are called B1, B2, G1, and G2 based on their fluorescence under UV light (blue or green) and relative chromatographic mobility during thin-layer chromatography (Bennett & Klich, 2003).

Aflatoxins, identified in the early 1960s, were found to be toxic compounds (Wild & Turner, 2002). Aflatoxin B1 is predominant and the most toxic and potent hepato-carcinogenic natural compound ever characterized (Squire, 1981; Bhatnagar *et al*., 2001). The conditions favouring formation of the aflatoxins have been described, as their metabolism, toxicity, DNA adduct formation, mutagenic, and carcinogenic activity (Eaton & Groopman, 1994). The immuno suppressive properties of aflatoxin B1, particularly on cell-mediated immunity, have been demonstrated in various animal models (Ali, Mohinddin & Vikram Reddy, 1994; Neiger, Johnson, Hurley, Higgies, Rottinghaus & Stahr, 1994; Pestka & Bondy, 1994). A major metabolate of aflatoxin B1 is aflatoxin M1 which is usually excreted in the milk and urine of

dairy cattle and other mammalian species that have consumed aflatoxin contaminated food or feed (Gourama & Bullerman, 1995).

The types of aflatoxin; the most predominant types and their effect are four major aflatoxins: B1, B2, G1, G2 plus two additional metabolic products, M1 and M2 that are of significance as direct contaminants of grains (FAO, 2002). The aflatoxins M1 and M2 were first isolated from milk of lactating animals fed aflatoxin contaminated rations; hence, the M designation. The B designation of aflatoxin B1 and B2 resulted from the exhibition of blue fluorescence under UV-light, whilst the G designation refers to the yellow green fluorescence of the relevant structures under UV-light. The chemical structures of these toxins are presented in Figure 2.

It should be noted that it is difficult to eliminate aflatoxins completely from food after they have developed, although some reduction can occur during processing. Aflatoxins persist under extreme environmental conditions and are even relatively heat stable at temperatures above 100 ^{o}C the boiling point of water (Jacobsen *et al*., 1993). Despite the importance of groundnuts as food and feed, the presence of aflatoxins has the potential to limit its use especially in human diet. Aflatoxin contamination is considered as one of the most important groundnut quality problems in Africa (Kaaya *et al.,* 2006). Mycotoxigenic fungi and aflatoxin contamination in groundnuts starts at the farm level and contamination occur in both pre and postharvest phases.

The pioneering effort in the survey of aflatoxin content of foods and food products in East Africa especially Uganda was undertaken in early 1966 (Lopez & Crawford, 1967). The content of aflatoxin was estimated in groundnuts sold for human consumption in the country. About 15% of the

samples examined contained more than 1 ppb of aflatoxin B1 and three percent contained more than 10 ppb. Studies conducted in 2000 in Kumi and Mayuge districts established that 48% of the groundnuts in the Kumi stored by farmers for up to seven months and 28% of those newly harvested tested positive for aflatoxins, with ranges of 0 – 22 ppb and 0 - 5 ppb, respectively (Kaaya, Warren & Adipala, 2000).

In Mayuge, 50% of the groundnuts stored for up to five months were positive for aflatoxins, with a range of 0 – 18 ppb. Another study conducted in 2003-2004 (Kaaya *et al.,* 2006) to determine the aflatoxin content of groundnuts from farms and markets (wholesalers and retailers) in Mayuge, Iganga and Mubende districts from St. Balikuddembe, Nakawa and Kalerwe the three busiest markets in Kampala indicated that aflatoxin levels increased along the chain up to retail markets. All forms of groundnuts obtained from retailers in markets had levels of aflatoxin significantly higher than the recommended 20 ppb by the WHO and FDA.

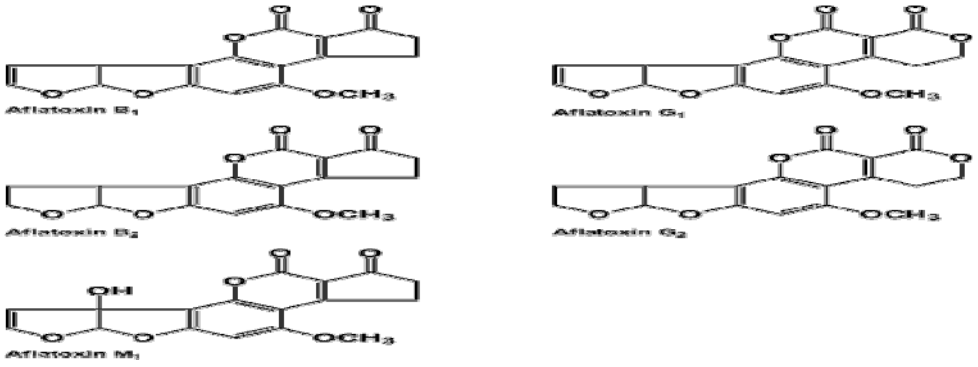


Figure 1: Chemical Structures of B1, B2, G1, B2, *G2*, and Aflatoxins M1

Source: Palmgren and Hayes (1987).

Factors influencing Aflatoxin Contamination of Grains

Aflatoxin contamination of foods and feeds highly depends on biological (biotic) and environmental (abiotic) factors that lead to mould growth, toxin production and can occur at both pre and post-harvest (Rosolem, Fernandez, Maringoni & Oliveira, 1997). For example, mechanical damage, insect and bird damage, drought, stress and excessive rainfall encourage pre-harvest mould growth and aflatoxin production (Miller, 1991). Strain variation in the fungus, interference by other micro-organisms, moisture, temperature, pH, the gaseous environment and preservatives are also important factors. The incidence and levels of fungal infection and aflatoxin contamination reported vary markedly from one geographical area to another (Smith & Moss, 1985; Kaaya *et al.,* 2006). In most instances, however, aflatoxins are formed after harvest, particularly when harvesting takes place during floods, or unseasonal rains or when there is improper storage of insufficiently dried agricultural commodities. The following factors have been singled out as those that mainly encourage mould growth and aflatoxin production in grains and kernels.

Moisture Content

The amount of moisture in a grain affects both grade and storability and has a critical effect on mould growth and mycotoxin production. It is one of the most important considerations in determining whether aflatoxin will develop in groundnuts after harvest. Storage fungi grow at moisture contents in equilibrium with relative humidity ranging from 65-70 to 85-90 percent. *A. flavus* will only grow when the moisture content of grains exceeds 90%, at 80-85% relative humidity and above.

Soil moisture stress has also been reported to enhance pre-harvest aflatoxin contamination of produce. Groundnuts exposed to drought stress in the field have been reported to have more *A. flavus* infected kernels than in irrigated plots. Excessive drought causes strains on pods and testas thus providing entry points for infection by fungi while excessive moisture weakens the pods and testas causing the same effect (Okello *et al*., 2010).

Temperature

The effect of temperature is difficult to separate from the effect of moisture. Under favuorable temperature and relative humidity conditions, aflatoxigenic fungi grow on certain foodstuffs, most commonly cereals, grain legumes and nuts. Production of aflatoxins is optimal at relatively high temperatures, so contamination is most acute and widespread in warm, humid climates. Under tropical conditions typical of Africa, stored products are more susceptible to *Aspergillus* species than other fungi, as many *Aspergilli* are favoured by the combination of low water activity and relatively high storage temperatures (Pitt & Hocking, 1997). *Aspergillus flavus* grows best between 10 ^{0}C and 45 ^{0}C at a relative humidity of 75% or more although the optimum conditions for aflatoxin production are between 25 ^{0}C and 30 ^{0}C, at 85% relative humidity (FAO, 1998).

Handling and Drying

Mechanical damage pre disposes kernels to invasion by storage fungi, including *A. flavus.* Under any given environmental conditions fungal growth is several times faster in damaged compared to intact kernels. Cracks and

breaks in grains are caused mainly during harvesting and shelling, although insect and rodent feeding may also be responsible for breaks in the pericarp (Sauer & Tuite, 1987).

Traditional groundnut drying techniques in developing countries like Nigeria or Mali involving field and bare ground drying are a major source of fungal contamination. They are slow, time consuming and labour intensive involving lots of crop handling (that the resource limited farmer may not adequately accomplish), and due to rains that normally persist at harvesting and drying times, it is difficult to achieve the recommended moisture level for safe storage. In addition, the crop is persistently exposed to soil contamination which is the source of fungi (Kaaya, Kyamuhangire & Kyamanywa, 2007; Okello *et al*., 2010).

The fundamental reason why commodities are stored dry is to increase storability and in part, prevent growth of storage fungi. If commodities are incorrectly stored, that is, in an improperly dried state or under high humidity with inadequate protection, fungi will inevitably grow. Duration of storage is an important factor when considering mycotoxin formation. The longer the retention in storage the greater will be the possibility of building up environmental conditions conducive to aflatoxigenic mould proliferation in groundnuts (Kaaya *et al*., 2000).

Storage structures commonly used by farmers in Africa are traditional and may not maintain an even, cool and dry internal atmosphere; they do not provide adequate protection from insects and rodents; are not easy to clean and above all, are not water proof. All of these conditions favour mould growth and aflatoxin production.

Insect Infestation or Damage

Insect infestation during storage is one of the major problems facing many farmers in African Countries. Insects and mites may damage stored grain, but they also interact with fungal colonization in many different ways. Fungal spores can be carried by insects. Toxin-producing fungi can infect growing crops, as a consequence of insect damage and may produce toxins prior to harvest or during harvesting and storage. During storage, insects, due to their metabolic heat and water, can increase the water activity and temperature of grain to levels suitable for fungal growth. Thus, it is important that insects are controlled both pre and postharvest (Hell, Cardwell, Setamou & Peohling, 2000).

Nutrition and Health effects of Aflatoxins

Aflatoxin (especially aflatoxin B1) recognition as potent carcinogens in animals and humans has made them subjects of government legislation as well as valuable tools in the study of cancer. There are a range of possible consequences of exposure to aflatoxins, largely determined by the dose, the duration of exposure, and the animal involved. In all cases, the young of species are much more susceptible than the adults, and nutrition can be an important factor.

Acute illness and death may occurs a result of consumption of foods contaminated with very high levels of aflatoxin. Individuals die as a result of jaundice and liver failure. In 2004, more than 200 people died in Kenya and more children died in 2010 as a result of consuming maize contaminated with

aflatoxins (Media reports). No animal species is resistant to acute toxic effects of aflatoxins, (Williams, Jolly, Styles, Jolly & Aggarwel, 2004).

Chronic Illnesses or Cancers

The International Cancer Research Institute identifies aflatoxin as a Class 1 carcinogen. This classification is the basis for the regulation of this toxin to exceptionally low levels in traded commodities (US 10 ppb in grain; and 0 ppb in milk; EU 4 ppb and 0 ppb in milk). Aflatoxin is predominantly perceived as being associated with liver cancers. The metabolites, especially those of aflatoxin B1 are capable of binding to protein, DNA and RNA thus interfering with the normal cellular functions resulting in initiation of carcinogenesis, mutagenesis or necrosis of the liver. For developing countries, the synergistic effects of aflatoxin compound the risk due to Hepatitis B virus (HBV), which is the other predominant cause of liver cancer (Okello *et al*., 2010).

Immunology

Aflatoxins have been reported to reduce immunity in humans and animals. It is as a result of their interference with activities of important cells that boost immunity in the body. Consequently, aflatoxins have been strongly linked to HIV/AIDS and malaria in Africa (Okello *et al.,* 2010).

Nutritional Illnesses

In animals it is established that aflatoxin in the diet decreases the rate of growth and other measures of productivity. In children, especially those

below three years, aflatoxin exposure enhances stunting and underweight. Aflatoxins have also been implicated in the slowed rate of recovery from protein malnutrition (kwashiorkor). Generally, from the animal health perspective, aflatoxins cause growth reduction due to protein synthesis interference and micronutrient (vitamins A, B12, C, D and E; minerals zinc, selenium, iron and calcium). This could be the reason why these toxins have been related to several nutritional-related illnesses in humans. Therefore, contamination of produce by aflatoxins puts consumer's health at high risk health hazards (Kaaya *et al*., 2010).

Economic effects of Aflatoxins

Aflatoxins in groundnuts, and indeed in all crops, can have direct economic effects resulting in loss of produce or decrease in market value. As well as indirect economic effects from loss of animals, increased costs of veterinary and human health care services, costs for-borne disease surveillance and food monitoring. Presence of high levels of aflatoxins in groundnuts may make it unacceptable for marketing, causing financial loss to the smallholder farmers or struggling retailer.

Depending on the market, economic losses may reach 100%, when the entire produce or product is rejected by the market if aflatoxin levels are higher than acceptable standards. It is estimated that Africa loses over 670 million US Dollars annually due to requirements for European Union aflatoxin standards for all food exports and world over, billions of dollars are lost by farmers and traders due to aflatoxin contamination (Otsuki, Wilson, & Sewadeh, 2001; Guo, Holbrook, Cleveland, Nieman, Scully, 2009). It is

therefor; very essential that all parties involved in the process of producing and marketing groundnuts should ensure that contamination from mycotoxins is minimized as much as possible (Table 2). Aflatoxins have been known to be highly carcinogenic, and historical evidence from Africa has further reinforced the concerns over aflatoxins. Disease burden on farmers and the citizenry pose direct economic costs to persons and government concerned safety standards, policy and testing for aflatoxins in groundnuts (Kaaya *et al*., 2000).

Table 2: Examples of types of Economic Losses associated with Aflatoxin (and other mycotoxins) Contamination

Bearer	Economic losses and costs
National level	
Primary producer	- Outright food and feed loss
	- Less income from contaminated food
	- Reduced productivity of livestock
Intermediary	- Less income from products refused, condemned or sold at a discount
	- Increased storage, transport, and packing costs
	- Potential loss of market
	- Increased costs due to surveillance and control

Table 2: Cont'd

National government	- Lower forex from reduced exports - Increased costs due to surveillance and control - Increased costs of shipment, sampling and analysis of products for export - Increased need for expenditures in human health and livestock care services -Increased costs for training, communication and extension programs
Consumer (human or livestock)	- Impaired health and productivity capacity - Possible higher medical and veterinary costs
International level	- Loss of market value or market - Trade distortions

Source: Jemmali (1987)

Maximum Tolerable Levels and Enforcement

The level of aflatoxin poison in groundnuts or other crops varies from country to countries. There are many countries that do not have clearly set standards on aflatoxin contamination based on aflatoxin levels in most local foodstuffs. The National Bureau for Standards, in collaboration with other bureau of standards from the East African Belt has however set a limit of 10 ppb for all foods and feeds but only currently certifies products intended for export. Other countries have different maximum tolerable levels of aflatoxin contamination with the EU having the most stringent standards, (Table 3).

Table 3: Maximum level of Total Aflatoxin in foodstuffs

Country	Product	Maximum tolerable limit (ppb)
EU1	Groundnuts – Ready to eat	4
	Groundnuts – for further processing	15
USA	Groundnuts (all products)	20
India	Groundnuts (all products)	30
Kenya	Groundnuts (all products)	10
Uganda	Groundnuts (all products)	10

Source: Okello (2010)

UNBS is the government agency charged with ensuring that all products are safe for consumption. Enforcement of maximum tolerable levels of aflatoxins would however be a very challenging process as most groundnut products in the country are traded informally. Peanut butter, flour, roasted nuts, and grain are mostly sold unpackaged or in inadequate packaging implying that enforcement of any maximum acceptable levels standards would prove very complicated. The UNBS however recognizes this problem and is already in the process of securing laboratory and human capacity to test levels of aflatoxin contamination in foodstuffs (Okello *et al*., 2010).

Ecology and Aflatoxin Management in Groundnut Production

Site Selection for Groundnuts Production

Groundnut is not suited to growing in very dry areas or at altitudes above 1500 m (around 5000 ft). Optimum temperatures are 27 - 30 °C for vegetative growth and 24 - 27 °C for reproductive growth. Between 450 mm and 1250 mm of evenly distributed rainfall is required annually for good

growth and yield. Early maturing small seeded varieties require 300-500mm while medium to late maturing large seeded varieties need 1000-1200mm rainfall. All soils other than very heavy soil are suitable for growing groundnut, but the best are deep, and well drained sandy, sandy loam or loamy sand soils. The latter facilitate the forcing of the developing fruit into the soil (pegging). Groundnut will not grow well or fix nitrogen in acidic or infertile soils. The soils should have a pH (H2O) between 5.3 and 7.3. Soil testing; would determine if there is a need to apply fertilizer and or soil conditioners to assure adequate soil pH and plant nutrition to avoid plant stress, especially during seed development, which makes peanuts more susceptible to fungal infestation (Okello *et al*., 2010).

Soil Amendments

Application of lime (0.5 t/ha), farm yard manure (10 t/ha) and cereal crop residue (5 t/ha) at the time of sowing, either singly or in combinations with lime and farmyard manure, helped reduce *A. flavus* seed infection and aflatoxin contamination in groundnuts by 50-90%. Lime, a source of calcium, enhances cell wall thickness and pod filling and decreases fungal infection (Rosolem *et al.,* 1997). Organic supplements, such as farmyard manure and crop residues, favour growth of native microbial antagonists and suppress soil- and seed-borne infections (Karthikeyan, 1996). These three components also improve the water-holding capacity of the soil, minimizing the effect of end-of-the-season moisture stress, and thereby reducing the fungal colonization and aflatoxin accumulation in the peanut seeds. Lime and farmyard manure are cheap and easily available in most developing countries.

Crop Rotation

The continued cultivation of groundnuts on the same land may lead to a build-up of high populations of *A. flavus* or *A. parasiticus* in the soil. Whilst will increase the probability of infections and aflatoxin contamination. A rotation of 3 years or longer can usually reduce disease, pest and weed problems. Because of the incidence of pests and soil-borne diseases, groundnut should not be grown after cotton, although cotton can be used in rotation after groundnut. Other legumes, tobacco, tomatoes and certain other vegetables may cause a build-up of nematodes and soil-borne diseases and, therefore, should be avoided in rotation with groundnuts. Crops such as cassava, sweet potato and sunflower can also be used while crops such as maize should be avoided in rotations as they are susceptible to *Aspergillus* infection. Although a number of crops are used as intercrops with groundnut, results from intercropping research have been inconsistent, so any advantages or disadvantages are not known (Okello *et al*., 2010).

Groundnut Seeds Bed Preparation

Good land preparation provides suitable soil conditions for rapid and uniform germination, early weed suppression, good root penetration and growth, and steady pod formation, filling and seed development. Land should be prepared early, before the rains start, so that sowing can take place early in the rains.

All previous crop residues and weeds should be completely removed or buried, and seed beds should be smooth to provide good soil-to-seed contact after sowing. Farmers who use tractors are advised to turn the soil deep to

bury residue and weeds, using a disc plough, 3-4 weeks before planting. In wet, low lying areas it may be worth considering using ridges in which to plant groundnuts. The use of ridges can prevent water logging, and improve weed control and harvesting. Ridges should be made at, or just before, sowing and they should be flat-topped (Okello *et al.,* 2010).

Varieties of Groundnut to be Planted

Groundnut varieties which are genetically more resistant to the growth of the fungus and the production of aflatoxins should be chosen, for example Serenut 2 one to be selected for planting. Drought tolerant varieties also have been found to have greatly reduced aflatoxin contamination. Additionally, choosing varieties which are resistant to diseases and pests can help reduce the incidence of aflatoxin contamination (Okello *et al*., 2010).

Groundnut Seeds Selection

Careful seed selection is recommended before sowing. Groundnut pods intended for seeds should be hand-shelled 1-2 weeks before sowing and only good quality seed should be selected for sowing. Immature, damaged, skinned, mouldy, small or shriveled seeds should be sorted and discarded (should not be fed to animals like chicken). It is good practice to purchase certified seed at regular intervals, preferably every 2 -3 years. The seeds must be free from contamination, irrespective of the sources of supply (Okello *et al.,* 2010).

Groundnut Seeds Treatment

Controlling of seedling blights caused by soil bacteria and fungi, and also other fungal diseases, using fungicide treatment is recommended. The seeds can also be treated with an insecticide and fungicide mixture. Thiram gives good protection and can be applied as a dust at 120g of thiram per 100 kg of seed. The dust must be uniformly mixed with the seed. This will reduce seed borne infections during seedling germination. This reduces injury to seed and allows initial vigorous growth (Okello *et al*., 2010).

Sowing or Time of Planting Groundnut Seeds

With the current weather changes in Africa, the planting date is difficult to standardize. However, farmers should plant as soon as there is adequate moisture in the ground to ensure good germination. Timely planting dates should be selected to take advantage of periods of higher rainfall, avoiding end of the season drought effects. Seeds should be sown at a depth of 5 - 6 cm. Seeds must not be sown immediately after heavy rains since they imbibe too much water, which causes rot. This also results into excessive soil compaction which may hinder germination. Long duration (Igola 1, Serenut 1R) varieties should only be planted with the first rains in the first season. Short duration varieties (Serenuts 3R, 4T, 5R, 6T) can be planted in either season. Early planting generally improves yields and seed quality (Okello *et al.,* 2010).

Planting Density of Groundnut Seeds

Groundnut spacing depends on the growth habit of the variety. The recommended spacing ensures that there is good plant population. The recommended space between rows is 45 cm while the recommended spacing per seed is 7.5 - 10 cm for bunch types (e.g. Red Beauty) and 10 - 15 cm for semi-erect types (e.g. Igola 1, Serenut 1 and Serenut 2).

Row spacing can be reduced from 45 cm to 30 cm, if desired, and this will allow earlier ground cover and help prevent serious weed problems. Wider spacing will produce fewer yields per hectare. It is important to sow groundnut seed in rows and at the right spacing as this helps to reduce the incidence of rosette disease, ensures a more uniform pod maturity, better quality seed and maximizes yield. Planting groundnut plants closer together results in individual plants setting fewer pods, but over a short period of time. Overall, this will ensure that the pods will be of a similar age and stage of development and, therefore, make it easier to decide when to harvest (Okello *et al.,* 2010).

Irrigation

Drought conditions favour aflatoxin contamination. Avoid end-of-season drought with supplementary irrigation or timely planting groundnut varieties with maturity period fitting in the rainfall cycle. Drought resistant varieties would help withstand the end or mid-season drought.

Harvesting Management Practices

Damage to pods at the time of harvest should be avoided as much as possible since this can lead to rapid invasion of the kernels by *A. flavus* or *A. parasiticus* which lead to aflatoxin contaminations. Excessive moisture should be removed from the pods after harvesting through shaking.

Timing of Pulling

It is very important to harvest the crop at optimum maturity, as excessive numbers of over mature or very immature pods at harvest can be reflected in high levels of aflatoxin in the product. Also delays in harvesting will result in poor quality seed due to mould infections and subsequent aflatoxin contamination of the seeds or pods.

Harvesting Indicators

As the pods mature, the inside portions become brown to black, while immature pods retain a fresh white appearance. The cellular layer just below the outer layer of the pod undergoes several colour changes during the maturation phase. This cellular layer is called the mesocarp. It changes in colour from white to yellow to orange to brown and finally black as the pod matures. This colour distinction can be used to estimate crop maturity with the 'hull scrape 'method.

Harvesting Techniques

Two major harvesting techniques are used in Uganda; hand and hoe or ox drawn plough. Whichever method is used care should be taken not to injure

the seeds and pod. Hand harvesting or hand pulling are most suitable for erect/semi erect groundnut varieties (Serenut 4T and 6T) in sandy, loam soils which are well drained and are commonly used during the rainy season when the soils are moist and soft. The entire crop branches are building together as the crop is being lifted. Hand harvest is done only when there is enough moisture in the soil (Okello *et al.,* 2010).

Hoe Plough

Used for spreading groundnut varieties (Serenut 3R), on heavy soils and during dry conditions. With the application of this method (hoe plough) is very effective in lifting the entire crop from soils with reduced pod loss. A forked hoe or plough causes less pod or seed damage than unforked ones. This harvesting technique is practiced mainly in the second rains when drought usually set in at harvesting time. Mechanical damage during harvesting with a hoe is a big problem in groundnuts. When pods are damaged, the moulds will enter and produce toxins. The situation becomes worse when drying takes place on bare ground (Okello *et al.,* 2010).

Cleaning and Selection at Harvests

Freshly harvested groundnuts should be cleaned and sorted to remove damaged nuts and other foreign matter. It is important to shake the plant after lifting/harvesting to remove soil from pods and avoid forming optimum conditions for the aflatoxin development. Damage to pods at the time of harvest should be avoided as much as possible since this can lead to rapid invasion of the pods by *A. flavus* or *A. parasiticus*. Groundnuts should be

handled as gently as possible and every effort made to minimize physical damage at all stages of harvesting and transportation procedures. Individual plants that die from attack by pests (termites, nematodes) and diseases (wilts, pod rots, rosette) should be harvested separately as their produce is likely to contain aflatoxin (Okello *et al.,* 2010).

Post-harvest Management Practices

The Processing of Groundnut during and after Harvesting

However, during harvesting, transportation, processing, drying, storage and marketing, groundnut and their products may become contaminated with mycotoxins from sources such as soil, harvesting tools, processing tools, drying and storage facilities, and marketing environment. In Africa, the traditional processing methods and practices are labour intensive and very inefficient using rudimentary tools. The risk of such contamination can be greatly increased as a result of poor traditional practices.

Drying of Unshelled Groundnuts

In most instances, aflatoxins are formed after harvest, particularly when harvesting takes place during end-of-season rains. The drying stage is all-important to reduce attack and damage from insects and fungi. Traditional drying techniques in Africa involving bare ground drying are a major source of fungal contamination (Okello *et al*., 2010b). They are slow, time consuming and labour intensive involving lots of crop handling, and due to rains that normally persist at harvesting, it is difficult to achieve the recommended moisture level for safe storage. Some farmers do not dry groundnuts

immediately after harvest, due to labour constraints. Thus, they heap the nuts either in the field or in houses. Sometimes farmers store wet groundnuts in bags for a few days waiting for sunshine. These practices, coupled with the inefficient and slow drying process under humid conditions enhance aflatoxin contamination greatly.

Shelling of Groundnuts

Mechanical damage to foodstuff during shelling, threshing and winnowing makes them much more vulnerable to invasion by storage moulds, including *A. flavus.* Under any given environmental conditions fungal growth may be several times faster in damaged compared to intact nuts. Cracks and breaks in groundnut pods and testa are caused mainly during shelling by trampling or use of machines. There are two types of groundnut shellers now used in other African countries. The hand operated the motorised shellers. The latter normally use electricity and can be a simple type that can handle small volumes of groundnuts or big type that handle several bags of groundnut per hour (Okello *et al.,* 2010).

Postharvest Storage

The fundamental reason why groundnuts should be stored dry is to increase storability and in part, prevent growth of storage fungi. If groundnuts are stored incorrectly, that is, in an improperly dried state or under high humidity with inadequate protection, fungi will inevitably grow. Duration of storage is an important factor when considering aflatoxin formation. The longer the retention in storage the greater will be the possibility of building up

environmental conditions conducive to *A. flavus* proliferation and production of aflatoxin (Odogola, 1994; Waliyar *et al*., 2007; 2008). Additionally, in Africa groundnuts are stored in two forms: In shells or pods (unshelled) and in shelled form (as kernels). Storing groundnuts in shells or pods is more recommended because shells offer protection against mould infection. When stored in kernel form, groundnuts deteriorate very fast because they pick-up moisture and are easily invaded by moulds, insects and rodents (Okello *et al.,* 2010).

In most parts of Africa, however, traditional means of crop storage are not yet improved as evidenced by the storage structures, whether traditional or modern. They should provide protection from insects, rodents, and birds; maintain an even, cool and dry internal atmosphere; easy to clean and should be water proof and protected from flooding. These recommendations were made in view of *A. flavus* infection and aflatoxin production in stored groundnuts and other produce. The maximum moisture content for storage of groundnuts (unshelled) is 9% whilst that for shelled groundnuts is 7% (Odogola, 1994). At these moisture contents, if the relative humidity is maintained at 70% and temperature 25 – 27 ^{0}C, there is guarantee for safe storage of the nuts for approximately one year.

Mahogany

Mahogany is plant which belongs to the *melicaeae* and the genius *swietenia* and is also of the chinaberry family. *Khaya A. Juss,* is the acyual source of the African mahogany and it is related to the American mahogany of the genus Swietenia which is the original source of mahogany. The bark of the mahogany is extremely bitter and is used as a botanical against fungi, insects

and for medicinal purposes, the fruit contained flavonoids and saponins (Taylor *et al*., 2010).

This botanical has an anti- inflammatory and antibacterial prosperity. Research has shown that all parts of mahogany are very essential in combating lot organisms. The oils induce cytotoxicity, damage the cellular and the organelle membranes, DNA and reactive oxygen species. Such activity is mostly induced by phenols, aldehydes and alcohols (Bakkali *et al*., 2008). Mahogany also contained antiavral, antifungal and bactericidal properties (Abdelgaleil, 2001).

Neem

Neem is botanical that is widely spread in the world; it can be easily multiplied by seed or stem. The extract, powder, cake and the oil of neem have a very huge activities range inhibiting and deleterious against fungi, insects, and nematodes. It has been reported that neem extracts was used in several ways in plant protection, foliar treatments on post-harvest produces such as grains (Schmuttere, 1990).

The products of neem as an anti-feedants, anti-oviposition, repellent and growth regulatory properties (Biol, 2006); neem also has systematic properties (Radwanski, 1977) which normally provide the bitter selectivity towards non-phytophageous against organisms (Rodriguez et al., 1987). Neem is not hazardous to mammals and it has been used in India for lot of years as traditional medicine by farmers for its pesticidal, fungicidal and anti-feedant properties (Jotwani et al., 1981). It has also been recorded by Brahmachari, (2004), that more than hundred years Indian farmers have been using the leaves to protect growing and their harvested crops. It has also been revealed

that all parts of neem tree possess high medicinal values, which can be very effective in managing fungi, insects, and nematodes. Generally, neem is active against broad spectrum of pests, inter-specific toxicity of individual oil and as well as compounds highly active in the management of both pre- and post-harvest organisms (Isman, 2000).

Ginger

Ginger (*Zingiber officinale Roscoe*) is the botanical name for ginger, a tropical herbal plant found in abundance in Asia. It belongs to the family of *Zingiberaceae*. It is widely used as a botanical, spice in traditional and modern cookings. Biochemically, the main active components in ginger are gingerol, shogaol and zingiberene, of which 6-Shogaol having the most potent antioxidant and anti-inflammatory properties (Marsden, DiplCH AHG, MSOM Lac., Messonnier, S., DVM and Cheryl Yuill).

Ginger extract has been extensively studied for its pharmacological and biological activities such as anti-emetic, anti-inflammatory, anti-bacterial, anti-convulsion, analgesic, anti-ulcer, antitumour, anti-fungal, Anti-thrombotic, antidiabetes, peripheral circulatory stimulant, promotive secretion of saliva and gastric juices, increase tone of and peristalsis in intestines, and anti-allergen. Ginger is well-known tropical herb whose root is used in both Traditional Chinese Medicine and Western Herbal Medicine. It can be used as fresh root or may be as extract, or it may be prepared as a tincture, powder for treatment against fungi and insects.

Garlic

Garlic (*Allium sativum*) has traditional dietary and medicinal applications as an anti-infective agent. In vitro evidence of the antimicrobial

activity of fresh and freeze-dried garlic extracts against many bacteria, fungi, and viruses supports these applications.

During the early steps involved in identifying the active constituents of garlic were the discovery that the compound allicin (allyl 2-propene thiosulfinate) is formed when garlic cloves are crushed and that its formation depends upon the action of the enzyme alliinase of the bundle sheath cells upon the alliin of mesophyll cells. Methyl and allyl sulfide derivatives of allicin are formed by the steam distillation of mashed garlic to produce garlic oil (GO), (Ross, O'Gara, Hill, Sleightholme & Maslin).

Garlic is now a member of the Alliaceae and is related to onions (Allium cepa), chives (Allium schoenoprasum) and ornamentals like star of Persia (Allium cristophii). Although many plants include “garlic” as part of their common names, only plants in the genus Allium with the specific epithet sativum are true garlics. Plants like garlic chives (Allium tuberosum) have a mild garlic flavor but are not really garlic. Elephant garlic (Allium ampeloprasum), which closely resembles true garlic but has very large cloves and a milder flavor, is actually a type of leek.

The “stinking rose,” garlic may be known for its odor as much as its flavor, but garlic is actually odorless until its cells are ruptured by being “bruised, cut or crushed”. Garlic signature scent comes primarily from sulfur compounds. When a garlic clove is cut, alliin, an “odorless, sulfur-containing amino acid derivative” reacts with the enzyme alliinase to form allicin and other sulphur compounds. Allicin breaks down into diallyl disulfide, which is largely responsible for garlic’s odor and serve as a repellent against fungi and

insects. Garlic is an effective antibiotic, an anti-viral and anti-fungal agent, and probably an immune system enhancer

CHAPTER THREE

MATERIALS AND METHODS

The study consisted of one field survey and four laboratory experiments. To achieve the set objectives of the study survey was conducted in August 2014.

Experiment One

The objective of this study was to determine groundnut sellers' perception of fungal contamination, source of groundnut, transportation and storage.

Administration of Questionnaires

One enumerator was trained on how to administer the questionnaires. The training encompassed the meaning and proper interpretation of each item on the interview schedule. The structured questionnaires interview schedule was prepared in English and then translated into local language (Twi) to the respondents and their responses were ticked or written on the schedule. All 60 of the groundnut sellers responded to the questionnaires and the questionnaires were administered in August 2014.

Formal Survey

Since the character of the survey was exploratory with time restriction, preference was given to a rapid appraisal (RA) study relying on participatory appraisal technique (PAT). Structured questionnaires were used, primarily to bring to light the sellers' perception of the importance of groundnuts and aflatoxin contamination resulting from *Aspergillus* spp. attack. The use of structured questionnaires survey was helpful in order to obtain base line information in respect of transportation, sale, source of groundnut, alternative use of mouldly groundnut, and storage management of groundnuts. This experiment basically employed seed sampling and the use of structured questionnaires to obtain data from groundnut sellers. The sellers were selected for the assessment of their knowledge level on best practices of groundnut sale. However, the questionnaires were made up of both open and closed ended questions.

Study Area

The survey was conducted in six market districts of the Central Region. The areas sampled included, Cape Coast, Fosu, Mankessim, Jukwa, Kasoa and Swedru.

Figure 2: A map of the Central Region in Ghana showing areas where Samples were collected

Population

The respondents for this experiment were all groundnut sellers in six markets from Cape Coast, Mankessim, Fosu, Swedru, Kasoa and Jukwa in the Central Region.

Sampling Method and Sample Size

Two phase sampling techniques were used for the questionnaires survey. The quota sampling technique was conducted to selection ten (10) sellers for each district market and random sampling technique was the second method employed. Ten groundnut sellers were selected from each of the six district markets making up to sixty (60) groundnut sellers as the sample size. At each of the market 16.7% of the respondents were interviewed.

Table 4: Sample size of Respondents from each of the Six Markets in the Central Region

Location/ District	Frequency	Percentage
Cape Coast (Cape Coast Municipal)	10	16.7
Fosu (Assin North District)	10	16.7
Mankessim (Mfantseman District)	10	16.7
Jukwa (Twifo Heman Lower Denkyira)	10	16.7
Kasoa(Agona East)	10	16.7
Swedru (Awutu Senaya Municipal)	10	16.7
Total	60	100.0

Experiment Two

The objective of this experiment was to identify the mycoflora of the groundnut samples from the six markets in the Central Region using the Blotter Method Mathur and Kongsdal, (2001).

Collection of Groundnut Samples

The samples were collected from the six different major markets in the Central Region of Ghana, where the field survey was conducted. The samples were collected from the sellers who were interviewed during the field survey. The markets included: Cape Coast Metropolitan, Kasoa, and, Mankessim Districts, which lie in the Coastal Savannah zone and Swedru, Jukwa and Fosu Districts located in the Forest zone.

Four samples were collected per market per district, making a total of 24 samples from the six markets in the region. Drawing the primary,

composite and as instructed in Mathur and Kongsdel (2001) submitted samples, the working groundnut samples were obtained. The working samples were then placed in sterile polythene bags and transported to the laboratory for analyses.

Plating of Groundnut Samples

The groundnut samples were taken to the laboratory of Biotechnology, Seed and Science Division (BSFSD) of Crops Research Institute, CSIR-Kumasi, Ghana for seed health testing. The blotter method recommended by the International Seed Testing Association (ISTA, 1966) and (Mathur *et al*., 2001) was used for the study. Ten seeds of each sample was plated in plastic Petri dishes (9.0cm diameter) lined with three moistened blotters in 40 replicates, making four hundred seeds per sample per market as recommended by ISTA (1966).

Incubation of Plated Groundnut Samples

The plates were incubated at 28± 2 ^{o}C under 12 hours of alternating cycles of NUV (near ultra violet) light provided by Phillips black tubes and darkness for 7 days according to the recommendation by ISTA (1966) in a completely randomized design.

Plate 1: Plated Groundnut Seeds in an Incubation Room

Identification of Fungal Pathogens on Groundnut Samples

Examinations of the seeds for fungal growth were done on the eighth day of incubation. The fungi developing on the samples were identified under the stereoscopic microscopic on the basis of their habit characters as described in the seed health testing manual (ISTA, 1966). In doubtful situations,

identification was done under a compound microscope (ADP 2100 series, Satorius and Adam equipment, New Jersey, USA) for confirmation.

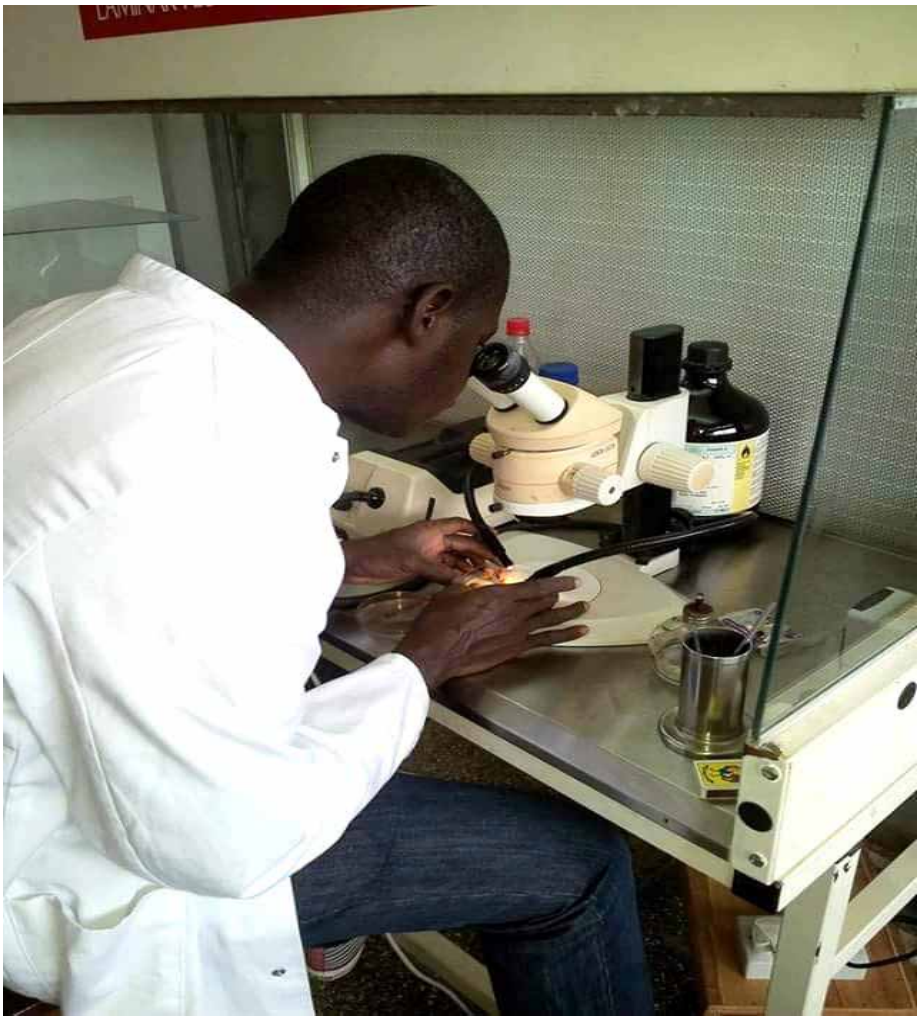


Plate 2: Examination of Groundnut Samples for Fungal Organisms under a Microscope and in Lamina Flow Chamber

Experiment Three

This experiment was aimed at determining the diversity of *Aspergillus flavus* isolates from the groundnut samples based on cultural and morphological characteristics.

Study Area

The study was conducted at the Plant and Molecular Laboratory, CSIR-CRI, Fumesua, Kumasi.

Preparation of Potato Dextrose Agar (PDA)

Potato dextrose agar (PDA) was prepared by stirring 39g of dehydrated PDA in one liter of distilled water and autoclaving at 1.05kg/cm^2 pressure at 121^{0}C for 15 minutes. Solidified 15ml PDA in a Petri dish was inoculated at the center with a 1cm disc of agar medium bearing the mycelium of the test fungal.

***Aspergillus flavus* isolates**

The cultures of *A. flavus* were obtained from the blotter method. Three day old cultures of *A. flavus* isolates kept on slant were used to inoculate the various PDA plates.

Pure cultures of *A. flavus* from the 24 samples were obtained by transferring fungal colonies to new PDA plates and incubating the plates for 6 days. The plates were wrapped with sterile plastic paper tapes and placed in the incubation room at 28-30^{0}C in a completely randomized design with three replications per treatment. The radial diameters of unamended plates were measured with a ruler every day for the period of six days of incubation.

Identification of the *Aspergillus Flavus* Isolates

Isolates were identified to species (*A. flavus)* level based on cultural and morphological (phenotypic) features as described by Cotty (1994a) and Okuda, Klich, Selffert and Ando, (2000).

Experiment Four

The objective of this experiment was to identify the efficacy of botanicals on the radial mycelia growth of *A. flavus in vitro*.

Study Area

The study was carried out at the Plant Pathology Section, Plant and Molecular laboratory at the Crop Research Institute, Fumesua, Ashanti Region.

Preparation of Plant Extracts

Aqueous of the botanicals neem (*Azadirachta indica*) mahogany leaves (*Khaya anthotheca*), ginger (*Zingiber officinalis*) and garlic (*Allium sativum*) together with synthetic fungicide Benlate were used in a PDA media in order to determine their efficacy on inhibiting *A. flavus* growth. The method applied was that of Asare-Bediako *et al.,* (2007) and Shovan *et al*., (2008)

Garlic, ginger, neem and mahogany leaves were collected fresh. Each was washed thoroughly with fresh running tap water, then with alcohol (75%) and with six changes of sterilized distil water (SDW) as described by Rajamanic *et al*., (2012) The botanicals were then homogenized with a blender and then poured into air tight bottles. The extracts were filtered through cheesecloth to obtain aqueous extracts as stock solutions.

Amendment of the Botanical Extracts and the PDA

Potato dextrose agar (PDA) was prepared as described. The warm PDA (200 mL) was amended with one tablet of amoxicillin (200 mg) in order to check bacterial contamination and then with, 10, 15 and 20 mls of each

plant extract to give the three levels of concentrations of 10, 15, and 20% (v/v) of each plant extract. Fungicidal suspensions of different concentrations (10, 15 and 20 v/v or 100, 200 and 300 ppm) was prepared by dissolving requisite quantities of Benlate in warm PDA. Approximately 15 ml of each molten PDA amended with the plant extracts or Benlate suspension was poured into each 9.0 mm Petri- dish and allowed to set.

Plating of *Aspergillus Flavus* Isolates on the amended PDA

The three day old cultures of isolates of *A. flavus* kept on slant were used to inoculate the various PDA plates. Pure cultures were obtained from the three day old *A. flavus* isolates. Inoculum plugs were obtained with a sterile cork borer from the growing margin. Pure cultures of *A. flavus* from the 24 samples were obtained by transferring fungal colonies to the new PDA plates and incubating the plates for 7 days. The plates were wrapped with sterile plastic paper tapes and placed in the incubation room at 28-30 ^{0}C in a Completely Randomized Factorial design with three replications per treatment. Three PDA, Benlate plates were amended and used as controls in the experiment. The radial diameters of the amended and unamended plates were measured on the seventh day of the incubated isolates.

Plate 3: Extraction and Wrapping of Isolated *A. flavus* in a perti- dish on PDA

The percentage inhibition of *A. flavus* mycelial growth were calculated based on the colony diameter on control plates and fungicide treated plates using the following formula as stated by Sunder and Ishnaveni (1995), with slight modification:

% inhibition $= \frac{Y-A}{Y} x\ 100$

Where,

Y= mycelia growth on the control plate.

A= mycelia growth on the fungicide treated plate.

Experiment Five

The aim of this experiment was to determine the types and quantity of aflatoxins (AFT) in the groundnut samples.

Sample Extraction

This experiment was conducted at the aflatoxin laboratory of the Department of Food Science of the Kwame Nkrumah University of Science and Technology, Kumasi.

To minimise sub-sampling error in *A. flavus* test or AFT analysis, 25g of all the groundnut samples were weighed and ground in a blender at high speed for 2 minutes. Each sample was mixed thoroughly with 5 g of sodium chloride and collected in transparent plastic bags. The cover was removed from the blender jar and pour 50 mL extracts was poured into fluted filter paper and collected in clean vessels. The filtrate was then collected for analysis by high performance liquid chromatography (HPLC) method.

Extraction Dilution

Filtered extracts of the groundnut samples were analyzed using the HPLC method (AOAC, 2004) with a slight modification. The test portion, regarding HPLC analysis, was extracted using 125 mL of 70% methanol (80ml/20 mL). Filtered extracts (15mL) were poured into clean containers and diluted with 30 mL of distilled water and thoroughly mixed.

Chemicals and reagents, such as 2 µg mL-1 solutions of AFB1, AFG1 and 0.5µgml-1 of AFB2 and AFG2 and MycoSep column 226 (AflaZone) were purchased from Romers Labs, USA. HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany) and trifluoroacetic acid (TFA) was obtained from Sigma Aldrich Chemical USA. All other chemicals and organic solvents were at least of analytical grade.

Aflatest Affinity Chromatography

Aflatest immune- affinity columns (IACs) were used to clean up the samples. The filtered extracts (15 ml) using pipete were passed through the Aflatest column and additional 10 mL distilled water was added into column and elute was finally diluted by water before being analyzed by HPLC.

Mobile Phase

Methanol solution with the 6:4 (v/v) ratio in addition to each liter of mobile phase, a volume 350 ml Nitric acid (4 mol/L) and 120 mg potassium bromide was added to the elute and dissolved when using post column.

Column

The column involved using Phenomenex Hyper Clone HPLC system (BDS C18 150 x 4.60 mm, analytical column 5 um) at a temperature of 40 ^{o}C, and flow rate of 1ml/min.

Derivatisation

Aflatoxins B2 and G2 are naturally much more fluorescent than aflatoxins B1 and G1. Hence aflatoxin B1 and G1 fluorescence were increased for HPLC fluorescence detection by derivatisation.

Post Column Derivatisation (Bromination)

Electrochemically generated bromine (KOBRA cell) follows the instructions for the installation of the cell as supplied by the manufacturers and operate using the following parameters: - flow rate: 1.00 mL/min or appropriate flow rate based on column type and size, and a current of 100 μA.

Detector

Fluorescence detector, with a wavelength (λ) = 360 nm excitation filter and a wavelength of λ > 420 nm cut-off emission filter, or equivalent fluorescence at 360 nm excitation and 440 nm emission. As a recommended settings for adjustable detectors are Ex. = 360 nm, Em = 435 nm for the detector.

Statistical Analysis

The survey data was analysed into descriptive statistics in the form of frequency distributions and bar charts using Statistical Package of Service Solutions (SPSS) version 21 (IBM). Data collected were subjected to analysis of variance (ANOVA) and the means separated with the least significant difference method at 5% level of probability and DMRT at 5% level using GenStat Release 4th edition. The IBM SPSS V21 was used to analyze the levels of aflatoxins into percentage and multiple comparisons within and between markets was done using Tukey test at 95% Confidence Interval. Graphs work was done using MS Excel software.

CHAPTER FOUR

RESULTS

This chapter presents the results of the field survey and five (5) laboratory experiments conducted to determine the fungal contaminants on groundnut samples.

Experiment One

Sellers' perception of fungal contaminants, source of Seeds, transportation, sale, alternative usages of groundnut and Storage management

Demographic Characteristics of the Respondents

Table 5 shows the gender of the respondent groundnut sellers. All the groundnut sellers (100%) were females and none was male (0%). The findings further showed that 13.3% of groundnut sellers had attained secondary education (Table 5). Majority of the respondents were found to have acquired basic education with the values of 26.7% and 23.3% for Junior high school and Primary education, respectively. Only 1.7% of the sellers had Tertiary education. About 35% of them had no formal education (Table 5).

Majority of the groundnut sellers (35%) had 1-3 years, experience, in the sale of groundnuts, followed by 23.3%, who had 4-6 years, experience.

Those with 7-9 years and 10-12 years, experience were 13.3% each, 6.7% and more than 15 years, experience, 6.7%, (Table 5).

Table 5: Demographic Characteristics of the Respondent Groundnut Sellers

Variable	Frequency	Percentage
Gender of the respondents		
Female	60	100
Male	0	0
Total	60	100
Educational background		
No formal Education	21	35.0
Primary Education	14	23.3
Junior High School	16	26.7
Senior High School	8	13.3
Tertiary	1	1.7
Total	60	100.0
Experience in sale of groundnut		
1-3	21	35.0
4-6	14	23.3
7-9	8	13.3
10-12	8	13.3
13-15	4	6.7
> 15	5	8.3
Total	**60**	**100**

Source: Field survey, 2014

All (100%) of the respondents had not heard about aflatoxin through the media, local radio, international radio, extension officer, health worker, neighbor, television, newspaper, buyers, traders and other sources.

Sources of Groundnuts

Data presented in Table 6 shows, the locations where respondents purchased their groundnuts. The respondents (sellers) obtained their groundnuts from various sources. Most sellers (38.3%) purchase their groundnuts from Techiman; followed by those who obtained theirs from Tema (25%) and Accra (21.7%). Very few of the sellers obtained their groundnuts from other sources namely, Swedru, Fosu, Cape Coast and Ejura.

Table 6: Sources of Groundnuts

Location	Frequency	Percentage
Accra	13	21.7
Cape Coast	2	3.3
Ejura	1	1.7
Fosu	3	5.0
Techiman	23	38.3
Tema	15	25
Swedru	3	5.0
Total	**60**	**100.0**

Source: Field survey, 2014

The Groundnut Traders

This figure despites data on the traders of groundnuts, groundnut seller purchased their product from both farmers and traders. Figure 3 shows, that most groundnut sellers 68.3%, purchased bulk of their groundnuts from farmers, where as 31.7% of the sellers purchased their groundnuts from traders.

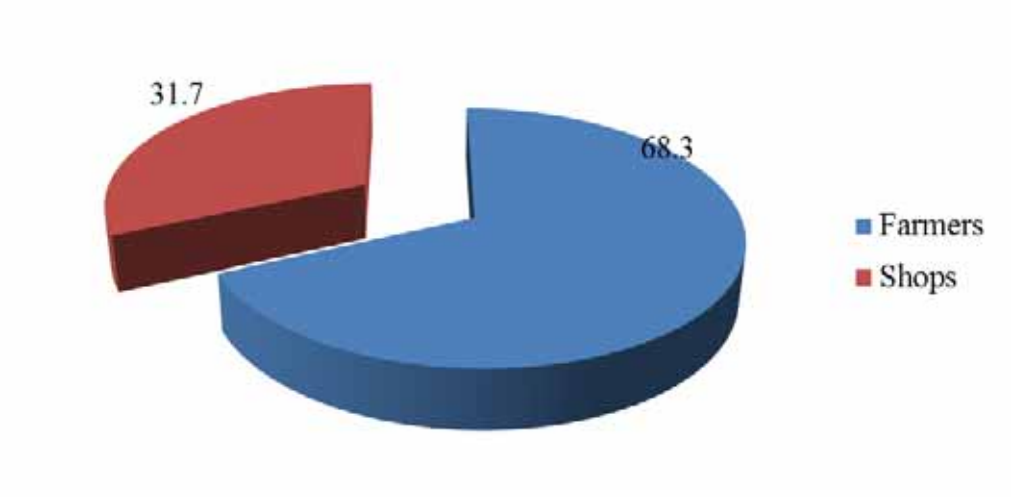


Figure 3: Groundnut Traders

Forms in which Groundnuts are Purchased, Transported and days spent during Transportation

Table 7 show, that majority of the respondents purchased groundnuts that are unshelled. Table 7 also shows the means of transporting groundnuts from the point of purchase to the point of sale. Majority of the sellers (76.6%) transport their groundnuts in closed vans, whilst 13.3% use open vans and 10.0% by wheelbarrows and porters. For majority of the sellers, it takes about 1-2 days for their groundnuts to reach the sale point.

Table 7 shows, times of the day that groundnuts are transported from the purchased area to the sale points. Majority (86.7%) of the sellers are

unaware of the actual time the groundnuts are transported whilst, 6.7% of the sellers transport their groundnut during the afternoon. Sellers that transport groundnut in the morning and night are 5.0% and 1.7%, respectively. The results showed time taken for consumers to purchase all the groundnuts. The result indicates that more sellers (76.7%) could sell off their stock within two weeks whilst the other (23.3%) could do so within 3-4 weeks.

Table 7: Form in which Groundnuts are Purchased and Transported

Form of groundnuts	Frequency	Percentage
Pods	60	100
Shelled	0	0
Total	60	100
Transportation of groundnut		
Open Van	8	13.3
Closed Van	46	76.7
Others (Wheelbarrow/porters)	6	10.0
Total	60	100
Number of days taken for groundnuts to reach the selling point		
1-2	60	100
3-4	0	0
5-6	0	0
Others	0	0
Total	60	100

Table 7: Cont'd

Time of the day groundnut seed are transported		
Morning	3	5.0
Afternoon	4	6.7
Night	1	1.7
Total	8	13.3
Unknown	52	86.7
Total	60	100
Time to sell		
1-2 Weeks	37	76.6
3-4 Weeks	23	23.3
Others	0	0
Total	**60**	**100.0**

Source: Field survey, 2014

Table 8 shows, the type of storage structures used by sellers to store groundnuts. Most sellers (46.7%) stored their groundnut in their metal containers. Others stored theirs in the market (13.3%) and in short metal containers (18.3%) as shown in plates 4a and b. whereas, (21.7%) stored in wooden box, plate 5a with spaces insects and pests can easily get in the and destroyed the groundnuts.

Table 8: Type of Storage Structures

Storage	Frequency	Percentage
Metal Container	28	46.7
Metal container	11	18.3
Wooden Box	13	21.7
Others	8	13.3
Total	**60**	**100.0**

Source: Field survey, 2014

Plate 4a: Blue Metal Container used to Store Groundnuts in Swedru Market

Plate 4b: Light Green Metal Container used as Table and Storage for Groundnuts in Cape Coast Metropolitan market

Plate 5a: Wooden box used as Market Table and Storage of Groundnuts in Mankessim Market

Plate 5b: Open Market Storage (others) with Polyethylene Bag containing Groundnuts with Transparent Plastic in Cape Coast Metropolitan Market

Other Physical Contaminants (storage matters) of Groundnuts

It is shown in Table 9 that 81.7% of the respondents store groundnuts with beans, 61.7%, store with maize, 50.0% of the seller's stored groundnuts with rice and 40.0% stored groundnuts with millet. Others stored groundnuts with flour (36.6%), and 6.7% pepper as shown in Plate 6. None of the respondents stored their groundnut on pallets; neither did their storage facilities have proper ventilation and ceiling tile. Table 9 also shows that, all the respondents admitted that the groundnut is stored whilst in polyethylene bags.

Table 9: Other Physical Contaminants (storage matters) of Groundnuts

Other crops sold with groundnuts and food items	Frequency	Percentage
Beans	49	81.7
Flour	22	36.7
Maize	37	61.7
Millet	24	40.0
Pepper	4	6.7
Rice	30	50.0
Sugar	22	36.7
Total	188	313.5

Plate 6: Stored Groundnut with other Produces and Foodstuffs

Foreign Matters as Physical Contaminants and Alternative Usage of Groundnuts

Table 10 shows that all of the respondents (100%) noticed moulds on the groundnuts. Majority of sellers (58.3%) notice mould mainly in the dry

season whereas (8.3%) of them observe mould in the rainy season. However, 33.3% indicate that evidence of mould on groundnuts is found all year round.

Also all the respondents (100%) stated that the presence of moulds on the groundnuts adversely affects their sale. Preponderance of the respondents (100%) sees broken groundnut seeds in their groundnuts as shown in Plate 3. Majority of the groundnut sellers (71.7%) process their mouldy groundnuts into paste whereas 23.3% of the respondents dispose of theirs. Table 10 shows the foreign material sellers encounter in their groundnut. Majority of the respondents (73.3%) reported of groundnut shells, this was followed by pebbles (46.7%), weed seeds (43.7%), muds (33.4%) and ants (38.3%).

Table 10: Sellers Perception of Mould on Groundnut

Variable	Frequency	Percentage
Do you see mould?		
Yes	60	100
No	0	0
Total	**60**	**100**
Time of the year the mould is noticed		
Rainy Season	5	8.3
Dry Season	35	58.3
Both rainy & dry seasons	20	33.3
Total	**60**	**100**

Table 10: Cont'd

Do moulds affect your sale?		
Yes	60	100
No	0	0
Total	60	100
Do you notice broken groundnuts		
Yes	60	100
No	0	0
Total	60	100
Alternative usages of groundnuts		
Disposal	17	28.3
Groundnuts paste	43	71.7
Total	60	100
Contaminants		
Ants (bug, insect)	23	38.3
Muds	20	33.4
Pebbles	28	46.7
Shells	44	73.3
Weed seeds (plant debris)	26	43.3
Total	**141**	**235**

Source: Field survey, 2014 Multiple responses

Plate 7: Broken and Unbroken mixed Groundnut Seeds

Experiment Two

Mycoflora on groundnut samples from six markets in the Central Region

The fungal organisms revealed by the blotter method were: *Aspergillus flavus, Aspergillus niger*, *Rhizopus* spp., *Penicilium* spp., *Botryodiplodia theobromae* and *Macrophmina phaseolina.* The most dominant organisms of the groundnut samples were *Aspergillus flavus, Rhizopus* spp. and *Aspergillus niger* (Figure 4). *A. flavus* recorded the highest infection in all of the six district markets, followed by *Rhizopus* spp. *A. niger* recorded the third highest infection of all the samples ranging from 2%- 43% in Cape Coast and 2%- 42% in Fosu.

Rhizopus spp. was present on all the 24 groundnut samples collected from the six Districts in the Central Region. Cape Coast markets had the highest counts of infections ranging from 2%- 77%. Infection of samples from

Fosu, Jukwa, Kasoa, Mankessim, and Swedru ranged between 40%- 50%, 35%- 48%, 30%- 40%, 26%- 36%, 40%- 50% respectively, of infections in the samples. The highest counts of *A. niger* infection on the samples were found in Jukwa District with the mean infection of 45%- 55%. This was followed by samples from Kasoa, Mankessim and Swedru with the mean infection ranging between 26%- 39%, 25%- 37%, 26%- 39% respectively.

A. flavus and *Rhizopus* spp. ranked as the first and second highest with values of 5%- 77% and 2%-77% respectively. It was followed by *Penicillium* spp. which was the fourth highest in all the samples collected from the six Districts. Swedru obtained the highest values of *Penicillium* spp. infections ranging from 45% - 55%. This was followed by samples collected from Fosu with *penicillium* spp. mean infections of 39% - 50%. Cape Coast and Jukwa obtained a mean infection of *Penicillium* spp. ranging from 30% - 38% and 25% - 37% respectively, whilst *B. theobromae* and *M. phaseolin* ranked fifth and sixth. The least infection of *Penicilium* spp. was obtained from the groundnut samples collected from Kasoa and Mankessim with the mean values ranging from 10% to 15% and 15% to 23%, respectively (Figure 4).

Kasoa recorded mean range of 1% to 3% Botryodiplodia theobromae infections on the samples and while the rest of the Districts mean ranged from less than 1% to 2% of infection of B. theobromae from the groundnut samples. There was no infection of *Macrophomina phaseolina* found on the samples in Cape Coast. Percent infection of groundnut obtained from Jukwa means ranged from 2 to 5%, of the count of *M. phaseolina* infection. The rest of the district markets samples had less than 1% to 2%.

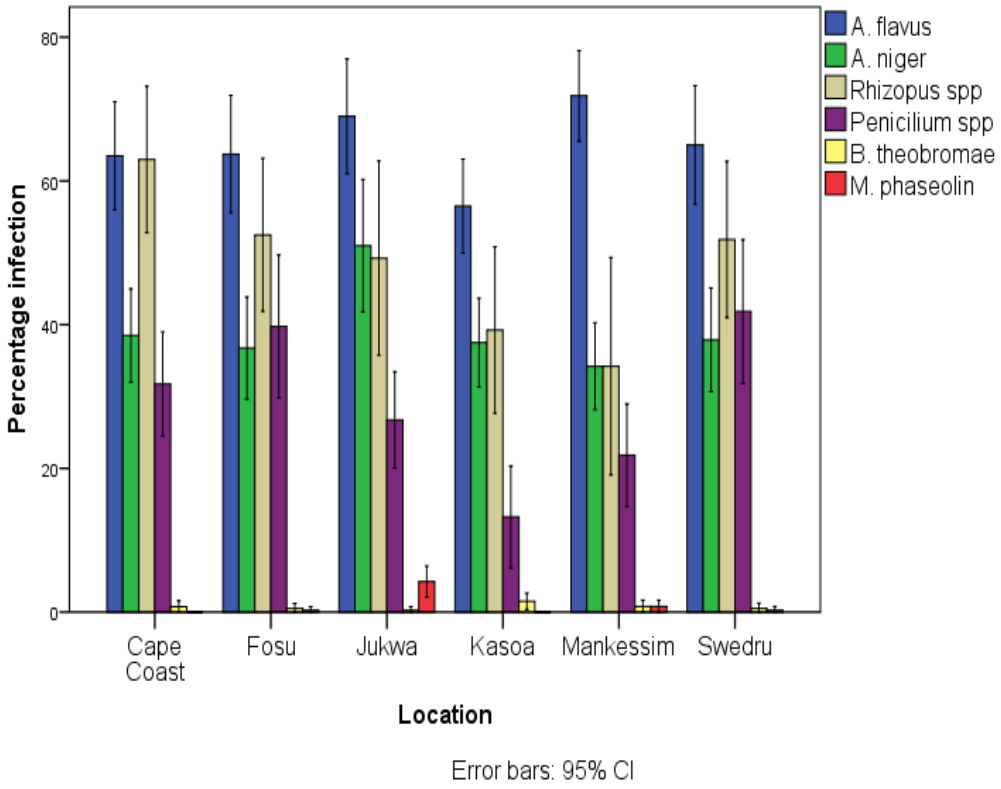


Figure 4: Fungal Infections of Groundnut Samples from the six District Markets (%)

Experiment Three

Cultural and Morphological Characteristics of *A. Flavus* Isolates

In figure 5 is a graphical presentation radial growth of the *A. flavus* isolates from all the six district markets in the Central of Ghana. There were rapid increases in radial growth as the progressed. The isolate were similar in growth starting from day one where they all had an average radial growth of 1.0 cm. Beyond day one, growth was still similar in the isolates until day 3. At day 6 the highest growth of 8.0 cm for Cape Coast, Jukwa, Fosu, Mankesssim, Swedru and 8.5 cm for Kasoa had been achieved by the isolates. There was no diversity in the radial growth in length of *A. flavus* isolates.

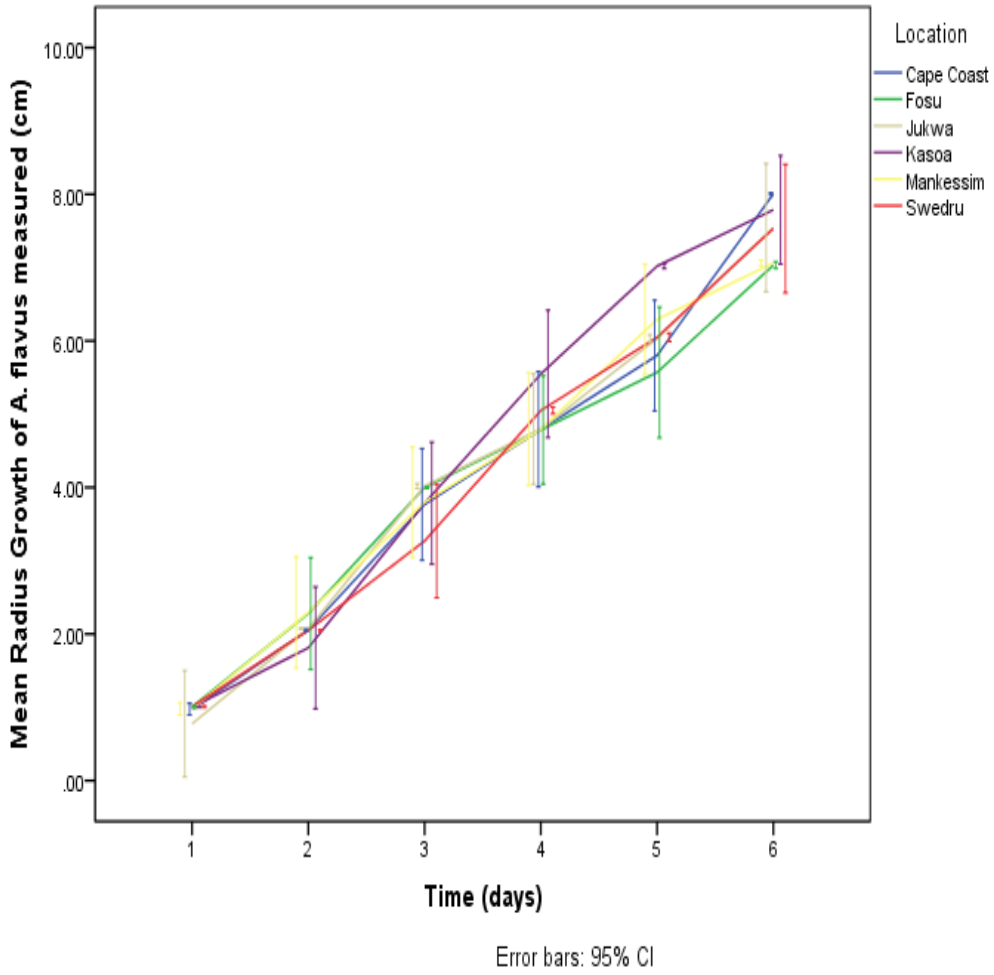
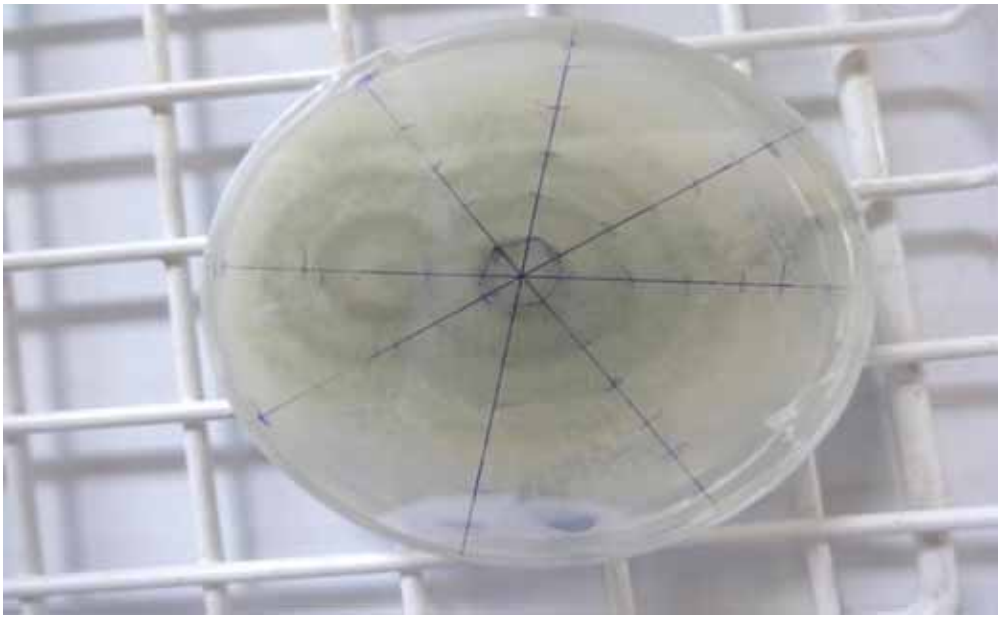


Figure 5: Radial Growth in Length (cm) of *A. flavus* on Groundnut Samples collected from Six Districts

Characterization of *A. flavus* Isolates

The fungal colonies of *A. flavus* isolated from the groundnut samples are presented in Plates 9 to 10. Colonies of *A. flavus* on PDA at room temperature of 27- 31 ◦C obtained a diameter of 4.0- 5.2 and 4.1- 5.3 cm in seven (7) days. The colonies consisted of a dense felt of yellowish-green mycelia becoming green with age and time, in concentric rings (Plates 8). The reverse side of the agar was initially creamish-yellow and with time and age turned yellow. Conidial head usually appeared radiate; conidiophores arose separately from the horizontal hyphae and were hyaline, coarsely roughened,

with globose vesicles and phialide borne on metulae. The conidia, globose to subglobose in shape were usually roughened, yellowish-green in colour, measuring 3.5µm to 3.6 µm in diameter (Plates 9 and 10).



Plates 8: Six-day old Culture of *A. flavus,* on Potato Dextrose Agar (PDA) plate (xx2/3). Note the Yellow Margin Coloration of the Green Colony

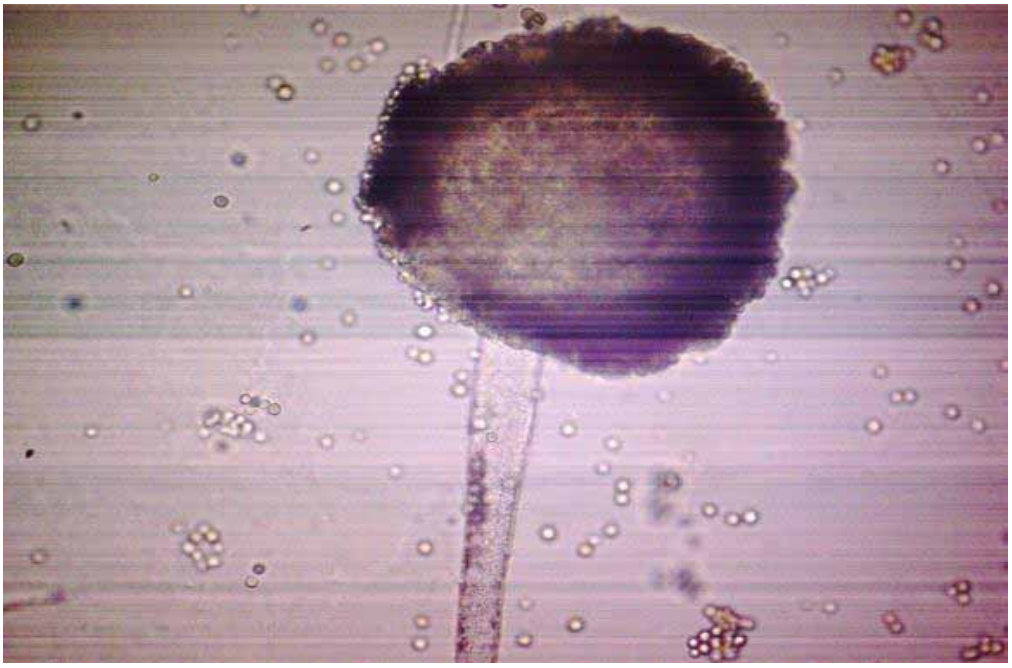


Plate 9: Conidiophore with Conidial Head and Spores of *A. flavus* under the Light Microscope (x400)

Plate 10: The Zonation in the Colony Conidial Head of *A. flavus* under the Light Microscope (x400*)*

Experiment Four

Means effect of Botanical Disinfectants on the Growth of *A. Flavus* Isolates *in Vitro*

Data on *in vitro* effect of plant extracts against *A. flavus* mycelia growth. It shows which one of the amended medium was effective in inhibiting the mycelia growth of *A. flavus* isolates. Table 11 result shows, the efficacy of plant extracts on the radial growth of *A. flavus*. There were no significant differences at concentration 10 for garlic, ginger and neem. Similarly, there were no significant difference between Benlate and mahogany at concentration 10 but there were significant differences among garlic, ginger, neem and Benlate and mahogany. Whilst, at concentration 15 garlic and neem were not significantly different whilst ginger, Benlate and mahogany shows

significant differences inhibiting mycelia growth of *A. flavus* among the treatments applied. However, at concentration 20 there were no significant differences among garlic, ginger, neem and Benlate except mahogany.

It is evident that the mean effect of plant extracts on *A. flavus* mycelia growth inhibition *in vitro* shows that garlic (90.0) recorded the highest mean followed by neem (80.5), Benlate (75.8) and Ginger (71.9), respectively. Whereas, mahogany (49.9) recorded the lowest mean of all the treatments *in vitro* on the mycelia growth of *A. flavus* isolates inhibition.

Table 11: Percent Inhibition of *A. flavus* Mycelia Growth

	% inhibition of *A. flavus* mycelia			Mean
Treatment	10	15	20	
Garlic	90.0a	90.0a	90.0a	90.0
Ginger	80.1a	45.7c	90.0a	71.9
Neem	80.0a	80.3a	80.3a	80.5
Benlate	65.9b	79.5ab	82.1a	75.8
Mahogany	52.3b	48.4bc	49.0b	49.9
		Lsd = 13.78		

Means with the identical letters are not significantly different from each other by DMRT at 5% level. Arcsine transformed means of *A. flavus* mycelia growth inhibition

Experiment Five

The Levels and Types of Aflatoxin in Groundnut Samples from Six Market Centres

Table 12 shows the mean levels of aflatoxin determined from groundnut samples from six markets in the Central Region. The highest level of aflatoxin (9.1 ppb) was obtained from the Cape Coast market whilst the lowest level of aflatoxin (1.8 ppb) was determined from Jukwa market. However, ANOVA on the levels of aflatoxin in the groundnut samples did not show significant difference ($p > 0.05$) among the different markets.

Table 12: The Levels of Aflatoxin determined from Groundnut Samples from Six Different Markets in the Central Region

Source of groundnut (Market)	Amount of aflatoxin (ppb)
Cape Coast	9.1
Fosu	4.1
Jukwa	1.8
Kasoa	7.2
Mankessim	5.4
Sweduru	6.5

Arcsine transformed of means of aflatoxin levels

The results of the HPCL analyses of the 24 groundnut samples collected from the six market centres in the Central Region revealed the presence of at least one of the four types of aflatoxins, namely Aflatoxin G1, G2 , B1 and B2 (Figure 6). Aflatoxin G1 (100%) and G2 (100%) were detected in the groundnut samples from all the six markets in the Central

Region. Aflatoxin B1 was detected in all the six markets but it was most frequent in groundnut samples from Fosu (75%), Mankessim (75%) and Swedru (75%), followed by Kasoa (50%) whilst it was less frequent in Cape Coast (25%) and Jukwa (25%) markets. Aflatoxin B2 was detected in groundnut samples from Fosu, Jukwa, Kasoa, Mankessim and Swedru but not in Cape Coast. Groundnut samples from Fosu, Jukwa, Kasoa and Mankessim had 50% occurrence of aflatoxin B2 whereas that of Swedru had 25%.

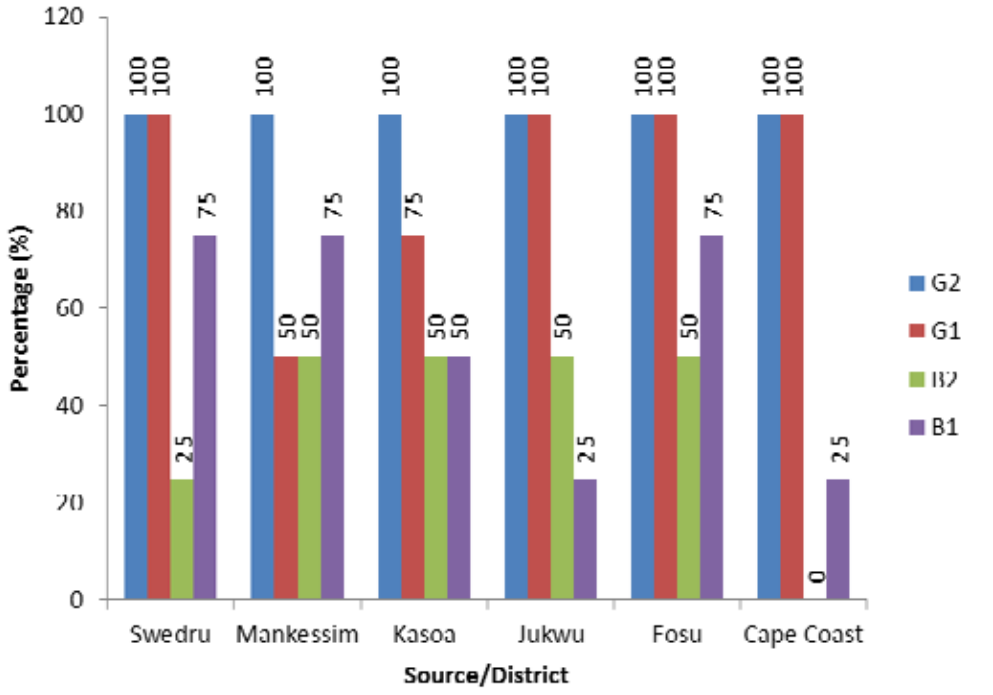


Figure 6: Types of Aflatoxin in Groundnut Samples from Six Market Centres in the Central Region

CHAPTER FIVE

DISCUSSION

Sellers Perception of Fungal Contamination, Source of Seeds, Storage and Preservation

It was found that all the groundnut sellers observed mould on the groundnuts. The storage condition in the study areas were (100%) without pallet, no ventilation and no ceiling tile and sellers stored groundnut seeds with other produce. Most of the sellers stored groundnuts in metal containers, wooden and metal boxes and in the open market. All the types of storage structures were very conducive for insect's pests and attack the buildup of heat. There were lots of inert materials found in the groundnuts by sellers. According to Neergaard, (1979), poor storage conditions can lead to seed contamination.

The results are also in agreement with reports from Hell *et al.,* (2003), well known of *A. flavus* spp. and subsequent production of aflatoxins in maize is dependent on a number of factors such as temperature, humidity, insect injury, broken seed, poor handling during harvest and storage. Also there was a significant positive correlation between moisture content of maize seed, *A. flavus* population and aflatoxin production as reported by Oyebanji and Efiuwevwere, (1999). As it was indicated in these findings, are also consistent with the report of Kaaya *et al*, (2000); who stated that if commodities are incorrectly stored, under improperly dried condition or under high humidity

factor then mycotoxins producers will inevitably increase. The duration of products store, the retention in storage and greater possibility of the building of environmental conditions that could be very conducive for aflatoxigenic mould proliferation in the groundnuts. Hell *et al,* (2000), reported that during storage, insects, due to their metabolic heat and water, are capable of increasing the water activity as well as temperature of the grain to levels that is very suitable for the growth and development of fungi infection.

In this work, sellers admitted encountering all sorts of contaminants, including weeds and pebbles in the groundnuts, which could be a good mean of transferring infections. This is in agreement with report that, in storage, fungi played a negative role in the quality of seed or the deterioration of grain Christensen and Lopez, 1963, Christensen and Kaufmannn, 1969. And Sauer *et al.,* (1992), Frisvad (1995) have reported that fungal infection of seeds before and after harvest remains a huge challenge of food safety in most parts of the African continent. This is linked with infections ranging from mouldy smell, moustiness and contamination (McAlpin *et al.*, 2002), Bankole and Adebanjo (2003). It was also reported by Coker (1989), Connif (1995) that infections occur particularly in warm and tropical region of the world, for instance, in Africa, where proper and accurate screening methods are lacking.

This could be the reason why the infections of the fungal organisms were high as indicated in the findings. Smith and Moss (1985), Kaaya *et al*., (2006), reported that the incidence and levels of fungal infection and aflatoxin contamination vary markedly from one geographical locality to another. In this study, findings of poor storage facility in the area where the seeds were obtained, is in conformity with Okello *et al.* (2010b), Neergaard, (1979)

reported that, high amount of moisture in grain affects the grade and storability, and it has a very critical impact on the growth of mould as well the production of mycotoxin. It is also in agreement with Pitt and Hocking, (1997), who observed that under tropical environment stored products are more susceptible to *A. flavus* which grows better than other fungi. Many *Aspergilli* are favoured by the multiplicity or the combination of low water activity and relatively high storage temperatures. It is reported by Miller, (1991), that *A. flavus* is common and widely spread in nature and it is often found in areas where certain grains are grown under hash environmental or stressful condition, with insects, birds as well as, biotic and abiotic factors. He also stressed, that mould is found widely on inadequately dried food or grain in the subtropical and tropical climate throughout the world.

The sellers in the six markets, had not heard about aflatoxins by either the following means media of communication through the local radio, TV, newspaper, by neighbours, buyers or traders, and as well as, extension officer or others sources. This contradiction findings of Narrod, Roye, Mahaku, DeGroote and Tionge., (2012), who reported that 14.7% of their respondents in Kenya had information on aflatoxins, through local radio, 48.% by extension officer, 2.8%, neighbor, 2.8%, TV 2.2%, newspaper, 0.6%, buyers or traders, 0.2% and other sources 2.8%. The authors also reported that 39.3% of the farmers did not know about aflatoxin. In this study 100% of the respondants had had no information on aflatoxin at all. This could be due to the fact that there has been no outbreak of aflatoxins or aflatoxicosis cases and the lack of adequate awareness on the part of the blame government or essential agencies whilst, the opposite holds true for Kenya.

From the findings of the study all the respondents mentioned they do not dry groundnut seeds after purchased have been made. It is reported by Areke, (2010) that it is very critical to control aflatoxins but control stations often do not exist at the pre-harvest level or on farms. After harvesting these critical control stations, may be well identified for aflatoxins produced by fungi during drying and storage. For instance, the critical control stations could be at the end of drying activity and one limit would be the moisture content or water activity since groundnut is an oily seed.

It was found in this work that, 21.7% of the sellers detected mud on the groundnuts and whilst other inert materials or contaminants were also encountered in the groundnut samples. This finding is similar to Jacobnsen, Coppock and Mostrom, (1998), who reported that dusts were collected from Georgia near a combine harvester which was heavily infested with aflatoxins with content ranging from 2,030 ppb-41,200 ppb. It was also shown that dusts on the elevator contained maize seeds that were contaminated with aflatoxins ranging from 621 ppb-1480 ppb. The findings showed that aflatoxins are produced when dust is present in the grain. This could be the link for contamination in the sample from the markets.

In stated in the work, 38.3% bugs, mud 33.4% and other includes pebbles 46.7%, shells 73.3%, and were found on the groundnuts as mentioned by the respondents. It is in strong agreement with Neergaard, (1977), who reported that seeds may be contaminated by spores, galls or fruiting bodies and plant residues. This could be the reason why there were lots of fungal organisms or contaminants on the samples.

The respondents mentioned that, they can purchase the groundnut seeds unshelled from all the purchasing sites. It has been reported by Okello *et al.*, (2010a), that storing groundnuts in shells or pods is more recommended because shells offer protection against mould infection. They also indicated that when groundnuts are stored as kernel or in shelled form, the groundnut deteriorate very fast, for they pick-up moisture and are easily invaded by moulds, insects, rodents and other contaminants.

In this study, 23.3% groundnuts were stored in metal containers, 31.7% in the market and 45.0% in shops. These three structures did not meet best storage facility due to high temperature, in the storage as well as, the lack of ventilation. This finding is in sharp contrast of the reports of Okelle *et al.*, (2010a), who stated that most parts of Africa have traditional means of crop storage that are not yet improved as evidence by the storage structures. Whether traditional or modern structures, these should maintain on even, cool and dry internal atmosphere free from contaminants. Authors also reported that groundnut and their products may become contaminated with mycotoxins from transportation vans, storage facilities and market locality.

Fungal Organisms on Groundnut Samples from Six Markets in the Central Region

The position of storage fungi in quality of seed or the deterioration of grain have been assessed (Christensen & Lopez, 1963); Christensen and Kaufmannn, (1969). During the examination of the groundnut samples using the blotter method, there were mould, dust, plant debris, weed seeds, broken seeds and insects found in the samples. In the blotter method of this study,

there were different types of fungi from a wide range of genera that were observed on all the groundnut samples collected from the six (6) major markets in the Central Region.

The results showed that six different fungi that were encountered, on the groundnut samples. The six were: *A. flavus, A. niger, Rhizopus* spp., *Penicullum* spp. *A. flavus* ranged the highest infections counts and *Rhizopus* spp. ranged the second highest. Whilst, *B. theobromae*, *M. phaseolin*a were the fifth and the sixth ranked highest infections found on the groundnut samples.

The high contaminations of *Rhizopus* spp. encountered confirms to the work done by Danquah, (1973), who also reported a high contamination of *Rhizopus* spp. in seed samples examined. However, the result obtained for *Penicillium* spp. is in sharp contract with work done by Danquah, (1973), who reported a low contamination of *Penicillium* spp.

The results here also show low infection of *Macrophomina phaseolina* and *Botryodiplodia theobromae* in all the district markets. There were fungi infections on the samples observed from this study like *Macrophamina phaseolina,* similar, result was obtained by Danquah, (1973).

Worked on Bambara groundnut (*Voandzeia subterranean*) Danquah, (1973*)* reported of encountering fungi, *A. flavus, A. niger, Rhizopus* spp*, Penicillum* spp*, Tricothecium mroseum, M. phaseolina*, and *Curvularia lunata* as fungal contaminants.

The result also provides very interesting and useful information on seed borne-fungi of groundnut samples as the thematic crop of this study. In this work it was found that *A. flavus* ranked 55% followed by *Rhizopus* 60% to

65%. Whereas, *A. niger* ranked third with 45% to 55% *Penicillium* ranked 25% to 35%. Danquah (1973) also found *A. flavus, Penicililum* spp. on most of the seeds with *M. phaseolina* which are known fungi on leguminous plants Ashanti Region. Mohana and Reveesha (2007), reported that the species of *Aspergillus* (35-100%), *Penicillium* (13-72%) and *Rhizopus* (32-40%) were present in high percentages, associated with sorghum, maize and rice seeds. These findings were contrary to this study in term of the percentages, but it could be due the different crops used, and the environmental effect.

A. Flavus Isolates from Groundnut Samples based on Culture and Morphological Characteristics

There was no diversity observed on the *A. flauvs* radial mycelia growth in length. It is shown that all *A. flavus* pure isolates when inoculated on the un-amended PDA; began to grow rapidly and increase in mycelia radius growth over time. There were a total of 24 *A. flavus* isolates obtained from the six markets district in Central Region. Mycelia growth of *A. flavus* on groundnut samples was 8.5 radial growths in length. Radial growth of A. flavus of groundnut from Fosu market and the rest of the district markets recorded 8.0 cm each after sixth (6) day.

Cultural and morphological characteristics of all the *A. flavus* isolates samples were observed keenly under the Compound microscope. They were all yellowish-green becoming green with age and time. These reports are in confirmation with the findings of Gourama and Bullerman (1995), who stated that *A. flavus* colonies are initially yellow, turning to yellow-green or olive green and appearing dark green with smooth shape and some having radial

wrinkles.

The *A. flavus* colony diameters were noticed to increase with time in the incubation period. The result revealed the growth to be physiological, agent of the change in size of growing cells of the *A. flavus* mycelia, population of the cells, tissues, organs of the organism (Moore, 1979). In fact, it is represented increase growth of the fungal organism (Cochrane, 1958). *A. flavus* radial growth pattern is similar to that reported by Cochrane (1958). The authors reported the linear radial growth of the fungal organism on the PDA surface. The authors stated that the constant radial growth rate was held in tight unless poison or toxic metabolites buildup. Cochrane (1958) reported that, the radial growth pattern of *A. flavus* fungi was unrestricted or uncontrolled.

Furthermore, the result indicated that the morphological basis of the radial growth development of the fungal occurred only at the tips of the hyphal. However, the radial growth was significantly different between the fungal organism isolates. The mean radial colony diameters could be attributed to the nature or the way of inoculums, and the radial growth media being used. This is in agreement with the findings of Sampson *et al.,* (1995) and Bediako *et al.,* (2002). Sampson (1995) indicated that it is very vital to culture fungal organism species on the best or appropriate growth media to be able to achieve the typical radial growth and sporulation. For instance, it was reported by Cochrane, (1958), that the mycelia growth colonial morphology showed differences with the species even within the genus and with the medium. Cochrane (1958) elucidated that the radial growth is more rapid with a heavy than small light inoculums. The report stressed that large inoculums of

the isolates may be required in order to starts growth on an unfavorable media. Most of the isolates had higher but different radial mycelia growth rates. On the sixth day the mycelia growth covered the entire Petri-dishe. It is indicative that some to the *A. flavus* isolates time of growth rate vary with time medium. This is in contrast to Lilly and Barnett (1969), who reported that time, was a function for spore germination, growth and subsequently infections of the host.

In Vitro Test of the effect of Botanical Disinfectants on the Growth of *A. flavus* Fungal Isolates

In vitro test of the effect of plant extract disinfectants on the growth of *A. flavus* fungal isolates

This study revealed that significantly the highest mean was 90.0 *A. flavus* mycelia growth of garlic. The second highest mean 80.5 inhibition of *A. flavus* was attained by neem and followed by Benlate 75.8 and mahogany appeared significantly inferior inhibition with a mean of 49.9. Concentration 15 of garlic and neem were not significantly different whilst ginger, Benlate and mahogany shows significant differences inhibiting mycelia growth of *A. flavus.* Though, at concentration 20 there were no significant differences among garlic, ginger, neem and Benlate except mahogany. Mallmoud *et al.* (1999) reported that neem leaf extract at 5%, inhibited growth of *A. flavus.* It was highly sensitive to antifungicidal properties at all concentrations. It followed definite pattern, the higher the concentrations more effective it becomes. *A. flavus* mycelia growth was significantly inhibited by garlic. This

is similar to the finding by Sultana *et al. (*2012), who indicated that it could be due to antifungal, possible toxic compounds properties embedded in the garlic.

Benlate significantly inhibited and slowed the growth of *A. flavus* mycelia growth *in vitro.* This could be due to its fungicidal properties. Benlate is known as a systematic fungicide, is used to normally disinfect seeds and for plant propagation materials against pathogens (Heitefuss, 1989). It is in line with the report of Fry (1982), that benomyl is a systemic fungicide and effective against Ascomycetes. It could be probably due to the fungicidal properties or the fungistatic action of the neem leaf extract as has been observed by Singh and Singh, (1980). Whereas, the aqueous neem leaf extract has been reported to inhibited at various levels, *A. niger* and *A. flavus* in groundnut Bansal and Sobti, (1993). *A. flavus* was inhibited on the PDA amended with all three levels of neem extract indicating that the fungal organism is intolerant or insensitive to the highest concentraction of aqueous neem leaf extract. A similar, observation was made by Bhatnager, Zeringue and MaCormmick, (1990), who noticed that neem leaf extract did not inhibit vegetative growth of *A. flavus in vitro* at all concentration (100) percent. The stimulative effect of neem may be due to the sporulation of *A. flavus*, it could be due to the nutrients given by the neem leaf extract as reported by Singh and Singh (1980). Other researchers have also indicated the ability of neem to stimulate the growth of fungi reported by Singh and Pandey (1967), *Penecillium aphanidermatum* was stimulated to grow in natural soil amended with neem oil cake reported by Rao and Salam (1976).

Aflatoxins Quantity and Types in the Groundnut Samples

Aflatoxins cannot be seen with the eyes or determined by flavour in any contaminated crops that are susceptible to the pathogens that produces them. The conventional method used is HPLC for the detection, levels and types of Aflatoxins. The result of the current project shows low concentrations of aflatoxins which in actual fact represent no health risk for the population in the study areas, the level of aflatoxins are all below the tolerance level set by the Ghana Export Promotion Authority and other institutions (GEPA, 2015).

The four major types of aflatoxins examined were B1, B2, G1 and G2. These four major types of aflatoxins have been indicated by FAO, (2002a) as very important aflatoxins that have direct contamination of grains. The four type's AFT contamination has also been reported by Mazahen (2007).

The maximum level of aflatoxin in foodstuffs in Kenya and Uganda is 10 ppb which is the maximum tolerable limit for all groundnuts (all products). In USA and India 20 ppb, 30 ppb, respectively, are their aflatoxins threshold levels for mastication in groundnuts (all products) as reported by Okello, *et al.* (2010b). However, Ghana aflatoxin tolerable level is 15 ppb in raw shelled groundnuts and 20 ppb of aflatoxin in groundnut products, by Ghana Bureau of Standards, Florkowski and Kolavalli (2012).

On the other hand, the highest concentrations of aflatoxins were below those reported by Jacobsen (1998), fall under permissible level. Similarly reports by (Ahmad & Ahmad, 1995; Fufa & Urga, 1996; Reddy, Reddy & Bagasra, 2001; Bircan, 2005).

Moreover, no animal species is resistant to acute toxin effects of aflatoxins (Williams, *et al.*, 2004). It was observed in this study the sellers

noticed all kinds of inert materials, contaminants including dust on the groundnuts. It was reported that aflatoxins produced in other stored cereal grains were seen as “grain dust”. This could be at the reason why these findings also indicated some levels of aflatoxins across the six markets. There are many contributions about minimising aflatoxins detoxification by using dietary clay and isothermal adsorption of aflatoxin content as recorded by Grant and Phillips (1998), Phillips (1999).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The study has revealed the following findings:

1. All the respondent groundnut sellers were females (100%), mainly with no formal education (35%) and basic education (primary 23.3%; Junior high school 26.7%) and mostly with 1 to 6 years experience in the sale of groundnuts (58.3%).
2. Most of the respondents purchase their groundnut from Techiman (38.3%) and Accra (21.7%). The groundnuts were mainly bought from farmers (68.3%) and the rest from shops (middle men) (31.7%), all in unshelled form (100%).
3. Majority of the respondents (76.7%) transport their groundnuts in closed vans, taking between 1 to 2 days to reach selling points (markets).
4. Most sellers store their groundnuts in metal containers (46.7%), wooden boxes (21.7%) or metal boxes (18.3%). The sellers store groundnuts with other produce such as beans (81.7%), maize (61.7%), rice (50%) and millet (40%).
5. All the sellers store their groundnuts in undisinfected warehouses with no ventilation, no ceiling tile and on bare floor without pallets.

6. All the sellers have been experiencing mouldy grains in the groundnut, mainly in the dry season or both rainy and dry season. This mouldniness negatively affect the sales of the sellers. Most of the sellers (71.7%) convert the mouldy groundnut into paste whereas others dispose of them.
7. The sellers do not dry the groundnut after purchase before retailing.
8. All the respondents experience broken grains. They also experience other inert materials including weed seeds, shells and pebbles.
9. The groundnut samples from the various markets were found to be infected with six different organisms. They were: *Aspergillus flavus, Aspergillus niger, Rhizopus* spp. *Botrydioplodia theobromae, Macrophomina phaseolina* and *Penicillium* spp.
10. *A. flavus* followed by *Rhizopus* spp. were the most common fungi infecting the grains in all the markets surveyed.
11. The radial growth of all the *A. flavus* isolates from the six markets increased from day 1 up to day 6, recording radial growth of between 6 to 8.5cm.
12. The isolates were yellowish-green becoming green with age and the reverse side of the agar was Creamish-yellow and with age turned yellow.
13. There was a strong radial growth inhibition of *A. flavus* mycelia growth with the garlic and neem amended PDA amended extracts *in vitro*.
14. Mahogany extract was the least effective against *A. flavus* mycelia growth *in vitro*.

15. The higher the concentration of the botanicals the better their effectiveness against *A. flavus* growth *in vitro.*
16. Aflatoxin level of (9.1 ppb) was obtained from Cape Coast market and the lowest level of aflatoxin (1.8 ppb) was detected from the Jukwa market, although all the groundnut samples had below the aflatoxin tolerable limit for consumption. ANOVA on the levels of aflatoxin in the groundnut samples did not show significant differences ($p > 0.05$) among the different market centres.
17. All four types of aflatoxin (B1, B2, G1 and G2) were detected in majority of the groundnut samples collected from the six different markets.

Conclusions

It is indicated from the findings that all the sellers or respondents do not have any information on aflatoxins contaminations. Whilst majority of the sellers had no formal education, majority of the sellers 35% had experienced in the sale of groundnuts. Whereas, all the sellers were females and bulk of them purchased the groundnut seeds from Techiman, most of them from farmers and then transported the groundnuts in closed vans. From the results, however, it was demonstrated that there were lots of contaminants on the groundnuts and it was also stored with other foodstuffs.

From the experiment it can be concluded that *A. flavus* recorded the highest counts in all samples collected from the six markets and followed by *Rhizopus* spp. *Botryodiplodia theobromae* and *Macrophomina phaseolina* recorded the lowest counts from the six markets of the Central Region.

It can also be concluded that groundnut samples pre-treated with garlic powder obtained high efficacy on reducing most saprophytes on the samples whilst neem and mahogany used as botanical powders recorded low effectiveness in reducing saprophytes. From the result it was indicated that unamended PDA media showed the highest length in *A. flavus* radial growth.

The results of the study demonstrated that amended media with garlic were able to inhibit the growth of *A. flavus in vitro* whilst, neem and mahogany performed poorly in inhibiting the *A. flavus* growth. *A. flavus* isolates were inhibited of the growth of the radial mycelia (90.0%) of all the garlic amended plates and mahogany inhibited poorly (48.9%).

Aflatoxin contents detected in the entire groundnut samples fall below the permissible aflatoxin levels of Ghana (20 ppb) in the six markets. However, highest level of aflatoxin (9.1 ppb) was obtained from Cape Coast market and the lowest level of aflatoxin (1.8 ppb) was detected from Jukwa market. In addition, all the four types of aflatoxin (B1, B2, G1 and G2) were detected in majority of the groundnut samples.

Recommendations

Based on the findings of the research work, it is recommended that:

1. Other botanicals should be evaluated in the forms of extract to see the levels of efficacy on *Aspergillus flavus.*
2. The lack of adequate information on the importance of mycotoxins on man and animal health in Ghana, diagnostic survey of seed mycroflora is very necessary to accurately know the status of aflatoxins and other mycotoxins in the country. Blotter Seed Health Testing methods should

be used on groundnut seeds from other markets in Ghana to determine the seed health quality.

3. Further studies should be conducted through the length and breadth of Ghana to know the types and total levels of aflatoxin content in groundnut as well as other crops that can be contaminated by fungi producing aflatoxins. The prevention of aflatoxins formation in the agricultural commodities at the farm and the market levels through better management practices including seed treatment, resistant varieties of planting material, pest control, crop rotation and good pre-harvest and post-harvest techniques (drying, storage, bagging system) should be studied.
4. The possible combinations of the various disinfectants should be tried. It is so because no single disinfectant could completely inhibit the radial mycelia growth of *A. flavus* fungal organism *in vitro* (100%).
5. The groundnut sellers go down for storage should be well improved.
6. There should be a cost effective analysis of technologies on the risk of reducing strategies for all types of value chain actors as well as massive on the risks of aflatoxins.
7. Whereas, it will be more lucrative for further research to be conducted on groundnut paste.
8. National government and stakeholders should strengthen the regulatory competence, massive awareness and good infrastructures in Central Region of Ghana, to ensure food safety and improved public health. The approach should be multidisciplinary and should involve Seed

Pathologists, Toxicologists, Mycologists, Biochemists, Food Scientists and Socio Economists.

REFERENCES

Abdegaleil, S., Okamura, H., Iwagawa, T., Sato, A., Miyahara, I., Doe, M., Nakatani, M., (2001). Khayanolides rearranged phragmalin limonoid antifeedants from senegalenesis. *Tetrahedron, 57*, 119- 126.

Ahmand, I. F., & Ahmed, S. K. (1995). *Contamination of red chilli with Aflatoxin B1 in Pakistan*. Boston: Free Press

Akyaw, M., Owusu Danquah, O. A. (1977). *Safe handling to control stroage insects and fungi of seed*. Kumasi: Crop Research Institute.

Ali, M. V., Mohinddin, S. M., & Vikram, R. M. (1994). Effect of dietary aflatoxin on cell mediated immunity and serum proteins in broiler chicken. *Indian Veterinary Journal, 71,* 760-760.

Anon, (2015). *European Union supports groundnut value chain in Ghana*. Retrieved on June 21 , 2015 from www.traqueghana.org/index.php

AOAC Int. (2004). *Associate reference's manual on development, study, review, and approval process*. New York: AOAC Internal Publication.

Asare-Bediako, E. (2012). *Studies of microorganisms affecting sprouting of minisetts of white yam (Dioscorea routundata, poir) CV. Pona in Ghana*. Unpublished master of philosophy thesis submitted to the Department of Crop Science. University of Cape Coast, Cape Coast, Ghana. pp. 79

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils: A review. *Food Chem,. Toxicology, 46*, 446-475.

Bankole, S. S., & Adebanjo, A. (2003). Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. *African Journal Biotechnology, 2*, 254-63.

Bansal, R. K., & Sobti, A. K. (1993). An economy remedy for the control of two species of Aspergillus on groundnut. *Journal of Indian Phytopath*, *43*, 451-452.

Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews, 16*, 497-499.

Bennett, J. W., Leong, P. M., Kruger, S., & Keyes, D. (1986). Sclerotial and low aflatoxigenic morphological variants from haploid and diploid *Aspergillus Parasiticus*, *42*, 848- 851.

Bhanagar, D., Cotty, P. Y., & Cleveland, T. E. (2001). Genetics and biological control of aflatoxigenic fungi. In Wilson, C. L., Droby, S. (Eds) *Microbial food contamination* (pp. 208-240). Boca Raton: CRC Press.

Bhatnager, D., Zeringue, H. J., & McCormmick, S. (1990). *Neem leaf extracts inhibit aflatoxin biosynthesis in aspergillus flavus and aspergillus parasiticus.* Boca Raton: CRC Press

Biol, J. K. (2006). *Antifeedant sand toxicity effect of some plant extracts on "Thaumetopae Solitaria"* Presember: Frey Ondo Kuz Mayis University Press.

Bircan, C. (2005). The determination of aflatoxins in spices by immunoaffinity column extraction using HPLC. *International Journal of Food Science & Technology, 40*(9), 929-934.

Brahmachari, G. (2004). Neem-an omnipotent plant: Retrospection. *Chembiochem, 94*(1), 25-41.

Brown, R. L., Cotty, P. Y. Cleveland., T. E., & Widstrom, N. W. (1993). Living maize embryo influences accumulation of aflatoxin in maize kenels. *Journal of Food Protection,* 56, 967-971.

Calberg, E. J. (2008). *An economic evaluation of groundnut research in Uganda and Ghana*. Boston: Free Press.

Caliskan, S., Arslan, M., & Arioglu, H. (2008). Effects of sowing date and growth duration on growth and yield of groundnut in a Mediterranean type environment in Turkey. *Field Crop Research*, *105*, 131-140.

Cary, J. W., Klich, M. A., & Beltz, S. B. (2005). Charactersization of aflatoxin-producing fungi outside of Aspergillus section Flavi. *Journal of Mycologia*, *97*, 425-432.

Christensen, C. M. (1981). A synoptic key and evulation of species in the *aspergillus flavus* group. *Journal of Mycology, 73*, 1056-1084.

Christensen, C. M., & Kaufman, H. H. (1969). *Grain storage: The role of fungi in quality loss*. Minnesopolis: McGraw Hill.

Cochrane, W. V. (1958). *Physiology of fungi*. New York: John Wiley and Sons, Inc.

Coker, R. D. (1989). *Control of aflatoxin in groundnut products with emphasis on sampling analysis and detoxification*. New Delhi: ICRISAT.

Connif, P. (1995). *Official methods of analysis* (16th ed.). Arlington: AOVC International.

Cotty, P. (1988). Aflatoxin and sclerotial production by *Aspergillus flavus*: influence of pH. *Journal of Phytopathology*, *78*, 1250-1253.

Cotty, P. J. (1994). Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the population of *A. flavus* infection cotton bolls and on the aflatoxin content of cottonseed. *Journal of Phytopathology, 84*, 1270-1277.

Cotty, P. J. (2001). Cottonseed losses and mycotoxins. In T. L., Kirkpatrick, C. S., Rothrock, (Eds), *Compendium of cotton diseases part 1. infectious diseases* (pp. 9-13). Minnessota: The American Phytopathological Society.

Cotty, P. J., & Cardwell, K. F. (1999). Divergence of West African and North American communties of Aspergillus section Flavi. *Journal of Applied and Environmental Microbiology*, *65*, 2264-2266.

Cotty, P. J., & Jaime-garcia, R. (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contaminantion. *International Journal of Food Microbiology*, *45*, 167-173.

Cotty, P. J., Probst, C., & Jaime- Garcia, R. (1989). Virulence and cultural characteristatics of two Aspergillus flavus strains. *Journal of Phytopathology*, *79,* 808-814.

Danquah, O. A. (1973). *Survey and importance of seed-borne fungi of rice, sorghum, maize, cowpea and bambarra groundnut of Ghana.* Unpublihsed master of scicences thesis submitted to the Department of Crop Science, College of Agriculture, University of Ghana, Legon. Accra, Ghana.

Debrah, S. K., & Saliyar, F. (1996). *Groundnut production and utilization in Africa; Past Trends; Projections and opportunities for increased production*. Paper Presented at the 5th Regional Groundnut Workshop for West Africa., Accra, Ghana.

Dieneret, U. L., Cole, R. J., Sandeers, T. H., Paynes, G. A., Lee,L. S., & Klich, M. A. (1987). Epidemiology of aflatoxin formation by Aspergillus flavus. *Annual Review of Phytopathology, 25*, 246-270.

Dowd, D. L., & Groopman, J. D. (1998). Involvement of arthropods in the establishment of mycotoxigenic fungi under field conditions. In K. K., Sinha, D., Bhatnargar (Eds.), *Mycotoxins in agriculture and food safety* (pp. 307-350). New York: Marcel Dekker.

Eaton, D. L., & Groopman, J. D. (1994). *The toxicology of aflatoxins: Human health, veterinary, and agricultral significance*. San Diego: Academic Press.

FAO (2012). *Food and agricultural organization crop production statistics*. Retrieved February, 13 2013, from: http://www.fao.org/ag/agp/agpc.

FAO. (1998). *Mycotoxin preventation and control in food grain*. Rome: Food and Agricultural Organization of the United Nations.

FAO. (2002). *Evaluation of certain mycotoxins in food.*. Geneva: World Health Organization.

FAO. (2003). *World agriculture*. Retrieved on February, 13 2013 from http//www.fao.org/ag/agp/agpc/gcds/.

FAOSTAT (2010). *Groundnut world production*. Retrieved on February 13, 2013 from http://www.faostat.fao.org.

Fernandez Pinto, V., Patriarca, A., Locani, O., & Vaamonde, G. (2001). Co-occurance of aflatoxin and cyclopiazonic acid in peanuts grown in Argentina. *Food Additives and Contaminant, 18*, 1017- 1020.

Florkowski, W., & Kolavalli, S., (2012). *Aflatoxin: Quality institute in the groundnut value chain*. New York: International Food Policy Research Institute (IFPRI).

Frisvad, J. C. (1995). Mycotoxigenins and mycotoxigenic fungi in storage. In D.S., Jayas, N. D. G., White, & W. E. Nuir. (Eds), *Modern concepts in penicillium and aspergillus classification* (pp. 143-154). London: Plenum Press.

Fry, W. E. (1982). *Principals of plant disease management*. New York: Academic Press.

Fufa, H, & Urga, K. (1996). Screening of aflatoxins in shiro and ground red pepper in Addis Ababa. *Ethiopian Medical Journal*, *34*(4), 243-249.

Geiser, D. M., Pitt, J. I., & Taylor, J. W. (1998). *Cryptic speciation and recombination in the aflatoxin production fungus, Aspergillus flavus*. Proceedings of the National Academy of Sciences of the United States of America, 95, 388- 393.

Ghana Export Promotion Authority (GEPA) (2015). *Ghana Export Promotion Authority develops groundnuts training manual*. Retrieved on June 21, 2015, from http://vibeghana.com/2015/06/19/ghana-export-promotion-authority-develops-groundnuts-training-manual/.

Goto, T., Wicklow, D. T., & Ltd, Y. (1996). Aflatoxin and cyclopiazonic acid production by sclerotium-production *Aspergillus tamari* strain. *Applied and Environmental Microbiology*, *62*, 4036-4038.

Gourama, H., & Bullerman, L. B. (1995). *A. flavus* and *A. parasiticus:* Aflatoxigenic fungi of concern in food and feeds. *Journal Food Protection*, *58,* 1395- 1404.

Gregg, B. R., Gastel, A. J. G. van, Asiedu, E., Donkoh, F., & White, R. W. (1999). Good seed, extension and farmes. *WASDU, 2*, 85-89.

Guo, B., Sobolev, V., & Lynch, R. (2002). Impact of phytoalexins and lesser comstalk borer damage on resistance of aflatoxin formation. *Journal of Mycopathologia*, *14*, 23-25.

Guo, B., Yu, J., Holbrook, C. C., Cleveland, T. E., Nierman, W. C., & Scully, B. T. (2009). Strategies in prevention of preharvest aflatoxin contamination in peanuts: Aflatoxin biosynthesis, genetics and genomics. *Peanut Science*, *36*, 11-20.

Heitefuss, R. (1989). *Crop and plant protction: The practical foundation.* Chister: Ellis Horwood Ltd. .

Hell, K., Cardwell, K. F., Peohling H. M. (2003). Relationship between management practices, fungal infection and aflatoxin for stored maize in Benin. *Journal of Phytopathology*, *151,* 690-698.

Hell, K., Cardwell, K. F., Setamou, M., Peohling, H. M., (2000). The influence of stroage practices on aflatoxin on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. *Journal of Stored Product Research*, *36*, 365-382.

Hocking, A. D. (1991). Xerophillic fungi in intermediate and low moisture foods. In D. K., Arora., K. G., Mukerji, & E. H. Marth, (Eds), *Handbook of applied mycology: Foods and feeds*. New York: Marcel and Dekker.

Horn, B. W. (2007). *Biodiversity of Aspergillus section flavus in the United States*. New York: Sage.

Horn, B. W., Greene, R. L., & Dorner, J. W. (1995). *Effect of corn and peanut cultivation on soil populations of Aspergillus flavus and A. parasiticus in Southwestern Geogria*. Boston: Sage.

Horn, Greene, L. R., Sobolev, V. S., Dorner, J. W., Powell, J. H., & Layton, R. C. (1996). *Association of mycotoxins production with vegetative compartibility groups*. New York: Sage.

International Agency for Research on Cancer (IARC) (1993). *IARC monographs on the evulation of carcinogenic risk to humans*. Lyon: World Health Organization.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Production, 19*, 603-608.

ISTA (1966). *International rule for seed testing*. Wageningen: ISTA

Izge, A. U., Mohammed, Z. H., & Goni, A. (2007). Levels of variability in groundnut (*Arachis hypogaea L.*) to Cercopsora leaf spot disease-implication for selection. *African Journal of Agricultural Research, 24,* 182-186

Jacobsen, B. J., Bowen, K. L., Shelby, R. A., Diener, U. L., Kemppainen, B.W., & Floyd, J. (1993). *Mycotoxins* and *mycotoxicosis*. Retrieved on July18, 2014 from http://www.aces.edu/dept/grain/ANR-767.php.

Jacobsen, B. J., Coppock, R.W., & Mostrom, M. (1998*). Stored grain*. New York: Free Press.

Jemmali, M. (1987). *Trade and economic implication of mycotoxins: Needs for greater uniformity*. Bankok: FAO.

Jotwani, M. G., & Srivastava, K. P., (1981). Neem insecticide of the future. III-Chemistry, toxicology and future strategy. *Pesticides, 15*(12), 12-19.

Kaaya, A. N., Warren, H. L., & Adipala, E. (2000). Moulds and aflatoxin contamination of maiz and groundnuts in Mayuge and Kumi districts of Uganda. Kampala. *Muarik Bullentin*, *3*, 33-41.

Kaaya, A. N., & Warren, H. L. (2005). A review of past and present research on aflatoxin in Uganda. *African Journal of Food, Agriculture, Nutritution and Department, 5,* 69-76.

Kaaya, A. N., Eigal, W., & Harris, C (2006). Peanut aflatoxin levels on farms and markets of Uganda. *Peanut Science, 33*, 68-75.

Kaaya, A. N., Kyamuhangire, W., & Kyamanywa, S. (2007). Factors affecting aflatoxin contamination of harvested maize in the three agroecological zones of Uganda. *Journal of Applied Sciences*, *6*, 2401-20407.

Karthikeyan, A. (1996). Effect of organic amendments, antogonist Trichoderma Viride and fungicides on seed collar rot of groundnut. *Plant Disease Research, 11*,72-74.

Klich, M. A. (1998). Soils fungi of some low-altitude desert cotton fields and ability of their extracts to inhabit *Aspergillus flavus*. *Mycopathologia*. *124*, 197-100.

Klich, M. A., & Pitt, J. I. (1988). Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. *Transactions of the British Mycological Society*, *91*, 99-108.

Klich, M. A., Mullaney, E. J., Daly, C. B., & Cary, J. W.,. (2000). Molecular and physiological aspects of aflatoxin and sterigmatocystin

biosynthesis by *Aspergillus tamarii* and A. ochraceoroseus. *Applied and Environmental Microbiology, 53*, 605-609.

Lilly, G. V., & Barnett, H. L. (1951). *Physiology of fungi*. New York: McGraw-Hill Book Co. Inc.

Lopez, A. & Crawford, M. A. (1967). Aflatoxin aontent of peanuts sold for human consumption in Uganda. *Kampala Lancent, 2,* 1351-1354.

Mathur, S. B., & Kongsdal, O. (2001). *Common laboratory seed health testing methods for detecting fungi*. Frederksberg: Sage

Mazahen, M. (2007). Determination of aflatoxins in imported rice to Iran. *Food Chem Toxicol, 47*, 2064-66.

McApin, C. E., Wicklow, T. D., & Horn, B. W. (2002). DNA fingerprinting analysis of vegetative compatibility groups in *Aspergillus flavus* from a peanut field in Georgia. *Plant Disease, 86*, 254-8.

Miler, J. D. (1991). *Significance of mycocotoxins for health and nutrition*. New York: McGraw Hill.

Miller, J. D. (1991). *Significance of mycotoxins for health and nutrition.* ACIAR Proceedings No. 36, Australian Centre for International Agricultural Research.

MOFA-SRID (2013). *Agriculture in Ghana: Facts and figures*. Accra: Statistics Research and Information Distractrate (SRID).

Moore, T. C. (1979). *Biochemistry and physiology of plant hormones*. New York: Springerverlag.

Narrod, C. D., Roy, G., Mahaku, H., DeGroote, M., & Tiongc, D. (2012). *Exploring the scope of cost-effective aflatoxin rick reduction strategies in mazie value chains so as to improve market access of the poor in Kenya*. New York: Sage

Neergaard, P. (1979). *Seed pathology*. Copenhagen: Macmillan Press.

Neiger, R. D., Jonhson, T. J., Hurley, D. J., Higgies, K. F., Rottinghaus, G. E., & Stahr, H. (1994). The short-term effect of low concentrations of dietary aflatoxin and T-2 toxin on mallard duckings. *Avian Diseases*. *38*, 738-738.

Nutsugah, S. K., Oti-Oboateng, C., Tsigbey, F.K., & Brandenburg, R. L. (2007b). Assessement of early losses due to early and late leaf spots of groundnut (*Arachis hypogaea L.*) *Ghana Journal of Agricultural Science, 40*, 21-26.

Odogola, W. R. (1994). *Postharvest management and strorage of food legumes*. Harare: UNDP/OPS Regional Programme.

Okello, D. K., Biruma, M., & Deom, C. M. . (2010). Overview of groundnuts research in Uganda: Past, present and future *African Journal of Biotechnology*, *9*, 6448-6459.

Okuda, T., Klich, M. A, Selfert, K., & Ando, K. (2000). *Intergration of modem taxonomic methods for penicillum and aspergillus clasification*. Reading: Hardwood Academic Punlishers

Otsuki, S., Wilson, J. S., & Sewadeh, M. (2001). What price precaution? European harmonisation of aflatoxin regulations and African Groundnut Exports. *European Review of Agricultural Economics*, *28,* 263-284.

Oyebanj, I . A. O, & Efluvwevwere, B. J. O. (1999). Growth of spoilage mould and aflatoxin B production in naturally contaminated or artificially inoculated maize as influenced by moisture content under ambient tropical condition. *Biodegradation, 44*, 209-217.

Palmgren, M. S., & Hayes, A. W. (1987). *Aflatoxins in food*. New York: Academic Press.

Pande, N., Saxena, J., & Pandey, H. (2003). Natural occurance of mycotoxins in some cereals. *Mycoses*, *33*, 126-128.

Payne, G. P., & Brown, M. P. (1998). Genetics and physiology of aflatoxin biosynthesis. *Annual Review of Phytopathology*, *36*, 329-362.

Pestka, J. J., & Bondy, G. S. (1994). *Mycotoxin-induced immune modulation.* New York: Raven Press.

Peterson, S.W., Ito, Y., Horn, B. W., Goto, T. . (2001). *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*. *Mycologia*, *93*, 689-703.

Pildain, M. B., Frisvad, J. C., Vaamonde, G., Cabral, D., Vargga, J., & Samson, R. A. (2008). Two novel aflatoxin-producing Aspergillus species from Argentinean peanuts *International Journal of Systemaatic and Evolutionary Microbiology,* 58, 725.

Pitt, J. I., & Hocking, A. D. (1997). *Fungi and food spoilage* (2nd ed.). London: Blackie Academic and professional.

Radwanski, S. (1977). Neem tree 3: Further uses and potential uses. *World Crops and Livestock, 29*, 168-169.

Rajahmundry, A. P. (1993). *World neem conference*. New Delhi: Indian Society of Tobacco Sciences.

Rao, P. N. & SALAM, M. A. (1954). Curvularia specis from discoloured grains from Hyderabad. *Journal Indian Botany of Science, 33*, 268-271.

Raper, K. B., Fennell, D. I., (1965). *The genus Aspergillus*. Baltimore: Williams and Wilkins.

Reddy, S., Reddy, T., Andl, A., Bagasra, M. M., Lu, D. J., Epstein, E. E., Morrisey, S., & Millar. E. (2001). *Characterization of wnt gene expression in developing and postnatal hair follices and identification of Wnt5a as a target of Sonic*. London: Sage.

Rodriguez, K. R., Morgan, J. G., & Chet, I., (1987). Biological control of namatodes: Soil amendments and microbial antaonists. *Plant Soil, 100*, 237-247.

Roger, G. D. P. (2003). *New healthy foods*. Madrid: Marpa Arts Graficas.

Rollins, J. A., & Dickman, M. A. (1998). Increase in endogenous cyclic AMP levels inhabits sclerotial development in *Sclerotinia clerotiorum*. *Applied and Environmental Microbiology, 64*, 2539-2544.

Rosolem, C. A., Fernandez, E. M., Maringoni, A. C., and Oliveira, D. M. T. (1997). Fungal incidence on peanut grains as affected by drying method and Ca nutrition. *Field Crops Research,* 52, 9-5.

Saito, M., Tsuruta, O. (1993). A new variety of *A. flavus* from tropical soil in Thailand and its aflatoxin productivity. *Association of Mycotoxicology, 37*, 31-36.

Saito, M., Tsuruta, O., Siriacha, P., Kawasugi, S., Manabe, M., Buangsuwan, D. (1986). Distribution and aflatoxin productivity of the atypical

strains of *Aspergillus flavus* isolated from soils in Thailand. Proceedings of Japan Association of *Mycotoxicology*, *24*, 41-46.

Sampson, R. A., Hoekstra, E. S., Frisvad, J. C., & Flitenborge, O. (1995). *Introduction to food-borne* (4th ed.). Wageningen: Centraabureau voor Schimmel Cultures..

Sauer, D. B., & Tuite, J. (1987). *Condition that affect the growth of Aspergillus flavus and production of aflatoxin in stored maize*. Boston: Free Press.

Sauer, D. B., Meronuck, R. A.& Christensen, C. M. (1992). Micoflora. In Sauer, D. B. (Eds). *Storage of cereal grains and their products*. St. Paul: American Association of Cereal Chemists.

Schmutterer, H. (1990). Posterities and potential of natural pesticides from the neem tree, Azadirachta inidica. *Annu. Rev. Entimol., 35*, 271- 297.

Scott, P. M. (1991). *Methods of analysis for mycotoxins: An overview*. London: Elsevier Applied Science.

Shovan, K., Majumder, Mathew, D., Killer, Fouad, I., Boulos, Mark, C., Kelley, A., & Mahadevan-Jansen. L. (2008). *Comparsion of auto flluorescence, diffuse reflectance, and Roman spectroscopy for breast tissue discrimination*. New York: McGraw Hill.

Singeh, K., Frisvad, C., Jeas, T. U., & Mathur, S. B. (1991). *An illustrated manuel on identification of some seed-borne aspergillus, fusarian, penicillia and their mycotoxins*. Ryvangs: Danish Government Institute of Seed Pathology for Developing Countries.

Singh U. P, H. B. & Singh, R. B. (1980). The fungicidal effect of neem (*Azadirachta indica*) extracts on some soil-borne pathogens of germ (*Ciser arietinum*). *Mycologia, 72,* 1077-1093.

Singh, D. K., & Yadava, D. S. (1992). Production potential and economics of cowpea based intercropping systems under rainfed condition. *Indian Journal of Agronomy*, *37*, 424-429.

Singh, R. S. & K. R. (1967). Population dynamics of pythum *aphanidermatum* in oil cake amended soils. *Canada Journal of Mocrobiology, 13*, 601-610.

Smart, M. G., Wicklow, D. T., Caldwell, R. W., (1990). *Pathogenesis of Aspergillu ear rot of maize: Light microscopy of fungal spread from wounds*. New York: McGraw Hill.

Smart, M. G., Shotwell, O. L, & Caldwell, R. W (1990). Pathogenesis Aspergillus ear rot of maize: aflatoxin B1 levels in grains around wound inoculation sites. *Phytopathology*, *80*, 1283-1286.

Smith, J. E., & Moss, M. O. (1985). *Mycotoxins: Formation, analysis and significance*. Chichester: John Wiley & Sons.

Squire, R. A. (1981). Ranking animals carcinogens: A proposed regulatory approach. *Science, 214*, 877-880.

St. Leger, R. J., Screen, S. E., & Shamps-pirzadeh, B. (2000). Lack of host spercialization in Aspergillus flavus. *Applied and Environmental Microbiology*, *66*, 320-324.

Sundar, A. R., & Ishnaveni, D. (1995). In-vitro Antagonism of Trichderma spp. against t::O fungal pathogens of Cans. *Indian Journal of Plant Protection*, *232*, 152-155.

Tsigbey, F. K., Brandenburg, R. L., & Clottey, V. A. (2004). *Peanut production methods in Northern Ghana and some disease perspectives.* Accra: Publishers-Cp- Wood.

Tweneboah, C. K. (2000). *Modern agriculture in the tropics wirh special reference to Ghana.* Accra: Publishers-Cp- Wood.

Upadhyaya, H. D., Reddy, L. J., Gowda, C. L. L, & Singh, S. (2006). Identification of diverse groundnut germplasm: Sources of early maturity in a coer collection. *Field Crop Research, 97*, 261-271.

Waele, D. & Swanevelder, C. J. (2001). *Crop production in the tropical Africa*. Belgium: Goikink.

Waliyar, F., Ntare, B. R., Diallo, A. T., Kodio, O., & Diarra, B. (2007). *Onfarm management of Aflatoxin contaminantion of groundnut in West Africa: A synthesis report*. New York: International Crops Research Institute for the Semi-Arid Tropics.

Wicklow, D. T., & Shotwell, O. L., (1983). Intrafungal distribution of Aspergillus flavus and Aspergillus parasiticus. *Canadian Journal of Microbiology*, *29*, 1-5.

Wild, C. P., & Turner, P. C., (2002). The toxicology of aflatoxins as basis for public health decisions. *Mutagenesis, 17*, 471-481.

Williams, J. H., T. D. Jolly, P. Styles, J. K. Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and intervations. *American Journal of Clinical Nutrition*, *80*, 1106-1122.

World Book of Encyclopedia (1990). *World book of encyclopedia*. New York: Author

Yu, J., Cleveland, T. E., Nierman, W. C., & Bennett, J. W., (2005). *Aspergillus flavus* genomics: Gateway to human and animals health, food safety, and crop reisistance to diseases. *Revita Lberomericana De Micologia, 22*, 194-202.

APPENDICES

APPENDIX: A

Questionnaries for field survey

University of Cape Coast

School of Agriculture

Department of Crop Science

STUDIES OF *Aspergillus flavus* AND AFLATOXIN CONTAMINANTS OF GROUNDNUT (*Arachis hypogaea L.*) FROM SIX MARKETS IN THE CENTRAL REGION, GHANA.

Interview of seller's on groundnut storage, source and seed storage material in six markets of the Central Region, Ghana.

This research seeks to determine the way groundnut seed has been purchased by seller's in the Central Region. It also wishes to seek seller's level of knowledge adhering to best practices in groundnut sale and storage management. The purpose of the study is purely academic (as part of a Master of Philosophy/ M.PHIL. study at UCC) and does not in any way attempt to invade seller's privacy. You are highly assured that all the information provided will be treated confidentially. Kindly respond as honestly as possible.

Identification of the seller's/respondents

1. Respondent category: Seller [ ]
2. Town/ village/ market:----------
3. Name of district:----------

SECTION ONE: Social demographic characteristics of seller's respondent.

Please provide responses which best describes your situation. Tick [√]. In the boxes or write on the spaces provided.

4. Sex : M [ ] F [ ]

5. What is your level of education?

1. [ ] No formal education 2. [ ] Primary 3. [ ] JHS 4. [ ] SHS/TECH/VOC 5.[ ] Tertiary

SECTION TWO A: seller's knowledge on best practices of groundnut management against fungal contaminants.

6. Do you know of any contaminant on groundnut? 1. [ ] Yes 2. [ ] No

7. If 'yes', please describe it:

_____

8. Where do you notice the contaminants most? 1. [ ] Purchasing storage site2. [ ] Sale storage

Site

9. Do you see mould on groundnut during purchasing? 1. [ ] Yes 2. [ ] No

10. What time of the year do you notice more moulds on the groundnut? 1. [ ] Rainy season 2. [ ] Dry season 3. [ ] Both seasons

11. Consumers are concerned about moulds in your groundnut? 1. [ ] Yes 2. [ ] No

12. Does the presence of moulds affect the sale of your groundnut? 1. [ ] Yes 2. [ ] No

13. If yes, how? Please specify:

_____

14. Do you treat the groundnut? 1. [ ] Yes2. [ ] No

15. If 'yes', what type of chemical?

_____

16. If no why?

_____

17. Where do you store your groundnut?

_____

18. What other produce do you store the groundnut with?

_____

19. Do you spray the storage house? 1. [ ]Yes 2. [ ] No

20. If 'yes', what is the name of the chemical used?

_____

21. What is the condition of your storage house?

_____

22. Do you get any technical advice from any expert on groundnut seed management/sale? 1. [ ] Yes 2. [ ] No

23. If 'yes', what type of technical advice?

_____

24. From whom do you get the advice?

_____

SECTION THREE B: Seller's ways of obtaining groundnut seed/grain, preservation mechanisms.

25. Where do you purchase groundnut seed from?

_____

26. What type of seed do you buy?

1. [ ] Farmer saved seed 2. [ ] Own farm 3. [ ] Others (please specify):_____

27. How do the groundnut seeds look during purchasing? 1. [ ] pod 2. [ ] Shelled

28. How do you transport the groundnut after purchasing? 1. [ ] Open van 2. [ ] Closed van 3. [ ] Other: (please specify):

_____

29. If in an open van, what time of the day do you transport the groundnut?

1. [ ] Morning

2. [ ] Afternoon 3. [ ] Night

30. Do you dry the groundnut? 1. [ ] Yes 2. [ ] No

31. If yes, how? 1. [ ] Sun drying 2. [ ] Air drying3. [ ] Other (please specify):

_____

32. What material do you store your groundnut seed/grain in?

1. [ ] Jute/bag 2. [ ] Container 3. [ ] Basket 4. [ ] None 5. Other: (please specify)

_____

33. Where do you dry the groundnut seed/grain? 1. [ ] Drying floor 2. [ ] Attic 3. [ ] Others (please specify):

_____

34. Do you place the groundnut on pallet in the warehouse? 1. [ ] No

2. [ ] Yes

35. Do you see any inert materials (mud, weed seed, dead insect, pebble, shell) in the groundnut when purchased? 1. [ ] Yes 2. [ ] No

36. How long does it take before the groundnut reaches at your sale point?

1. [ ] One/two days 2. [ ] Three/four days 3. [ ] Five/six days

37. How long will it take for consumers to buy your entire groundnut?

1. [ ] One/ two weeks 2. [ ] three/ four weeks 3. [ ] five/ six weeks

4. [ ] Unknown

38. Do you travel to other market to sell? 1. [ ] Yes 2. [ ] No

39. Do you notice broken groundnut seeds? 1. [ ]Yes 2. [ ] No

SECTION FOUR: Sources of information on aflatoxins.

40. Have you heard about aflatoxin on local radio? 1. [ ] Yes 2. [ ] No

41. Have you heard about aflatoxin on international radio? 1. [ ] Yes

2. [ ] No

42. Have you heard about aflatoxin from Extension Officer? 1. [ ] Yes

2. [ ] No

43. Have you heard about aflatoxin from Health Worker? 1. [ ] Yes

2. [ ] No

44. Have you heard about aflatoxin from neighbor? 1. [ ] Yes 2. [ ] No

45. Have you heard about aflatoxin from newspaper? 1. [ ] Yes 2. [ ] No

46. Have you heard about aflatoxin from trader/ buyer? 1. [ ] Yes

2. [ ] No

47. Have you heard about aflatoxin from other sources? 1. [ ] Yes

2. [ ] No

48. Have you heard about aflatoxin from TV? 1. [ ] Yes 2. [ ] No.

49. What type of storage structure do you used? 1. [ ] Metal container 2.

[ ] Metal table container 3. [ ] Wooden table box 4. Other (open market)

APPENDIX: B

Percent inhibition of mycelia growth of *Aspergillus flavus*

Summary

Analysis of variance

Transformed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	10635.92	2658.98	28.38	<.001
Treatment.Conc_Mls	10	4972.03	497.20	5.31	<.001

APPENDIX 11

Residual	45	4215.87	93.69		
Total	59	19823.82			

Tables of means

Variate: transformed

Grand mean 73.6

Treatmen	Benlate	Garli	Ginger	Mahogan	Neem
	75.8	90.0	71.9	49.9	80.5

Standard errors of differences of means

Table	Treatment	Treatment Conc_Mls
rep.	12	4
d.f.	45	45
s.e.d.	3.95	6.84

Least significant differences of means (5% level)

Table	Treatment	Treatment Conc_Mls
rep.	12	4
d.f.	45	45
l.s.d.	7.96	13.78

Analysis of variance

Variate: transformed

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	10635.92	2658.98	28.38	<.001
Treatment.Conc_Mls	10	4972.03	497.20	5.31	<.001

Residual	45	4215.87	93.69
Total	59	19823.82	

Tables of residuals

Variate: transformed

APPENDIX 12

Summary of (ANOVA) table

The quantity of aflatoxin in the groundnut samples from the six market centres

Units residuals, s.e. 8.38, rep. 1

units	1	2	3	4	5	6	7
	-9.2	-9.2	9.2	9.2	-3.2	-3.2	-3.2
units	8	9	10	11	12	13	14
	9.7	-3.2	-3.2	9.7	-3.2	0.0	0.0
units	15	16	17	18	19	20	21
	0.0	0.0	0.0	0.0	0.0	0.0	0.0
units	22	23	24	25	26	27	28
	0.0	0.0	0.0	-3.0	9.9	-16.7	9.9
units	29	30	31	32	33	34	35
	-10.6	-7.7	-10.6	28.9	0.0	0.0	0.0
units	36	37	38	39	40	41	42
	0.0	-14.3	-16.1	19.2	11.1	-1.6	13.7
units	43	44	45	46	47	48	49
	-13.3	1.3	-2.2	13.1	-9.7	-1.1	3.9
units	50	51	52	53	54	55	56
	-2.4	-7.2	5.7	-8.0	-4.9	2.3	10.5

units	57	58	59	60
	-0.2	-7.4	-0.2	7.9

APPENDIX 13

Effects of plant extracts on inhibition of *A. flavus*

Tables of means

transformed

Grand mean 73.6

Treatment	Benlate	Garlic	Ginger	Mahogany	Neem
	75.8	90.0	71.9	49.9	80.5

Standard errors of differences of means

Table	Treatment	Treatment Conc_Mls
rep.	12	4
d.f.	45	45
s.e.d.	3.95	6.84

Least significant differences of means (5% level)

Table	Treatment	Treatment Conc_Mls
rep.	12	4
d.f.	45	45
l.s.d.	7.96	13.78

APPENDIX: C

APPENDIX 15

Summary of (ANOVA) table

		Sum of Squares	df	Mean Square	F	Sig.
Aflatoxin (Swedru)	Between Groups	17076.707	3	5692.236	4226.725	.000
	Within Groups	5.387	4	1.347		
	Total	17082.094	7			
Aflatoxin (Mankessim)	Between Groups	99654.015	3	33218.005	29680.796	.000
	Within Groups	4.477	4	1.119		
	Total	99658.492	7			
Aflatoxin (Kasoa)	Between Groups	63145.902	3	21048.634	93027.497	.000
	Within Groups	.905	4	.226		
	Total	63146.807	7			
Aflatoxin (Jukwu)	Between Groups	69.938	3	23.313	1428.037	.000
	Within Groups	.065	4	.016		

	APP16					
	Total	70.003	7			
Aflatoxin (Fosu)	Between Groups	31210.899	3	10403.633	97184.800	.000
	Within Groups	.428	4	.107		
	Total	31211.327	7			
Aflatoxin (Cape Coast)	Between Groups	5752.695	3	1917.565	2013.799	.000
	Within Groups	3.809	4	.952		
	Total	5756.504	7			

Multiple Comparisons

Tukey HSD

						95% Confidence Interval	
Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Aflatoxin (Swedru)	Market 1	Market 2	46.32000*	1.16048	.000	41.5958	51.0442
		Market 3	117.84500*	1.16048	.000	113.1208	122.5692
		Market 4	99.27500*	1.16048	.000	94.5508	103.9992
	Market	Market	-	1.160	.000	-51.04	-41.59

		APP17					
		1	46.32000*	48		42	58
	2	Market 3	71.52500*	1.160 48	.000	66.80 08	76.24 92
		Market 4	52.95500*	1.160 48	.000	48.23 08	57.67 92
	Market 3	Market 1	-117.8450 0*	1.160 48	.000	-122.5 692	-113.1 208
		Market 2	-71.52500*	1.160 48	.000	-76.24 92	-66.80 08
		Market 4	-18.57000*	1.160 48	.000	-23.29 42	-13.84 58
	Market 4	Market 1	-99.27500*	1.160 48	.000	-103.9 992	-94.55 08
		Market 2	-52.95500*	1.160 48	.000	-57.67 92	-48.23 08
		Market 3	18.57000*	1.160 48	.000	13.84 58	23.29 42
Aflatoxin (Mankessi m)	Market 1	Market 2	247.9850 0*	1.057 91	.000	243.6 784	252.2 916
		Market 3	261.2200 0*	1.057 91	.000	256.9 134	265.5 266
		Market 4	263.0050 0*	1.057 91	.000	258.6 984	267.3 116
	Market 2	Market 1	-247.9850 0*	1.057 91	.000	-252.2 916	-243.6 784

		APP18					
		Market 3	13.23500*	1.057 91	..001	8.928 4	17.54 16
		Market 4	15.02000*	1.057 91	.001	10.71 34	19.32 66
	Market 3	Market 1	-261.2200 0*	1.057 91	.000	-265.5 266	-256.9 134
		Market 2	-13.23500*	1.057 91	.001	-17.54 16	-8.928 4
		Market 4	1.78500	1.057 91	.433	-2.521 6	6.091 6
	Market 4	Market 1	-263.0050 0*	1.057 91	.000	-267.3 116	-258.6 984
		Market 2	-15.02000*	1.057 91	.001	-19.32 66	-10.71 34
		Market 3	- 1.78500	1.057 91	.433	-6.091 6	2.521 6
Aflatoxin (Kasoa)	Market 1	Market 2	-217.2850 0*	.4756 7	.000	-219.2 214	-215.3 486
		Market 3	-8.63000*	.4756 7	.000	-10.56 64	-6.693 6
		APP19				-	-

		Market 4	-116.91000*	.47567	.000	118.8464	114.9736
	Market 2	Market 1	217.28500*	.47567	.000	215.3486	219.2214
		Market 3	208.65500*	.47567	.000	206.7186	210.5914
		Market 4	100.37500*	.47567	.000	98.4386	102.3114
	Market 3	Market 1	8.63000*	.47567	.000	6.6936	10.5664
		Market 2	-208.65500*	.47567	.000	-210.5914	-206.7186
		Market 4	-108.28000*	.47567	.000	-110.2164	-106.3436
	Market 4	Market 1	116.91000*	.47567	.000	114.9736	118.8464
		Market 2	-100.37500*	.47567	.000	-102.3114	-98.4386
		Market 3	108.28000*	.47567	.000	106.3436	110.2164
Aflatoxin (Jukwu)	Market 1	Market 2	-.17500	.12777	.574	-.6951	.3451
		Market 3	2.35500*	.12777	.000	1.8349	2.8751
		Market 4	-5.70000*	.12777	.000	-6.2201	-5.1799
	APP20						
	Market	Market	.17500	.1277	.574	-.3451	.6951

	2	1		7			
		Market	2.53000*	.1277	.000	2.009	3.050
		3		7		9	1
		Market	-5.52500*	.1277	.000	-	-
		4		7		6.045	5.004
						1	9
	Market	Market	-2.35500*	.1277	.000	-	-
	3	1		7		2.875	1.834
						1	9
		Market	-2.53000*	.1277	.000	-	-
		2		7		3.050	2.009
						1	9
		Market	-8.05500*	.1277	.000	-	-
		4		7		8.575	7.534
						1	9
	Market	Market	5.70000*	.1277	.000	5.179	6.220
	4	1		7		9	1
		Market	5.52500*	.1277	.000	5.004	6.045
		2		7		9	1
		Market	8.05500*	.1277	.000	7.534	8.575
		3		7		9	1
Aflatoxin	Market	Market	1.50000*	.3271	.034	.1681	2.831
(Fosu)	1	2		8			9
		Market	-	.3271	.000	-	-
		3	145.8100	8		147.1	144.4
			0*			419	781
		Market	-6.72000*	.3271	.000	-	-
		4		8		8.051	5.388
						9	1
	APP21						
	Market	Market	-1.50000*	.3271	.034	-	-.1681

	2	1		8		2.8319	
		Market 3	-147.31000*	.32718	.000	-148.6419	-145.9781
		Market 4	-8.22000*	.32718	.000	-9.5519	-6.8881
	Market 3	Market 1	145.81000*	.32718	.000	144.4781	147.1419
		Market 2	147.31000*	.32718	.000	145.9781	148.6419
		Market 4	139.09000*	.32718	.000	137.7581	140.4219
	Market 4	Market 1	6.72000*	.32718	.000	5.3881	8.0519
		Market 2	8.22000*	.32718	.000	6.8881	9.5519
		Market 3	-139.09000*	.32718	.000	-140.4219	-137.7581
Aflatoxin (Cape Coast)	Market 1	Market 2	-72.36500*	.97581	.000	-76.3374	-68.3926
		Market 3	-21.20500*	.97581	.000	-25.1774	-17.2326
		Market 4	-19.16500*	.97581	.000	-23.1374	-15.1626
	APP22						
	Market	Market	72.36500*	.9758	.000	68.39	76.33

2	1		1		26	74
	Market 3	51.16000*	.97581	.000	47.1876	55.1324
		APP:I				
	Market 4	53.20000*	.97581	.000	49.2276	57.1724
Market 3	Market 1	21.20500*	.97581	.000	17.2326	25.1774
	Market 2	-51.16000*	.97581	.000	-55.1324	-47.1876
	Market 4	2.04000	.97581	.295	-1.9324	6.0124
Market 4	Market 1	19.16500*	.97581	.000	15.1926	23.1374
	Market 2	-53.20000*	.97581	.000	-57.1724	-49.2276
	Market 3	-2.04000	.97581	.295	-6.0124	1.9324

*. The mean difference is significant at the 0.05 level.