UNIVERSITY FOR DEVELOPMENT STUDIES

STUDY OF AFLATOXIN DISTRIBUTION AND OFF-FIELD PRACTICES THAT REDUCE ITS CONTAMINATION IN *ARACHIS HYPOGAEA* L_ UPPER EAST REGION_GHANA

VINCENT NINKUU

THESIS SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF PHILOSOPHY (M.PHIL) DEGREE IN BIOTECHNOLOGY

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BY

VINCENT NINKUU

[B.SC. APPLIED BIOLOGY (MEDICAL MICROBIOLOGY OPTION),

TEACHERS CERT "A"]

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THESIS SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF PHILOSOPHY DEGREE (M.PHIL) IN BIOTECHNOLOGY

FEBRUARY, 2017

CANDIDATE'S DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for another degree in this university or elsewhere. Works that were consulted have been duly acknowledged by way of references.

Candidate's signature: _____ Date: _____

Name: Vincent Ninkuu

Supervisors' Declaration

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis as laid down by the University for Development Studies.

Principal Supervisor's Signature: _____Date: _____

Name: Dr. Nelson Opoku

HOD's Signature: _____ Date: _____

Name: Dr. Nelson Opoku

ABSTRACT

Aflatoxin is a known secondary metabolite produced by *Aspergillus flavus* and *Aspergillus parasiticus.* They mostly contaminate oil seed crops such as groundnuts. Their impact on food safety and liver related diseases globally is becoming worrisome. This research was conducted in thirty (30) communities across four administrative districts in the Upper East Region of Ghana to determine the aflatoxin distribution in the region and the effect of post-harvest drying and storage treatments on the incidence of aflatoxin contamination in harvested groundnuts. Three drying techniques (Rack, Tarpaulin and Status Quo) methods were studied to determine the most effective technique that reduces aflatoxin to an acceptable level. FluoroQuant Afla reader was used for the aflatoxin analysis and fungal species isolation was done using PDA and tetracycline solution 1.2 mL / 240 mL of PDA. The study revealed that, Bolgatanga municipality had the lowest aflatoxin level of 10.63±6.10 ppb while Kassena/Nankana had the regional highest of 14.14±13.68 ppb. The study also revealed that groundnut dried on tarpaulin effectively reduced aflatoxin level by 58 % with a mean value of 8.40±0.67 ppb. Groundnuts dried on racks and farmers method (status quo) were found to have aflatoxin levels increased by 225.8 % and 53.73 % per the US standard with mean values of 48.87±19.75 and 23.06±14.66 ppb respectively. The fungal species isolated from the harvested groundnuts were; aflatoxicogenic species (*A. flavus, A. parasiticus*) and non aflatoxicogenic (*A. niger, A. fumigatus, and Rhizopus stolonifer*). In general, the data showed that aflatoxin levels in groundnuts can be reduced by drying the nuts on a tarpaulin.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFB1	=	Aflatoxin B_1
AFB2,	=	Aflatoxin B_2
AFG1,	=	Aflatoxin G_1
AFG2	=	Aflatoxin G_2
AFM1	=	Aflatoxin M_1
CCP	=	Critical Control Points
CDC	=	Center for Disease Control and Prevention (of U.S.A)
CGIAR	=	Consultative Group for International Agricultural Research
CLA	=	Carnation Leaves Agar
EU	=	European Union
FAO	=	Food and Agricultural Organization
FAQF	=	Food Allergy Quick Facts
FDA	=	Food and Drug Adminstration
g	=	gram
GDP	=	Gross Domestic Product
IARC	=	International Agency for Research on Cancer
IARC	=	International Agency for Research on Cancer
ICRISAT	=	International Crops Research Institute for the Semi-Arid Tropics
IFPRI	=	International Food Policy Reseach Institude
JECFA	=	Joint Expert Committee on Food Additives
LSD	=	Least significant difference
MDG	=	Millennium Development Goal
MT	=	Metric tons
NASA	=	National Aeronautics and Space Administration
OTA	=	OchratoxinA
OTB	=	Ochratoxin B

OTC	=	Ochratoxin C
PDA	=	Potato Dextrose Agar
ppb	=	Parts per billion
SAT	=	Semi-Arid Tropic
SD	=	Standard deviation
SNA	=	Spezieller Nährstoffarmer Agar
SSA	=	Sub-Saharan Africa
TSWV	=	Tomato Spotted Wilt Virus of groundnuts
USAID	=	United States Agency for International Development
USDA	=	United State Department of Agriculture
WHO	=	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

Arachis hypogaea is known in Africa, Asia, Europe, and Australia as groundnuts and in South and North America as peanut or monkey nuts. They are the edible seeds of a leguminous crop that grows to maturity underground. It is cultivated in almost 100 countries of which over 90 % are developing countries mostly in Sub-Saharan Africa (SSA). Groundnut is a staple food and valuable households cash crop for millions of people (CGIAR, 2005).

Groundnuts contain high edible oil content (40-50%), protein (25%) and are also a vital source of a variety of essential minerals and vitamins (Catherine *et al*., 2010). They can be directly consumed (raw), processed into meal/cake, confectionary products or snack food. The domestic value of groundnuts cannot be under estimated since every part of it is used in one way or the other; kernels mainly for human consumption, the vines are used as fodder for cattle consumption and the roots symbiotically fix nitrogen into the soil as rich nutrients for other plants (Catherine *et al*., 2010).

Global demand for groundnuts remains secured due to high snack food markets in the EU, North America and countries where groundnuts are a key food condiment, such as Indian, Mediterranean, Asian cuisines and Africa (ARD, 2008)

The important values of the groundnut as enumerated above is however adversely affected through fungal contamination that produces toxic secondary metabolites known as mycotoxin. Mycotoxins are secondary metabolic products of fungi that have become a global menace (FAO, 2002; USDA, 1998). Among these groups of toxins, aflatoxin have been marked as one with enormous economic importance because of

the adverse impact they exert on food security, human and livestock health as well as the marketability of the products (USDA, 1998). According to Ongoma, (2013) and FAO, (2002), aflatoxins are toxic metabolites of the fungi *Aspergillus parasiticus* and *Aspergillus flavus* whose niche is the soil. A number of authors have reported that several species of *Aspergillus, Fusarium, Penicillium* and *Alternaria* that are responsible for the production of several mycotoxins including aflatoxin are not only regarded as plant pathogens but are also sources of essential mycotoxins of critical concern in human and animal health (Abramson, 1997; D'Mello *et al*., 1997; Panigrahi, 1997 and Smith, 1997). About 25% of the world's crops are affected by these fungi and over 4.5 billion people are at risk of chronic aflatoxicosis (Ongoma, 2013). Countries located between latitudes 40^0 N and 40^0 S which encompasses all African countries are at greatest risk from aflatoxin mainly due their tropical climatic conditions (FAO, 2013; Strosnider *et. al*., 2006).

Several research works have been carried out on groundnut aflatoxin in Ghana and West Africa but the problem of proliferation by fungal strains on cash crops and staple food such as groundnuts and maize still remains a threat to humanity. This is as a result of little or no success attained by these researches to curb the loss due to aflatoxin contamination. It is however essential to shift from problem identification to finding post-harvest and storage antidotes to stall the activities of these fungal species that are rendering farmers hungrier, unhealthier and poorer.

The Northern part of Ghana which is made up of three separate administrative regions (Northern, Upper East and West regions) accounts for more than half of the groundnut producing areas in Ghana which altogether contributes 94 % of groundnuts produced in Ghana (FAO, 2013). The regions are located in the Guinea Savannah agro-ecological zone. The rainy season is mono-modal, starting in

April/May/June and ending in September/October with an annual rainfall varying between 900 and 1,100 mm (FAO, 2013). Optimum conditions for aflatoxin production are at 33 ^{0}C and 0.99 a_w; while that for growth of the afltatoxicogenics is 35 ^{0}C and 0.95 a_w (Pitt and Miscamble, 1995) These abiotic factors influence the development and contamination of groundnuts with mycotoxin (Magan *et al*., 2003). Fungal species are having their toll of harmful effects on groundnuts, preventing farmers from achieving their desire goal (Miraglia *et al*., 2009). *Aspergillus* and *Fusarium* species persist as a result of poor post-harvest treatment of farm products (Milani 2013). The consequence of the poor handling is mycotoxin notably aflatoxin proliferation that accounts for much of the reasons for product rejection even in the local markets (Milani, 2013). The prime focus of this research was to investigate post-harvest treatment interventions that could potentially reduce aflatoxin to acceptable levels and possibly stifle the growth and deleterious effect of these carcinogenic fungal species on groundnuts.

1.2 Problem Statement and Justification

West African countries including Ghana produce crops that are susceptible to aflatoxin contamination. These crops suffer rejection on the European market. The result of this rejection is poverty. The savanna ecological zone which embodies the three northern Regions is noted for the production of groundnuts which stands the risk of being contaminated with this toxin. Most homes, restaurants, Senior High Schools are served with groundnuts products such as soup, ‘nketia bugger (a confectionary made predominantly from groundnut), groundnut cake among others which have the potential of being contaminated with aflatoxins. Groundnuts and groundnut products

sold in the markets are without the level of aflatoxin concentration on them making the consumption of these products unsafe for man.

The EU which accounts for the largest groundnuts market has issued a stern warning on the need for Ghana to critically reduce the aflatoxin loads on its products (Ghanaweb, 2015). Rejection of Ghanaian groundnuts by the EU will result in the loss of foreign exchange to the tune of USD 6.4 million (Ghanaweb, 2015). The government's ambitious target of generating USD 5 billion from the non-traditional exports of which groundnut account for the above figure in 2013 sector by 2019 has been endangered (Ghanaweb, 2015).

These and many other reasons accounts for the need to investigate the aflatoxin contamination level of groundnuts grown in the Upper East Region of Ghana map-out the spots zone of aflatoxin in the Region and develop a lasting intervention model to reduce the level of contamination.

This will consequently curb the loss of yield to aflatoxin by arresting the activities of *Aspergillus parasiticus* and *Aspergillus flavus* as strains of fungi responsible for aflatoxin in groundnuts. This research will also isolate the *Aspergillus* and other fungal species responsible for the production of high and low values of aflatoxin.

1.3 Significance of the study

The first millennium development goal (MDG 1) which targets at the eradication of extreme poverty and hunger will not be attained if food security still remains a threat to humanity as a result of pre-harvest and post-harvest loss of yield to fungal species. Many homes in developing countries have not been able to meet their socio-economic needs due to the loss of yield of farm produce through moldy agents. The three Northern Regions produce nearly 94% of groundnuts as cash crop in Ghana (FAO, 2013), yet ranked as the poorest in the nation. This might partly be due to low prices

obtained for the cash crop (groundnut). The poor quality of the groundnuts may be potentially caused by poor post-harvest techniques such as bare ground drying, poor sorting, and storage conditions among others. These poor post-harvest treatment and storage techniques might have favored the growth of aflatoxicogenic and other fungal species. It is therefore essential to assess the aflatoxin loads in our commercial crop so as to design a post-harvest treatment technique that reduces aflatoxin levels. Knowing the aflatoxin level in the groundnut consumed in the Upper East Region will help other researchers in determining the risk level in terms of the disease it might cause. Prevalence data from Africa suggests that aflatoxin contamination in maize, groundnuts and sorghum is higher than the European Union aflatoxin standard (15 ppb) and that of USA (20 ppb) in many countries (Allameh *et al*., 2005; ICRISAT, 2010). Extensive research have been carried out on the impact of *Aspergillus* species, the precursor of aflatoxin on stored food commodities but little or no data is available on the strains of *Aspergillus* species responsible for the production of aflatoxin in groundnuts in the Upper East Region of Ghana. This research sought to determine the aflatoxin distribution in the Region and to isolate mycotoxin producing *Aspergillus* and other fungal species. This research also seeks to compare three drying (tarpaulin, rack and status quo methods) protocols relative to aflatoxin contamination, hence will contribute to knowledge and also serve as a policy document/protocol for the storage treatment to curb aflatoxin in groundnuts and also provide awareness of the high aflatoxin occurrence zones of aflatoxin in the Region.

1.4 Objective of the Study

The main aim of this study was to investigate the aflatoxin distribution of stored groundnuts in the region, assess the post-harvest treatment technique that effectively

reduce aflatoxin contamination in groundnuts in the Upper East Region and isolate the fungal species that accounts for the production of the mycotoxin.

1.4.1 Specific Objectives

- Investigate pre-intervention aflatoxin distribution of stored and fresh groundnut in the region.
- Isolate the mycotoxin producing *Aspergillus* and other fungal species from stored and fresh groundnuts.
- Map aflatoxin spots zones within the study areas
- Assess the aflatoxin concentration in groundnuts samples in different post-harvest interventions (tarpaulin, rack and status quo drying method stored in different bags; jute, plastic and status quo bags).
- Determine the moisture level of stored groundnuts in each treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 History and Domestication of Groundnuts

Groundnut (*Arachis hypogaea*) is believed to have originated from South America. *Arachis* was coined from the Greek words "arachos" meaning "a weed" and *hypogaea* means "underground chamber" and hence the name *Arachis hypogaea* (Gibbons *et al*., 1972). The earliest archaeological records of groundnuts dated back to 37450-3900 in Peru (Gibbons *et al*., 1972). Groundnuts were widely dispersed in the Southern and Central America during the European period of stay in the continent by the Arawak Indians whose presence was captured by archaeological data in 1300-2200 BP (Gibbons *et al*., 1972). The arrival and contact with groundnuts by Europeans resulted in its worldwide spread. The runner type of the Peruvian origin was taken to the Western Pacific, China, Madagascar and Asia (Naturland, 2000). Groundnuts finally found its way into the African continent through the Portuguese traders and to India via Brazil (Gibbons *et al*., 1972). South Eastern part of America was introduced to the Virginia type through slave trade from Africa (Gibbons *et al*., 1972). The growth of groundnuts was then accelerated and finally found its way into every corner of Ghana but Northern Ghana proved to be the home of groundnuts since the climatic and other prevailing conditions were optimum enough for their success (Atuahene-Amankwa *et al*., 1990).

Groundnuts became a commercial product after the American Revolution and sold by peasant farmers to local consumers leading to the establishment of first groundnuts market in 1833 at Wilmington, NC in the U.S (Johnson, 1964). The Civil War resulted into the astronomical increase in groundnuts consumption among the Soldiers (Johnson, 1964). In the later part of the 19th century, cultivation of groundnuts at

commercial level increased (Hammons, 1994). The crop became very popular and was mainly used for snack and roasted for sale by street vendors (Mc Gill, 1973). Invention of mechanic picker for harvesting and loss of cotton yield to cotton ball weevil boosted groundnuts production in 1900 in the US during the world wars (Johnson, 1964).

2.2 Taxonomy of Groundnuts

Arachis hypogaea belongs to the family Leguminosae. Its subfamily, tribe and sub-tribe are *Fabaceae*, *Aeschynomeneae* and *Stylosanthenae* respectively (Krapovickas and Gregory, 1994). The cultivated type belongs to the genus *Arachis*, which has 69 diploid and tetraploid species of South American origin (Krapovickas and Gregory, 1994). The *Arachis* is categorized into nine sections based on geographic distribution, morphology and cross-compatibility; *Caulorrhizae, Extranervosae, Erectoides, Procumbentes, Heteranthae, Trierectoides* and *Triseminatae* sections contain only diploid species *i.e.* 2n = 20 (Stalker and Simpson, 1995). The tetraploids (2n = 40) are more evolutionarily advanced and have evolved independently only in sections *Rhizomatosae* and *Arachis* (Smartt and Stalker, 1982). Due to difference in branching pattern and the occurrence of reproductive nodes on the main stem, *A. hypogaea* was sub-grouped into *hypogaea* and *fastigiata* subspecies (Krapovickas and Rigoni, 1960). *Hypogaea* comprises of different branching pattern as follows; erect/spreading habit of growth, absence of reproductive nodes on the main stem, fresh seed dormancy and extended maturation time (Smartt and Stalker, 1982). Virginia and Runner U.S. market types which are mostly grown in Africa including Ghana is classified into *hypogaea* whilst *hirsuta* is the Peruvian humpback or Chinese dragon type (Smartt and Stalker, 1982). *Fastigiata* possess reproductive nodes on the main stem, erect

growth habit, earlier maturity with little or absence of seed dormancy a sequential branching pattern (Smartt and Stalker, 1982).

2.3 World Groundnuts Production

The commercial and nutritional significance of groundnuts have enhanced it productivity, demand and utilization worldwide (ARD, 2008; FOA, 2007). Production of groundnuts has become a global business. World production level reached 35.7 million metric tons in 2004 and slightly dropped to 34 .9 metric tons (MT) in 2007. These values translates into about 8.7 percent of total oil seed production that stands at almost 410 million MT (FOA, 2007; FAOSTAT, 2007). China is in the lead of the production rank with over 40 percent of world's production. It is followed by the African continent with 24.65 percent and India with 18.2 percent. The United States contributes 5.4 percent to global groundnut output and 4.1 percent by Indonesia (FOA, 2007).

Table 2.1 World Groundnuts production in 2004

Country	Production (Mt)	Share of World Production (%)
China	14,385,000	40.3
India	6,500,000	18.2
Nigeria	2,937,000	8.0
USA	1,933,070	5.4
Indonesia	1,450,000	4.1
Sudan	1,200,000	3.0
Myanmar	715,000	2.0
Senegal	465,000	1.3
Viet nam	451,100	1.3
Chad	450,000	1.3
Argentina	418,571	1.2
Ghana	389,649	1.1
D. R Congo	363,850	1.0
Other	4,057,720	11.4
Total	**35,715,960**	**100.0**

Source: (FOA, 2007)

2.4 Importance of Groundnuts

2.4.1 Nutritional Profile of Groundnuts

Groundnuts are a vital edible oil source for millions of people living in the tropical climates of Africa. Groundnuts are known to be one of the ancient oil crop plant grown and consumed as snack, after roasting (Bansal *et al*., 1993; Jambunathan *et al*., 1985). The important contribution made by groundnut in diet of people in numerous countries cannot be downplayed. It serves as an excellent source of protein, fatty acids and lipid for human nutrition (Grosso *et al*., 1999). It confers additional protective growth and development to the consumer (Gaydou *et al.*, 1983; Grosso and Guzman,

1995; Grosso *et al*., 1999 and Grosso *et al*., 1997). Groundnuts provide the consumer with cheap source of high quality dietary protein and oil. This helps in curbing malnutrition among children in developing countries (Asibuo *et al*., 2008). Ayoola *et al*., (2012) conducted the proximate analysis on groundnut as illustrated below.

Table 2.2 Proximate composition of groundnuts on percentage dry weight basis

Compositions	Raw	Sun-dried	Roasted
Moisture content	7.48	3.4	1.07
Ash content	1.48	1.38	1.41
Crude fibre	2.83	2.43	2.41
Crude fat/oil	46.1	43.8	40.6
Protein	24.7	21.8	18.4
Carbohydrate	17.41	27.19	36.11

Source: (Ayoola *et al*., 2012).

Table 2.3 Mineral composition of the groundnut on dry weight basis

Mineral	Raw	Sun-dried	Roasted
	% dry weight	% dry weight	% dry weight
Sodium (Na)	0.71	0.69	0.57
Potassium(K)	0.47	0.51	0.55
Calcium (Ca)	1.18	1.24	1.35
Magnesium(Mg)	0.18	0.21	0.24
Iron(Fe)	0.4	0.47	0.47
Zinc(Zn)	0.44	0.42	0.5
Phosphorus(P)	0.68	0.65	0.69

Source: (Ayoola *et al*., 2012).

2.4.2 Oil Composition of Groundnuts

Common cooking and salad oil can be produced from groundnut. It is made up of 46 % primarily oleic acid (mono-unsaturated fats), 32 % primarily linoleic acid (poly-unsaturated fats) and saturated fats (primarily palmitic acid) comprised of 17 % (USDA, 2014 and Ozcan, 2010). Extraction of whole groundnuts through the use of simple water and centrifugation is being considered by NASA (National Aeronautics and Space Administration) for Advanced Life Support program for future long stay of human on space missions (Shi *et al*., 1998).

2.4.3 Health Benefits of Groundnuts

The medicinal value of groundnuts has maintained the competitiveness of its global demand (Segura *et al*., 2006). Groundnuts are nutrient packed foods with complex matrices of rich unsaturated fatty bioactive compounds such as fiber, minerals, high-quality vegetable protein, tocopherols, phenolic and phytosterol compounds (Griel and Kris-Etherton, 2006 and Segura *et al*., 2006). Epidemiologic researches revealed that intake of groundnuts have a high tendency of alleviating incidence of coronary heart disease, diabetes in women and gallstones diseases (Fraser *et al*., 1992; Hu and Willett, 2002). Limited research evidence also suggests that groundnuts is of beneficial effects on cancer, hypertension, and inflammation (Sabaté *et al*., 1993). Research on groundnuts indicates that, its consumption can drastically lower the cholesterol level of the consumers, even in the context of healthy diets (King *et al*., 2008). There is emerging evidence of beneficial effects on inflammation, oxidative stress, and vascular reactivity (King *et al*., 2008). Blood pressure, visceral adiposity and the metabolic syndrome also appear to be positively impacted by intake of the nuts (Lloyd-Jones *et al*., 2010). Contrary to expectations, epidemiologic studies and

clinical trials suggest that regular nut consumption is unlikely to contribute to obesity and may even help in weight loss (King *et al*., 2008).

2.4.3.1 Groundnuts Phytochemicals

Research reveals that polyphenols and other phytochemicals that provide health benefits to humans are found in groundnuts (BBC, 2005). Latest research also reports that groundnut's skins contains comparable polyphenolic content of many fruits (Lopes *et al*., 2011). The skins of groundnuts are a vital source of resveratrol, a phenolic, which is still under research for their potential on variety of effects on humans (Sanders *et al*., 2000).

2.5 Health Concerns of Groundnuts

The physiological response of humans to stimulus (food) varies greatly from one person to the other. Contact with some food items or derivatives could be enough to evoke the immunological response of some individual (Food Allergy Quick Facts (FAQF), 2012). Groundnuts are not excluded since it presents its own medical issues.

2.5.1 Allergies

Food Allergy Quick Facts (FAQF), (2012) reports that, approximately 0.6 % of the entire population in the United States have experienced mild to severe allergic reactions to the exposure of groundnuts. Symptoms of these allergies come in varying forms, some individuals experiences watery eyes to anaphylactic shock. This can result in fatality if remained untreated for an extended period of time (Food Allergy Quick Facts (FAQF), 2012). The allergenic effect of groundnuts correlates with how they are processed in countries where consumption is high (North America, China and Pakistan) (Maleki *et al*., 2003). Maleki *et al*., (2003) also reports that roasted groundnuts, a common practice in North America and Africa may cause the major groundnuts allergen (Ara h2) which is a notorious inhibitor of digestive enzyme called

trypsin. This has an inhibitory effect on digestion. That notwithstanding, this allergen has been proven to greatly protect Ara h1, which is another known major groundnuts allergen, from digestion, a property that also arises as a result of roasting (Maleki *et al*., 2003).

Høst *et al*., (2008) reported that, exposure to decaying groundnuts can critically increase the risk of allergies. Studies on animal (with limited or no evidence available from human subjects) suggested that groundnuts dose is an essential mediator of groundnuts sensitization and tolerance. Higher doses tend to lead to tolerance whilst lower doses however results in sensitization (Thompson *et al*., 2010). According to Lack *et al*., (2003), allergy associated with groundnuts has been linked to the use of the skin for preparations of groundnuts oil among children. It is however worth noting that, this evidence is not regarded as conclusive. Groundnuts allergies have also been linked to family history and soy products intake (Lack *et al*., 2003).

Though groundnuts allergy may persist throughout the individual's lifetime, Fleischer *et al*., (2003) report that 23.3% of children will outgrow an allergy.

2.6 Groundnuts Diseases

Many diseases affect groundnuts leading to loss of yield. Some of these disease can be transferred to humans (Thouvenel *et al*., 1976). Groundnut, like other crops are affected by viruses, fungi and nematodes among other pathogens. The buildup of these infectious agents leads to the contamination of yield with toxin (mycotoxins). Thouvenel *et al*., (1976) identified groundnuts as one of the most severely infected tropical plants in terms of fungal diseases.

2.6.1 Viral Diseases of groundnuts

Many studies have revealed that groundnuts are greatly affected by viral diseases. Groundnut clump virus identified by Thouvenel *et al*., (1976) results in loss of yield

and quality in groundnut. Groundnuts eye spot virus described by Dubem and Dollet, (1980) was known to reduce nuts quality. Tomato spot wilt virus of groundnuts was identified in 1985 (Dubern and Fauquet, 1985) alongside groundnut chlorotic spotting virus that causes dark green spots surrounded by a chlorotic halo on groundnut plant. Sometimes many leaflets show green line patterns (Dubern and Fauquet, 1985). All these diseases may have their effect on the consumer.

2.6.2 Bacterial Diseases of Groundnuts

Bacteria wilt of groundnut caused by *Pseudomonas solanacearum* is one of the important diseases that affect African groundnuts. This bacterium was first reported in Indonesia in 1905 and later in Georgia, USA in 1931. Presently, this disease is distributed across the length and breadth of the world in groundnuts cultivated areas such as Asia and Africa (Faujdar and Oswalt, 1992).

2.6.3 Nematode Diseases

Parasitic agents in the soil pose high health risk to most crop produce in contact with the soil such as groundnuts and yam. Several nematodes are parasitic and attack groundnuts. They are mostly root-knot nematodes (*Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne javanica*), root lesion nematode (*Pratylenchus brachyurus*), ring nematode (*Macroposthonia ornata*), sting nematode (*Belonolaimus longicaudatus*), and testa nematode (*Aphelenchoides arachidis*) (Thakur, 2014). Their infestation greatly affects yield quality and quantity (Thakur, 2014).

2.6.4 Fungal Diseases

Fungal diseases in groundnut are known to cause seed rots and diseases in seedling such as stem and root rot, wilts, blight, pod rot, and foliar diseases as well as early and late leaf spots (Faujdar and Oswalt, 1992). Many soil inhabiting fungi infect and damage the seed and germinating seedlings of groundnut. They may be identified by

fungal spores that give characteristic colorations to the seed. For instance, gray spores indicate *Rhizopus arrhizus*, black spores are *Aspergillus niger*, and green or blue spores are *Penicillium* spp. (Faujdar and Oswalt, 1992). Most species of *Aspergillus* and *Fusarium* produce mycotoxin and can cause aflaroot of groundnut foliar diseases. *Puccinia arachidia* Speg (rust) and leaf spot (*Cercosporidium personatum* (recently renamed *Phaeoisariopsis peraonata*) are the major foliar diseases of groundnut caused by fungi. Rust and late leaf spot are important diseases in India and most of the semi-arid tropic (SAT) regions. Early leaf spot is an important disease in Africa of which Ghana forms an integral part. Some fungal diseases, their symptoms and causative fungi are tabulated as follows;

Table 2.4 Groundnuts Diseases caused by fungi and their symptoms

Causative Fungi	Disease	Symptom
Aspergillus flavus	Aflaroot or yellow mold	Shriveled and dried seeds covered by yellow or greenish spores. Cotyledons show necrotic lesions with reddish brown margins. Seedlings are highly stunted, leaf size greatly reduced, with pale to light green color.
Fusarium solani and *Fusarium oxysporum*	Wilt	Lower end of tap root becomes brown to reddish brown. Secondary roots become brown and brittle. Leaves turn grayish green and plants dry.
Aspergillus niger	Crownrot/ collar rot	Germinating seeds are covered with masses of black conidia, rapid drying of plants. Later, whole collar region becomes shaded and dark brown.
Rhizopus arrhizus and *sclerotium rolfsii*	Seed, seedling and stem rot,	Sudden wilting of lateral branches that are completely or partially in contact with soil. White coating of fungus mycelium on affected plants.
Verticillum alboatrum	Vascular wilt	Wilting of leaflets and petioles, leaflets are curled and chlorotic.
Rhizoctonia solani	Root rot, break down of pod and wilt	Pre-emergence death of seedlings; shrunken, elongate dark brown areas on the hypocotyl. The decayed areas are covered with light-brown mycelium.

Source: (Faujdar and Oswalt, 1992)

Global food production especially developing countries in Africa are faced with fungal infection which consequently leads to many health complications and poverty. Fungal genera such as *Aspergillus* and *Fusarium* release toxic metabolites called mycotoxin in food crops, the result of which is mycotoxicosis and rejection of infested produce (Faujdar and Oswalt, 1992).

2.7 Taxonomy and History of *Aspergillus* Genus

Pier Antonio Micheli, an Italian biologist and priest was the first to catalogue *Aspergillus* in 1729 (Kenneth *et al*., 1965). The shape of the *Aspergillus* described by Micheli is like a holy water sprinkler (*aspergillum*) which is of Latin origin "a*spargere"* (to sprinkle) hence the generic name *Aspergillus* (Kenneth *et al*., 1965 and Powell *et al*., 1994). Mold that produce characteristic asexual spore-heads belong to the genus *Aspergillus.* These spores are the most important microscopic character used in *Aspergillus* taxonomy (Samson and Varga, 2009). About 250 species of this genus is known (Geiser *et al*., 2008). This number may rise in future due to rising application of the phylogenetic species based concept on DNA sequence data instead of visual morphological characters. According Bennett, (2010), *Aspergillus* is classified as follow;

Table 2.5 Classification of *Aspergillus*

Kingdom	Fungi
Division	Ascomycota
Class	Eurotiomycetes
Order	Eurotiales
Family	Trichocomaceae
Genus	***Aspergillus***

Source: (Bennett, 2010)

The description of the species *fumigatus* by Georg W. Fresenius in 1863(Lee) and the discovery of filamentous, *Aspergillus* species such as *A. niger* , *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. carbonarius*, and *A. alliaceus* as pathogenic paved way for extensive studies leading to discovery of many other species (Perrone *et al*., 2007).

2.8 Morphological Features of *Aspergillus* Genus

Aspergillus is a known cosmopolitan, filamentous and ubiquitous fungus that exists in nature. It can be identified by both their macroscopic and microscopic morphology (St-Germain and Summerbell, 1996).

2.8.1 Macroscopic Feature of *Aspergillus*

Macroscopic features which predominantly highlights the appearance and color of the species on a growth media are tabulated below;

Table 2.6 Macroscopic features of *Aspergillus*

SPECIES	SURFACE	REVERSE
A. clavatus	Blue-green	White, brownish with age
A. flavus	Yellow-green	Goldish to red brown
A. fumigates	Blue-green to gray	White to tan
A. glaucus group	Green with yellow areas	Yellowish to brown
A. nidulans	Green, buff to yellow	Purplish red to olive
A. niger	Black	White to yellow
A. terreus	Cinnamon to brown	White to brown

Source: (Rahul and Jha, 2014)

2.8.2: Microscopic features of *Aspergillus* species

The microscopic features that define specific *Aspergillus* species are illustrated below;

Table 2.7 Microscopic features of *Aspergillus* species

Species	Conidiophore	Phialides	Vesicle	Sclerotia	Cleistothcia	Hulle cells	Aleuriconidia
A. clavatus	Long, smooth	Uniseriate	Huge and clavate shaped	- Some(+)	-	-	-
A. flavus	Colorless, rough shape and short	Uniseriate Biseriate (some)	Round and radiate head	- Some(+)	-	-	-
A. fumigatus	(<300μm), smooth, colorless/ greenish	Uniseriate	Round/columnar head	- Some(+)	-	-	-
A. glaucus	Variable length, smooth, colorless	Uniseriate	Round, radiate to loosely columnar head	-	Yellow orange	-	-
A. nidulans	Short (<250 µm), smooth, brown	Biseriate and short	Round, columnar head	-	+ (red)	+	-
A. niger	Long, smooth, colorless or brown	Biseriate	Round, radiate head, compactly columnar head	-	-	-	- some(+)
A. terreus	long and smooth	Biseriate		-	-	-	-
A. Versicolor	Long, smooth, colorless	Biseriate	Round, loosely radiate head	-	-	- (some)strains	-

Source: (Rahul and Jha, 2014)

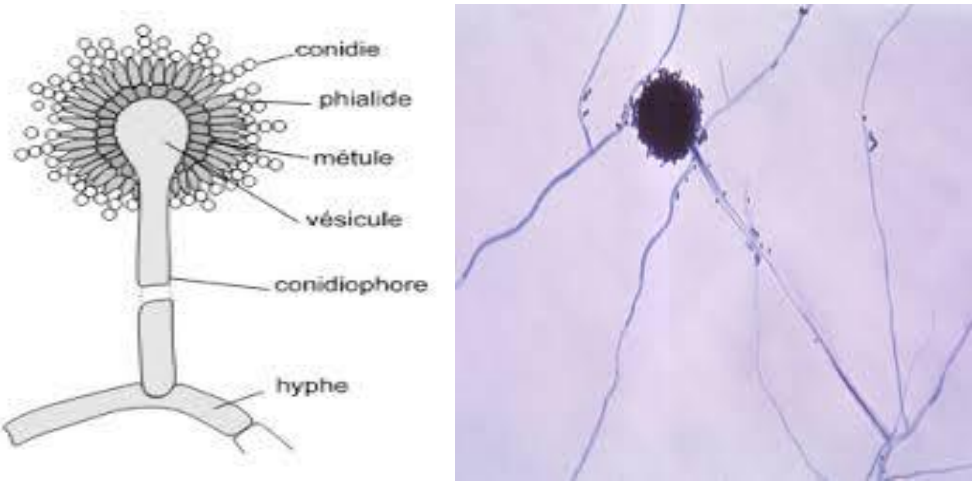


Plate 2.1: Microscopic Features of Some *Aspergillus* species

Source: (Gautam and Bhadauria, 2012)

2.9 Ecology of *Aspergillus*

Members of the genus thrive in a wide range of habitats. They exist in the soil, moist environments and in air (Diniz *et al*., 2012). According to Menezes *et al*., (2004); Mezzari *et al*, (2002) and Diniz *et al*., (2012) atmospheric air is the commonest agent of fungi dispersion, (particularly not in the case of only the spores), but also peculiar to fragments of the vegetative mycelium that matures into a viable component of the fungi during the aerial dispersion process. Fungi that possess some mechanism for their dispersion (Anemophilous fungi) have the potential to colonize varying habitat and most substrates in a unique mechanism (Smith, 2007). They expose their metabolites and propagules especially when they exist in schools, offices, hospitals, homes including all indoor environments (Mezzari *et al*, 2002). They are capable of surviving in fluctuation temperature, pH, oxygen concentration and even in highly saline environment (Smith, 2007 and Menezes *et al*., 2004).

Diniz *et al*., (2012) stated that *Aspergillus* are found commonly among decaying organic materials, in soil, stored grains, animal feed, and other materials. This might

accounts for the high occurrence of aflatoxin and other mycotoxins on food crops especially those that are in direct contact with the soil such as groundnut.

West African countries are favored for aflatoxin proliferation due to their climatic condition as Domsch *et al*., (2007); Pitt, (1994); and Diniz *et al*., (2012) also concluded that *Aspergillus* species which gives rise to aflatoxin are thermo-tolerant and exists in ranges (25 ^{0}C – 40 ^{0}C) and abundantly found in warm climates. These species are however less tolerant to water activity and temperate environment (Diniz *et al*., 2012).

2.10 Media for Culturing *Aspergillus* and other fungal species

The growth of fungal species in a laboratory condition require nutritive medium. Various media have been used with success in the culturing process of fungal species (Kumara and Rawal, 2008). Broad spectrums media are used for isolation of various groups of fungi as they enhance the vegetative and colony morphology, pigmentation and sporulation of these fungi species (Kuhn and Ghonnoum, 2003). This is however dependent upon the composition of the specific culture medium *i.e*. hydrogen ion concentration, light, temperature, water availability and surrounding atmospheric gas mixture (Kuhn and Ghonnoum, 2003; Kumara and Rawal, 2008; Northolt and Bullerman, 1982). Physical and chemical factors have a pronounced effect on diagnostic characters of fungi (St-Germain and Summerbell, 1996). Hence, it is often necessary to use several media whilst attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell, 1996).

Furthermore, findings for one species are not readily extrapolated to others, particularly for filamentous fungi, where significant morphological and physiological variations exist (Meletiadis *et al*, 2001). Some common media used in isolating most

fungal species include; Potato dextrose agar (a general purpose media), ¼ strength (PDA ¼) Carnation leaves agar (CLA), Komada modified media (K2) for isolating *Fusarium oxysporum* and *cubense*, Spezieller Nährstoffarmer agar (SNA), which is a specialized media for sporulation stimulation, production of microconidia in a stable way and suitable for chlamydospores detection (Ainsworth, 1971; Burguess *et al*., 1988 and Sun *et al*., 1978). An antibacterial agent (streptomycin sulfate 1.2 mL / 240 mL of PDA) has been an active ingredient in inhibiting the proliferation of bacteria cells. (Meletiadis *et al*., 2001)

2.11 Microscopic Identification of *Aspergillus* species

Microscopy is key in the identification of fungal strains. Conidial heads, vesicles, conidiosphore, sterigmata and colonies color are all enhanced by microscopy (Hocking and Pitt, 1989 and Raper and Fennel, 1965). The microscopic analysis involves the direct identification of the *Aspergillus* species on the medium by diligent manipulation with low power (Mislivec, 2000), Preparation of wet mount from the culture plate using lactophenol cotton blue and covered with cover slip and then examined using low power. In this microscopic examination, the distinctive features of each species are clearly shown namely, the nature of the hyphae, nature of conidiophore and the nature of the conidia (Samson *et al*., 1996).

2.12 Factors that Promote Mycotoxin Production by fungal species

Climate factors have great influence on plant, animal, and human epidemics (IPCC, 2007; Wint *et al*., 2002; Fitt *et al*., 2006; Thomson *et al*., 2006). Factors that promote the successful growth of these fungi have been categorized by D'Mello and MacDonald, (1997) as intrinsic and extrinsic, chemical, physical and biological factors, environmental and ecological (Zain, 2011; Sant'Ana, 2010). With much regards to this categorization, mycotoxin concentration is dependent on fungi type,

the substrate on which it thrives and more importantly the environmental/climatic influences on the fungi (Magan *et al*., 2003; Lacey, 1986). Among the above categorization, the following elucidated factors plays critical role in the buildup of these aflatoxin and other mycotoxins.

2.12.1 Nutrient Availability versus Fungal Growth

Mold species are incapable of producing mycotoxins without the appropriate concentration of nutrients available to it irrespective of its genetic ability to do so (Lacey, 1986). Mycotoxin production is correlated to the available nutrients to the fungi (Lacey, 1986).

Fungi in general require rich source of either organic or inorganic nitrogen, carbohydrates, trace element or some amount of moisture for effective growth and toxin production (Rachaputi *et al*., 2002). Lacey, (1986) established that substrate type impacts toxin production as *A. flavus* produces high concentration of aflatoxin when found on groundnuts as compared to when they are found on rice and other cereals.

Fusarium and *Alternaria* are both field fungi and contaminate grains before or during harvesting stage but are not effective storage fungi like *Aspergillus* and *Penicillium* grows at stressful water condition (Rachaputi *et al*., 2002). These storage fungi usually infect and increase the toxin level of produce stored in silos, bags and bans. The production of aflatoxin is usually skewed to higher concentration during off-season extended drought with high temperatures and humidity (Rachaputi *et al*., 2002).

2.12.2 Soil Types and Conditions

The Codex Alimentarius Commission- CAC, (2003) states that, soil is a good natural commodity that exerts the greatest influence on the incidence of fungi. Crops planted

in different types of soil may have significant variable levels of mycotoxin contamination (The Intergovernmental Panel on Climate Change (IPCC), 2007). For instance, groundnuts grown on light sandy soil favors rapid growth of fungi, especially under dry conditions (The Codex Alimentarius Commission (CAC) , 2003). Heavier soils on the other hand result in less aflatoxin contamination of groundnuts due to their high capacity of water holding which assist the plant to prevent drought stress (The Codex Alimentarius Commission (CAC) , 2003).

2.12.3 Climatic Conditions

Several environmental factors are key players in the growth of fungi and contamination of agricultural produce with their mycotoxin. The factors that are critical in rendering agricultural commodities unsafe are hot and humid conditions (Mclean and Berjak, 1987). The frequency of occurrence of mycotoxin, becomes higher in places that are characterized by hot and humid climate (Makun *et al*., 2009a and Makun *et al*., 2007). Although this does not preclude temperate zones, tropical climatic zones as pertains in Ghana are favorable for fungi growth and mycotoxin contamination. Mycotoxigenic fungi abundance in the tropics as a consequence of its warmer climatic condition results in major food spoilage (Mclean and Berjak, 1987). Many *Aspergillus* species thrives best with increased toxin production between 24 ^{0}C and 28 ^{0}C and a least moisture content of seed at 17.5 % (Ominski, 1994; Trenk and Hartman, 1970). Drought conditions constitute stressful factors to plants making them vulnerable to mold infection with ensuing increase in toxin production (Ominski, 1994). Report by the Codex Alimentarius Commission (CDC), (2004) reveals that, since droughts aids toxin contamination, it is probably the cause of the deadly outbreak of acute human aflatoxicosis in Kenya in 2004. Makun *et al*., (2009b) and

Adebanjo, (2000) in a similar research work, reported higher levels of mycotoxigenic mold contamination in the rainy season than in the dry harmattan season in Nigeria.

2.12.5 Pre-harvest Conditions

It has been reported that, the probability of pre-harvest contamination of groundnut with mycotoxin producing mold may be affected by the genotypes of the crop plant, drought limits, type of soil, available level of fertilizer, and the presence of insect activities (Doner and Holbrook, 1995). Abbas *et al*., (2002) and Payne, (1992) however, revealed that the most essential factor that favors fungal growth and toxin production occurs in the night with higher temperatures in the absence of the plants major source of energy (sun) that enable it to resist fungal infection. That notwithstanding, (Bruns, 2003) identified management practices that result in aflatoxin reduction such as timely planting, maintenance of plants optimal plant densities, proper plant nutrition, drought stress avoidance, controlling other plant pathogens, weeds and insect pests and proper harvesting.

2.12.6 Period of Harvesting

Harvest is the first stage in the production chain where moisture content becomes the most important parameter in terms of the management and protection of the crop (Bankole and Adebanjo, 2003). It also marks a shift from problems caused by plant pathogenic fungi, like *Fusarium*, to problems caused by storage fungi, like *Aspergillus* species (Atanda *et al*., 2013). Ideally, grains will always be harvested after a spell of dry weather when it is at good moisture content, so that immediate drying is not necessary. However (Bankole and Adebanjo, 2003), states that, this is not always possible hence inappropriate harvest time is a risk factor in Sub-Saharan Africa (SSA). Early harvesting reduces fungal infection of crops in the field and consequent contamination of harvested produce (Atanda *et al*., 2013). Even though

majority of farmers in Africa are well aware of the need for early harvesting, lack of storage space, unpredictable weather, labour constraint, need for cash, threat of thieves, rodents and other animals compel farmers to harvest at inappropriate time (Bankole and Adebanjo, 2003). According to (Kaaya *et al*., 2006), aflatoxin levels increased by about 4 times by the third week and more than 7 times when maize harvest was delayed for 4 weeks. Rachaputi *et al*., (2002) reported that lower aflatoxin levels and higher gross returns of 27 % resulted from early harvesting and threshing of groundnuts.

2.12.7 Pest Infestation

The invasion of insects on grains, nuts cereals causes deterioration and loss of quality, grade and marketability of these products. According to Avantaggio *et al*., (2002), damage of maize by insect invasion enhances *Fusarium* and *Aspergillus* contamination. Insects are capable of carrying mycotoxin-producing fungal spores from plant surfaces to the interior of the stalk or kernels or create infectious wounds through their feeding habits (Munkvold, 2003).

2.12.8 Post-harvest Handling

Post-harvest movement of food/feed commodities can be complex, passing between a number of intermediaries such as traders and intermediate processors, who may be situated at different geographical locations (Atanda *et al*., 2013). In the simplest case, produce may remain on-farm, in store or buffer storage for short periods of time before being passed directly onto the processor. In more complex cases it may pass through the hands of merchants or third party drying facilities (if harvested wet-grains) and held in storage for periods of time before finally arriving at the processors. At all times the produce can become susceptible to fungal contamination and

mycotoxin production if the storage conditions are not strictly controlled (Atanda *et al*., 2013).

2.12.9 Conditions and Duration of Drying Produce

Lanyasunya *et al.*, (2005) reported that drying of farm products to a lower moisture level is critical in stiffening fungal development, proliferation, and insect infestation. This results in longer shelf life of the farm product. This practice is however usually given little attention by most farmers in Northern Ghana especially on groundnuts which is mostly harvested during the peak of the rainy season (August/September). (Ayodele and Edema, 2010) examined the Critical Control Points (CCP) in dried yam chips production with particular attention to reducing mycotoxin contamination and identified the drying stage as a CCP. Aflatoxin concentration is known to increase in 10 folds within a 3-day period, when harvested maize on the field is stored with high moisture condition (Fandohan *et al*., 2005). This condition prevails in the Northern part of Nigeria due to lack of appropriate drying technology. About 10 – 13 % moisture levels is recommendable for stored products (Fandohan *et al*., 2005). Mestres *et al*., (2004) observed that grains that are not well dried before packaging are easily contaminated with aflatoxin and other mycotoxins.

2.12.11 Sanitation

According to (UNICEF/WHO, 2006), Ghana is ranked 48th dirtiest country out of 52 in Africa and 14th out of 15 West African countries only surmounting Niger. This data presents the precarious sanitation situation in Ghana of which the Upper East Region led the chart in open defecation in Ghana. Sanitation condition is amazingly proportional to mycotoxin proliferation in stored food (Atanda *et al*., 2013). Basic measures of sanitation such as elimination and destruction of debris from previous harvest can potentially minimize infection and infestation of farm produce in the field.

Sorting through hand picking or winnowing out damaged and infected grains from the food commodity can result in 40-80% reduction in aflatoxins levels (Atanda *et al*., 2013).

2.12.12 Traditional Processing Methods

In separate studies conducted by Fandohan *et al*., (2005); Lopez-Garcia and Park, (1998), to determine the correlation between aflatoxins and fumonisins contamination (against thorough cleansing of farm products through traditional processing of naturally-contaminated maize and maize-based foods), it was revealed that sorting, washing, winnowing, crushing combined with de-hulling of maize grains were effective in reducing mycotoxins to a significant level. Several scientific studies suggested however that, heat and pasteurization of produce do not completely eliminate mycotoxins concentration (Manorama and Singh, 1995).

2.12.13 Interaction between *Aspergillus* and Other Contaminants

Aspergillus parasiticus growing in the midst of some bacteria such as *Lactobacillius casei* and *Streptococcus lactis*, produce reduce amount of aflatoxin (Ominski, 1994). However, Meister *et al*., (1996) established that fungal metabolite such as cerulenin from *Ephalosporium caerulens* and *Acrocylindrium oryzae* and rubratoxins from *Penicillium purpurogenum* favors aflatoxin production even though they repress growth of aflatoxin-producing fungi. This type of favorable interaction between fungi in the same food matrix with regards to aflatoxin synthesis coupled with multi-occurrence of mycotoxins from the different fungi could have additive or synergistic effect on the health of the host (Van-Burik and Magee, 2001). This could worsen the aflatoxin concern because such simultaneous co-occurrence of fungi and mycotoxins

in African agricultural produce is a very common phenomenon (Makun *et al*., 2011; and Makun *et al*., 2007)

12.12.15 Breeding for Aflatoxin Resistance

The advent of Genetic Modification of Organisms (GMO's) has remediated loss of yield to some diseases (Brown *et al*., 2003). Resistance of corn to *Aspergillus* infections through transgenic means have been established (Brown *et al*., 2003) but the development of commercial hybrids is yet to hit the African soil where the problem really persists. Clements and White, (2004) opined that, there is difficulty in finding elite lines that maintain high yields and resistance within multiple environments. A report by Brown *et al*., (2001) however revealed that, tested transgenic maize inbred lines selected in West and Central Africa for moderate to high resistance to maize ear rot and aflatoxin were effective in the reduction of aflatoxin in comparison with the U.S. lines counterparts. With all the proven success in transgenic maize, Guo *et al.*, (2009) revealed that, resistance in groundnuts to aflatoxin contamination under all conditions has still not been achieved and breeding efforts including the use of microarrays is still in progress to aid in the identification of genes involved in crop resistance. Several approaches that involve the design and production of maize plants that reduce the incidence of fungal infection, growth and prevention of toxin accumulation are being reviewed (Brown *et al*., 2001; Guo *et al.*, 2009). These include the identification of resistance-associated proteins (RAPs) through proteomics as well as biochemical marker identification (Bhatnagar *et al*., 2008). Identification of aflatoxin accumulation resistance quantitative trait loci (QTL) and related markers is also under investigation (Warburton *et al*., 2009). In the long

term the identification of compounds that block aflatoxin biosynthesis would significantly enhance aflatoxin control.

2.13 Pathogenic and mycotoxin producing *Aspergillus* species

One species of fungi may produce many different mycotoxins and one mycotoxin type is capable of being produced by several fungal species (Reddy *et al.*,2009). Groundnut remains a major casualty to these mycotoxins proliferation especially aflatoxin in Africa. Aflatoxin and other mycotoxins are threat to human health by causing diseases such as liver cancer (Stenske *et al*., 2006).

The genomic comparison of *A. flavus* and *A. oryzae* confirmed that the two fungal species are closely related and might be of the same ecotype (Payne *et al*., 2006). *A. flavus* is an active member of the group one carcinogens (Bressac *et al.*, 1991) and infests crops such as groundnuts, maize, cotton seeds etc. *A. oryzae* in the other vain is an active ingredient in the fermentation industry and is classified as safe and non-pathogenic (Wogan *et al*., 1992). In Serra *et al*, (2005) research report on the isolation of mycotoxin producing *Aspergillus* species on grapes, mycotoxins producers represented 8.0 % of the grape mycoflora and potential producers of aflatoxins represents (0.3%), OTA, (6.0%), trichothecenes (1.2%) and patulin (0.5%). Ninety two percent of the remaining were described as non-mycotoxigenic and did not produce any known form of mycotoxin of health importance. *A. tenuissima* was found to produce very low toxin which was below the European and American mycotoxin acceptable level and therefore not considered as mycotoxigenic (Nielsen, 2003). *A. flavus* spores which are dispersed in the air eventually find their way into the soil and infest most crop products of which groundnuts are not an exception. This species is known to produce one of the most potent naturally existing carcinogen, aflatoxin B1 (Davis *et al*., 1966).

Table 2.8 List of some *Aspergillus* species and the type of mycotoxin they produce.

Mycotoxin	***Aspergillus* species**
Aflatoxin	*Aspergillus flavus, A. parasiticus*
Aflatrem	*Aspergillus flavus*
Austdiol	*Aspergillus ustus*
Brevianamide	*Aspergillus ustus*
Citreoviridin	*Aspergillus terreus,*
Cytochalasin E	*Aspergillus clavatus*
Cyclopiazonic acid	*Aspergillus versicolor*
Destruxin B	*Aspergillus ochraceus*
Ochratoxin	*Aspergillus ochraceus, Penicillium viridictum*
Oxalic acid	*Aspergillus niger*
Penicillic acid	*Aspergillus ochraceus*
Sterigmatocystin	*Aspergillus flavus*
Viriditoxin	*Aspergillus fumigates*
Destruxin B	*Aspergillus ochraceus*
Fumagilin	*Aspergillus fumigates*

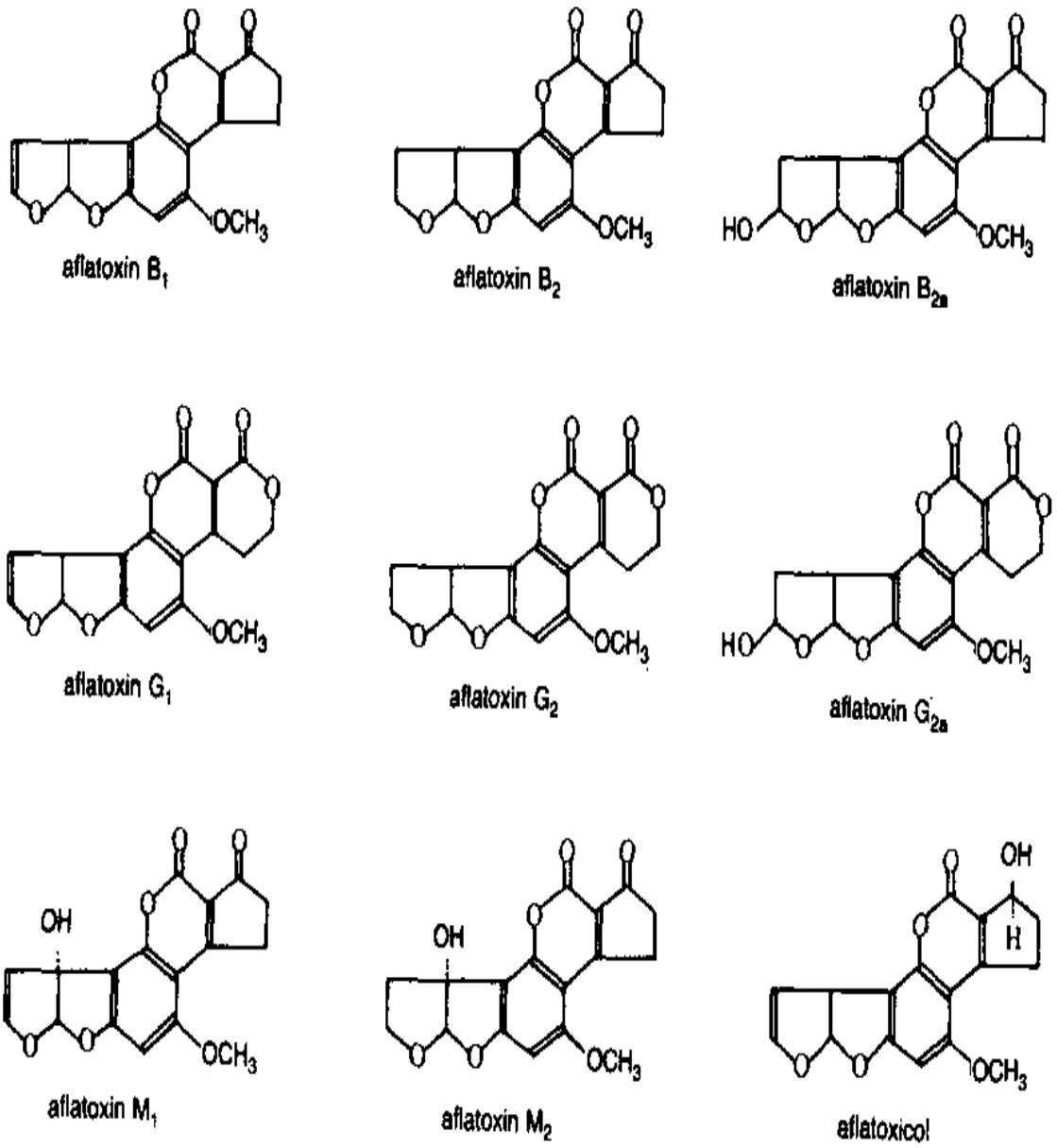
Source: (Hedayati *et al*., 2007; Blumenthal, 2004 and Azziz *et al*., 2005).

These and many more species of *Aspergillus* are responsible for the production of mycotoxin that accounts for their health risk. Among the toxins enumerated above in table 2.8, the following are known to pose the deadliest health risk to humans.

2.13.1 Aflatoxins

This group of mycotoxins is difuranocoumarin derivatives that are mainly produced by *Aspergillus* species notably *A. flavus* and *A. parasiticus*. Aflatoxin is categorized into B_1, B_2, G_1, $G_{2,}$ M_1, and M_2 and aflatoxin B_1 is the most potent carcinogen (Martins *et al*., 2001). It is directly correlated to adverse health impacts, (responsible for causing liver cancer, immune suppression, slows down pathological conditions), in most species of animals (Martins *et al*., 2001). *A. bombycis, A. ochraeoroseus, A. pseudotamari, and A. nomius* are also aflatoxin producers but rarely encountered in nature (Debtanu *et al*., 2014). Aflatoxins are dominant in food produced in the tropical and subtropical areas, such as groundnuts, cotton, , spices, pistachios and maize (Yin *et al*., 2008). In mycological terms, as contained in Debtanu *et al*., 2014), aflatoxin is associated with both carcinogenicity and toxicity causing diseases often referred to as aflatoxicosis. Molecular features of the various types of aflatoxin adopted from Cole and Cox, (1981) report are illustrated below;

Figure 2.1: Molecular structures of aflatoxin types

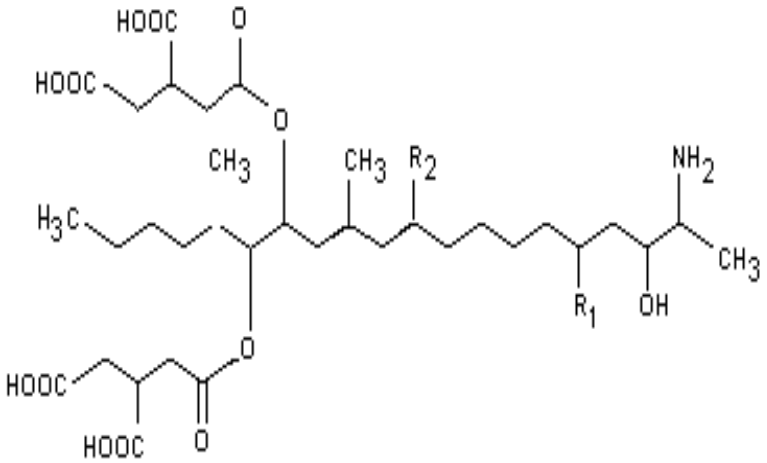


Source: (Cole and Cox, 1981)

2.13.2 Fumonisins

These were described and characterized in 1988 with a subdivision fumonisin B_1 as the most produced toxin. They can be synthesized by amino acid (alanin) into an acetate-derived precursor (Debtanu *et al*., 2014). They are produced by different strains of *Fusarium* such as *F. verticilloides* and *F. proliferatum* (Marasas, 1995).

Figure 2.2 Molecular structure of Fumonisins



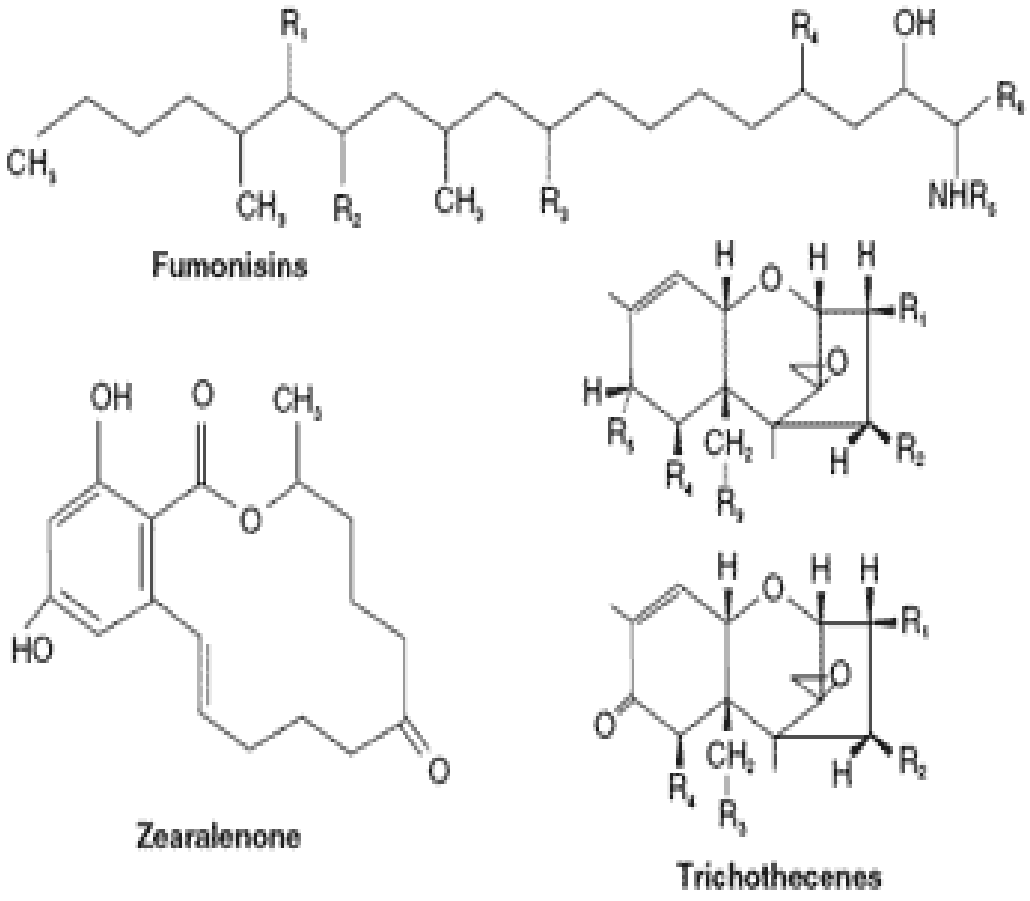
	R_1	R_2	Formula	CAS Number	Molecular mass
Fumonisin B_1	OH	OH	$C_{34}H_{59}NO_{15}$	116355-83-0	721.838
Fumonisin B_2	OH	H	$C_{34}H_{59}NO_{14}$	116355-84-1	705.839
Fumonisin B_3	H	OH	$C_{34}H_{59}NO_{14}$	136379-59-4	705.839
Fumonisin B_4	H	H	$C_{34}H_{59}NO_{13}$	136379-60-7	689.840

Source: (Debtanu *et al*., 2014)

2.13.3 Trichothecenes

This includes about a sixty member-family of sesquiterpenoid metabolites produced by *Fusarium, Phomopsis, Trichoderma, Myrothesium* and *Stachybotrys* genera. This toxin contains common 12-13 epoxytrichthene skeleton and an olefinic bond with various substituent side chains. High consumption leads to vomiting and alimentary hemorrhage. Humans can also suffer from dermatitis through direct contact (Harman *et al*., 2004). The molecular structure of Trichothecenes and other toxins adopted from (Cole and Cox, 1981) are illustrated Figure 4 below.

Figure 2.3: Molecular structure of Trichothecenes and other toxins

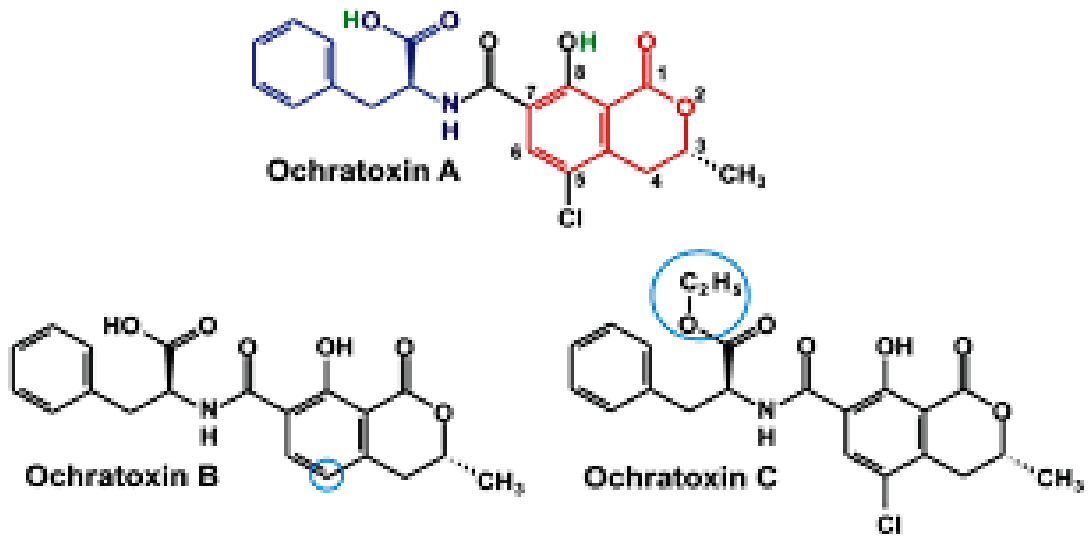


Source: (Cole and Cox, 1981)

2.13.4 Ochratoxin

The mycotoxin Ochratoxinis exists in three different forms of secondary metabolite (A, B, and C) (Bayman and Baker, 2006). All of these metabolites are primarily the products of *Penicillium* and *Aspergillus* species. These three forms are dichotomous since OTB (Ochratoxin B) is a non-chlorinated form of OTA (OchratoxinA) whilst OTC (Ochratoxin C) is an ethyl ester form of Ochratoxin A (Bayman and Baker, 2006). The major contaminant that produces ochratoxin is *Aspergillus ochraceus.* It contaminates a host of commodities including beverages *e.g.* wine and beer. *Aspergillus carbonarius* is the main species that contaminates vine fruit and releases its toxin during the juice formation process (Mateo *et al*., 2007). OTA has been tagged as a carcinogen and a nephrotoxin, and is linked to tumors in the human urinary tract, although research in humans is limited by confounding factors (Bayman and Baker, 2006 and Mateo *et al*., 2007).

Figure 2.4 Molecular structure of Ochratoxin



Source: (Bayman and Baker, 2006).

2.14 Plants and Animals Disease caused by Mycotoxins

Exposure of human and animal to mycotoxins can lead to acute or chronic diseases, and can be lethal in some cases (Richard, 2007). These mycotoxins can cause disease at various stages of plants development and may infect plants without causing symptoms (Desjardins and Plattner, 1998). Some *Fusarium* species such as *F. proliferatum* and *F. moniliforme* usually infect crop plants especially maize plants through wounds created by European corn borer (*Ostrinia nubilalis*) popularly named as "one million dollar bug" due to greater loss of yield that is attributed to it (Munkvold *et al*., 1997). These two species of *Fusarium* genera are effective in producing clinical significant fumonisins which is capable of causing cancer in laboratory rats, leucoencephalomalacia in horses pulmonary edema in swine, esophageal cancer in humans through consumption of food crop with high levels of fumonisins which is classified as a class 2B carcinogen by International Agency for Research on Cancer (IARC, 1993; Marasas, 1995; Rheeder *et al*., 1992).

Fusarium Head/Ear Blight of cereals which results in turning blighted areas brown and senescing prematurely is caused by several species of the *Fusaria* including *F graminearum* which accounts for loss of yield in wheat, oats barley etc. (Stack, 2003). Monitoring the diseases caused by *Fusarium* by isolating the responsible microbes in our staple foods would greatly help create a platform for further researches to mitigate or offset their deleterious effects on human and animal's health.

2.14.1 Human Mycotoxicoses

Mycotoxins detection in human foods and livestock feeds in Ghana and some other West African countries is not a recent development. These mycotoxins are most often detected in highly deleterious contents are compounded by synergistic interactions (Bankole and Adebanjo, 2003). Research finding revealed that victims of

mycotoxicosis who ingested contaminated food have their physiological mechanisms e.g. liver, kidneys and some specialized microorganisms in the gastrointestinal tract metabolizes the toxins (Bankole and Adebanjo, 2003). There is a close relationship between mycotoxin and human health implication on world wide scale. Aflatoxins is known to aggravate hepatitis B infection (JECFA, 2001).

Fumonisins in other vain have been established to be consistently responsible for esophageal cancer (Makaula *et al*., 1996). According separate research reports conducted by Adegoke *et al*., (1996); Oluwafemi and Da-Silva, (2009) and Onyemelukwe *et al*., (1992), aflatoxins have been found in the urine of liver disease patients in Zaria, in blood in Southern Nigeria, in organs of children who died of kwashiorkor in Western Nigeria, and in human semen in Benin city. Similarly Aflatoxin M_1 has been found in breast milk and in the blood of umbilical cord of babies in Nigeria (Adejumo *et al*., 2012). Even though aflatoxicosis is on the ascendency, legislation on Medical Ethics in Ghana restrained researchers from accessing individual case files. In some of these hospitals, the cases are alleged to have been documented as fungal infections, mycoses and mycotoxicoses and because this area of study is not yet very popular, many Africans are secretly dying of mycotoxicoses (Idahor, 2010; Idahor *et al*., 2010)

2.14.2 Livestock

Livestock consumes a chunk of these contaminated feed ingredients. They serve as a transit point of entry into humans and are best described as causative agents or suspected to be contributing factors in farm animal diseases that cause great economic losses (Ocholi *et al*., 1992). Feed ingredients are constituent nutrients for livestock ration. Some of the plants used in ration formulation like legumes, cereals, root nuts

and oil seed, crops are susceptible to mycotoxins contamination yet their deleterious effects are still a grey area (Ocholi *et al*., 1992). Reports by Ocholi *et al*., (1992) showed that mycotoxicoses in livestock are more severe in monogastrics than ruminants as a results of the detoxifying capabilities of some rumen microorganisms. It is was established that Young and pregnant animals are generally the most susceptible to mycotoxicoses (Ocholi *et al*., 1992). Under some conditions, the fungi may liberate potent mycotoxins at levels that may adversely affect livestock production. At moderate levels, effects may appear initially with more obvious symptoms within a few days to several weeks of ingestion of the contaminated foods or feeds. Mycotoxins could possibly have pervasive yet subclinical effects on performance and health in ruminants that may not be noticed (Ocholi *et al*., 1992). Performance losses of 5 – 10% are typical with consumption of moldy feeds even in the absence of mycotoxins (Ocholi *et al*., 1992).

On the other hand, mycotoxins contaminations increase production losses even when the mold is not readily visible. In horses, equine leukoencephalomalacia syndrome (a fatal mycotoxic disease occurring only in horses, donkeys and ponies) is characterized by the presence of liquefactive necrotic lesions in the white matter of the horse cerebrum (Atanda *et al*., 2013). Other pathological changes of this disease include lethargy, head pressing, in appetence, convulsion and sudden death. There are few reported suspected cases of aflatoxicoses in horses associated with *Penicillium purpurogenum* (Ocholi *et al*., 1992 ; Atanda *et al*., 2013).

Rabbits seem apparently unsusceptible to micro doses of mycotoxins especially when dosed orally for a relatively short period. Idahor *et al*., (2008) established that, there is gradual decrease in sperm production rates, final live weights, feed consumption and body weight gain concomitantly with increasing Fumonisin B1concentration in diet.

Similar studies revealed sufficient evidences of carcinogenicity and toxicity at micro doses of 1650-1990 µg Fumonisin B1per kg diet (Ogunlade *et al*., 2004). There were no negative effects on the rabbit's blood cellular components, serum protein metabolism and serum enzymes activities. It is also demonstrated that micro doses of Fumonisin B1can induce physiological and pathological damages in rabbits by reducing feed intake with resultant negative effect on body weight gain (Ewuola *et al.,* 2003). Pregnant New Zealand White rabbits are speculated to be very sensitive to the toxic effects of Fumonisin B1and that maternal toxicity was observed at daily gavage doses of 0.25mg/kg body weight (Ewuola *et al.,* 2003).

2.15 Possible Intervention Strategies/Regulations for Mycotoxins

SSA is known with ecological diversity and climatic contrasts with biophysical characteristics, agro-ecological zones and socio-economic conditions (Aregheore, 2005). This climatic condition enhances the development of fungal strains hence their negative effects (The Codex Alimentarius Commission (CAC) , 2003). The complete eradication of mycotoxin contamination in food commodities is unachievable, however good agricultural practices (GAP) represent a primary line of defense against contamination of cereals with mycotoxins, followed by the implementation of good manufacturing practices (GMP) during the handling, storage, processing, and distribution of cereals for human food and animal feed (The Codex Alimentarius Commission (CAC) , 2003).

(Vandegraft *et al*., 1975) reports that chemical preservatives that completely inhibit mold growth obviously could prevent mycotoxin formation. They however asserts that when preservative is only partly effective, or its effectiveness decreases with time, mold growth can occur, with possible formation of mycotoxins (Vandegraft *et al*., 1975).

CHAPTER THREE

MATERIALS /METHODOLOGY

3.1 Background/Research Design

This study was carried out in three phases as elaborated below;

3.1.2 Experiment One (Survey on Aflatoxin Levels in Groundnuts in storage from different households)

A survey on aflatoxin levels of groundnuts in storage was conducted in the study area. Samples of the stored groundnuts were taken to the laboratory. Aflatoxin analysis was carried out on the groundnut samples obtained from 500 House Holds (HH) in 20 communities across the Region.

3.1.3 Experiment Two (Fungal Isolation)

Aspergillus species that are responsible for production of aflatoxin and other fungal species in freshly harvested and stored groundnuts were isolated.

3.1.4 Experiment Three (Evaluation of drying and storage methods on aflatoxin levels of groundnuts the study area)

Fresh groundnuts samples from 20 household (HH) were analyzed for aflatoxin concentration before drying. Three drying and subsequent storage treatments were also investigated to determine their effect on the levels of aflatoxin in groundnuts. This was carried out with the help of 20 HH farmers from 10 communities in the Region. Moisture levels of groundnuts were also analyzed during storage to investigate the correlation between aflatoxin levels and moisture content.

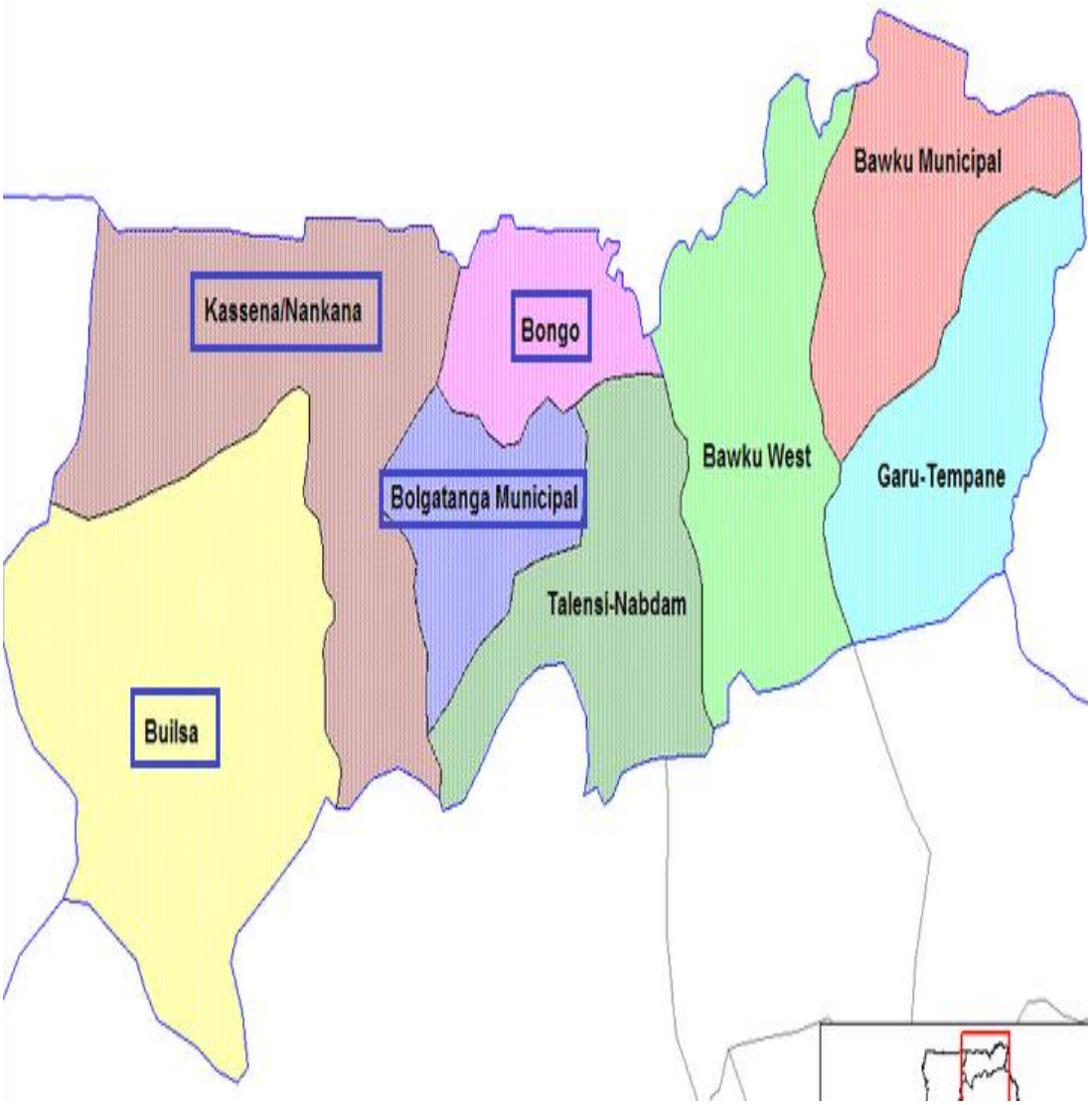
3.2 Survey Area

This research work was carried out in thirty (30) communities in the Upper East Region of Ghana. The Upper East Region is located in the northeastern corner of the country. It is situated in the North to Burkina Faso, East to Togo. The Region is located on Western part of the Upper West Region to Sissala District and to the south by Mamprusi in Northern Region. Bolgatanga is the administrative capital of the Region. The main source of livelihood in the region is agriculture which accounts for (65.9%) of all occupations (MOFA, 2010).

The soil in this Region is mainly developed from granite rocks. The soil is shallow and low in fertility. Valley areas have a soil that is sandy-loams and/or salty clays (MOFA, 2010).

The climate is characterized by a single rainy season that spans from May/June to September/October. The mean annual rainfall during this period is between 800 mm and 1,100 mm. The rainfall is erratic in duration. There is a long spell of dry season from November to April/May, characterized by cold, dry and dusty harmattan winds. Mean annual temperature ranges from as 14 ^{0}C to 35 ^{0}C (MOFA, 2010). The study was undertaken in thirty (30) groundnut farming communities in five (5) administrative districts of the Region. The communities include; Biu, Gaani, Kalsuko, Kapania, Kubimlugo, Tampola, Vonania, Wingo, Naaga, Gingabinia among others.

Figure 3.1: Map of Upper East Region showing Administrative Capitals of the studied Disticts.



Source: **(MOFA, 2010)**

3.3 Selection of farmers for Survey of aflatoxin levels in groundnuts.

Purposive sampling technique was employed to select groundnut farming households in the Upper East Region for the study. 500 groundnut HH farmers were selected across four districts in 20 communities for phase one (Pre-intervention studies) of the research. 20 other farmers were selected in 10 communities for phase three (Intervention) of study.

3.4 Distribution of intervention materials

Twenty (20) HH farmers were sampled from 520 for the phase three of the study. This was achieved by randomly picking 20 House Holds farmers through randomizing their House Hold Identity numbers. Each of the twenty selected farmers was provided with a tarpaulin for drying groundnuts. Farmers were also compensated for raising racks on their fields for drying uprooted groundnuts with leaves. Three plastic (fertilizer) and jute (cocoa) each were distributed among farmer for groundnut storage. Wooden pallets were freely distributed among farmers to keep groundnuts on in order to avoid them being in direct contact with the floor. Distribution of all these materials was done in the month of late July and early August 2014.

3.5 Intervention/Treatment

Three drying methods and three storage methods were evaluated in the study. The three drying methods were; drying on tarpaulin, drying on raised racks and drying on bare ground (farmers practice). The storage methods were storing in either jute bags, plastic bags or farmers old bags. Drying and storage treatments are illustrated in Table 3.1.

Table 3.1 Drying and storage of groundnuts evaluated

	Treatment		
Drying Protocol	**New Jute Bag**	**New Plastic Bag**	**Status Quo bag**
Tarpaulin Drying	Pallet Storage	Pallet Storage	status Quo Storage
Rack Drying	Pallet Storage	Pallet Storage	status Quo Storage
Status Quo Drying	Pallet Storage	Pallet Storage	status Quo Storage

Plate 3.1: Drying methods evaluated

A=Tarpaulin drying method, B= Rack drying method, C= Status Quo drying method

3.6 Sampling

3.6.1 Sampling of farmers stored groundnuts for aflatoxin contamination and occurrence of fungal species.

A total of five hundred (500) samples were taken from five hundred groundnuts farming households in twenty communities across four typical groundnuts farming districts in the Region. Groundnuts samples were taken from the sides, bottom, and middle and on top of the storage bags and composited. Analytical samples were taken from the composited samples for analysis of aflatoxin concentration to determine its distribution in the Region.

3.6.2 Sampling of fresh groundnuts on farmers field for aflatoxin analysis and fungal species composition before drying and storage.

Samples of fresh groundnuts were randomly taken from all quadrants of the field including the middle portion on the day of harvesting in the months of October and November, 2014. Representative samples of one (1) kilogram of nuts were obtained each farmer. The nuts obtained were labeled and sent to the laboratory for aflatoxin analysis.

3.6.3 Sampling of groundnuts after Drying

A good representative sample from the lots was randomly obtained from each of the three drying protocols (Tarpaulin, Rack and Status Quo drying methods) from the nineteen (19) farmers. A total of fifty seven (57) samples were obtained in late November and early December after drying was completed for moisture analysis

3.6.4 Sampling of groundnuts after storage

Representative groundnuts samples were randomly taken from top, middle, bottom and sides of each storage bag of the 3 drying treatments. Nine (9) samples of 200g each were taken from each of the nineteen groundnut farming households. A total of one hundred and seventy one (171) samples were obtained after three months of storage. These samples were sent to the laboratory for aflatoxin contamination determination.

Plate 3.2: Sampling groundnuts from farmers fields

3.7 Groundnuts samples transportation, preparation and refrigeration.

3.7.1 Fresh groundnuts samples transportation

Fresh samples of groundnuts from the field were transported in sterile sampling bags in an ice chest with ice blocks to maintain optimum condition that does not allow the buildup of aflatoxin and other mycotoxins.

3.7.2 Dry groundnut samples transportation

Dry groundnuts samples were kept in a transparent sterile bag and transported in a larger sample bag. No special treatment was given to these samples before they were transported since they were dried and transporting them in the dry weather would not alter the aflatoxin level that already existed.

3.7.3 Shelling of groundnuts

Prior to aflatoxin analysis, the groundnuts were shelled. Shelling was done manually in the laboratory with intermittent washing of hand and disinfecting with 80 % ethanol between samples to avoid contamination of samples of groundnuts yet to be shelled.

3.7.4 Refrigeration

Prior to the analysis, unshelled freshly harvested and dried groundnuts samples were stored in a freezer at -4 ^{0}C.

3.8 Laboratory analysis

3.8.1 Moisture analysis

The moisture contents of groundnuts after drying were determined using the oven drying method. Calculations of the moisture levels were done using the following equation;

$$W\% = \frac{(A-B)}{B} \times 100$$

Where %W = Percentage of moisture in the sample

A = Weight of wet sample (grams),

B = Weight of dry sample (grams)

3.8.2 Determination of Aflatoxin Concentration in groundnuts samples

3.8.2.1 Blending Process

A waring commercial blender was used to blend 200 g of groundnuts from which the analytical sample of fifty grams (50 g) was obtained. 100 ml of 86/14 acetonitrile/Deionized water was added and blended again for one (1) minute as recommended by RomerLabs, (2011)

3.8.2.2 Preparation of Diluents

25 ml of distilled water per a developer(diluent) concentrate was also prepared in accordance to the RomerLabs, (2011) protocol.

3.8.2.3 Calibration of the FQ Reader and Testing of Standards

As required by the protocol, the FQ Reader was calibrated using the internal calibration kits from the manufacturer after which groundnuts paste standards obtained from the US were tested to determine the efficiency of the fluoroquant reader. Three different standards were tested. These were samples with known concentration of aflatoxin.

3.8.2.4 Analyzing Aflatoxin Concentration in nuts

This was done using the Romer FlouroQuant (FQ) Reader and test kits and values or aflatoxin concentration level reported in parts per billion (ppb) in all lots by lot samples. The blended sample with the 86/14 acetonitrile/Deionized was filtered and 1000 µml extracted into a test tube. 1000 µml of developer concentrate was added. The mixture was corked and vortex for 10 seconds. The test tube was then cleaned

and the cork removed. The test tube and its contents was the inserted FQ reader slot for the aflatoxin level to be determined.

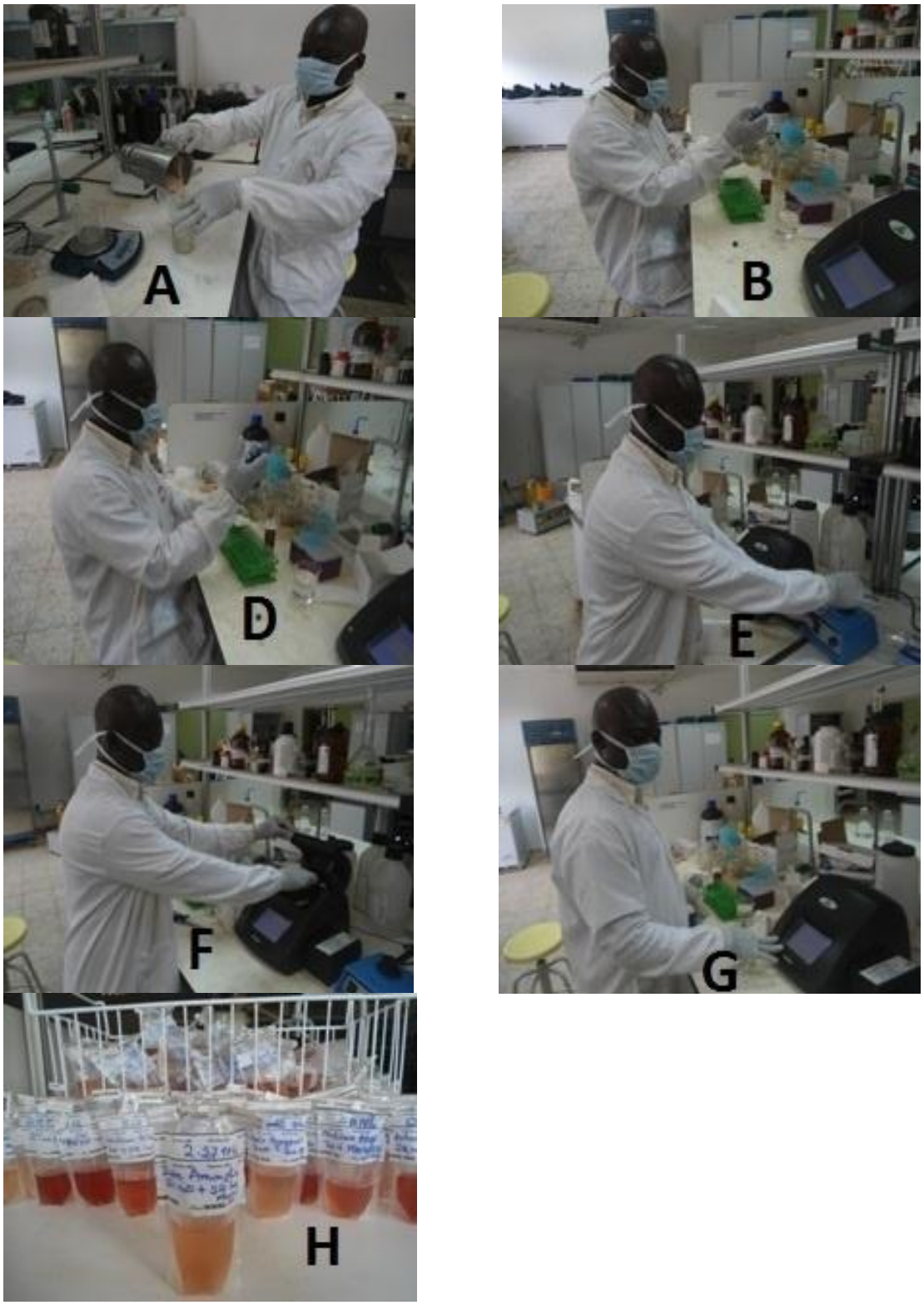


Plate 3.3: Analyzing Aflatoxin Levels in Groundnuts in UDS Spanish Laboratory Complex

Note: A-Filtering 50 g of groundnuts sample grounded with 100ml of 86/14 by volume Acetonitril/Water. **B –** Pipetting 1000 µml of groundnuts filtrate, **C –** Extracting filtrate using a syringe plunger, **D –** Adding 100 µml of developer (25 ml of water/per developer concentrate), **E –** Vortexing analytical extracts, **F –** Inserting cleaned curvet containing analytical sample into the FQ reader to determine the aflatoxin level, **G –** Reading the displayed value by the FQ reader, **H –** Aflatoxin level recorded on the sampling bag of remaining filtrates.

3.8.2.5 Waste Disposal

Sample extract solution and developer were transferred into liquid container for proper disposal. All other materials were disposed off in the normal solid waste system in the Spanish laboratories complex.

3.8.3 Fungal isolation and identification

3.8.3.1 Isolating and identification of fungal species associated with the groundnuts samples.

Fungal species isolation was done from both freshly harvested groundnuts from the field and stored nuts. This was to identify the variation of fungi that occur on the field and those that occur in storage. It was also used to predict fungal species responsible for higher aflatoxin proliferation and lower aflatoxin levels. Fungal isolation was carried out on samples with aflatoxin level within the US standard (0-20) ppb and samples with higher aflatoxin level than the US standard (> 20 ppb) from each of the studied communities.

3.8.3.2 Media Preparation

Potato Dextrose salt agar (39 g per 1 ml) was prepared according to the manufacturer's (Oxoid, Difco, USA) instruction, autoclaved at 121 ^{0}C for 15 minutes

and cooled to 45 ^{0}C to eliminate bubbles. Tetracycline solution was added to the media to selectively inhibit the growth of bacterial cells.

Prepared media was poured in the Petri dishes under sterile condition of lamella flow chamber to avoid contamination. Bubbles were eliminated from the poured media by flaming using bursen burner. Nose mask was worn to avoid breathing directly into the area of plating to avoid introducing bacterial and other contaminants into the media. Additionally hand gloves were worn to prevent hand contamination of media.

3.8.3.3 Isolation protocol/ Culturing of fungal species

Direct plating method of analysis of grounded groundnuts/shelled groundnuts samples were carried out to isolate the aflatoxin producing *Aspergillus* and other fungal spp (Warcup, 1960; Misiivec, 1984). The poured media on the Petri dish was divided into four quadrants. Shelled groundnut sample was then inoculated on each quadrant including the center. Each community sample was replicated ten times with a Petri dish taking five inoculums. Images of isolation are shown below.

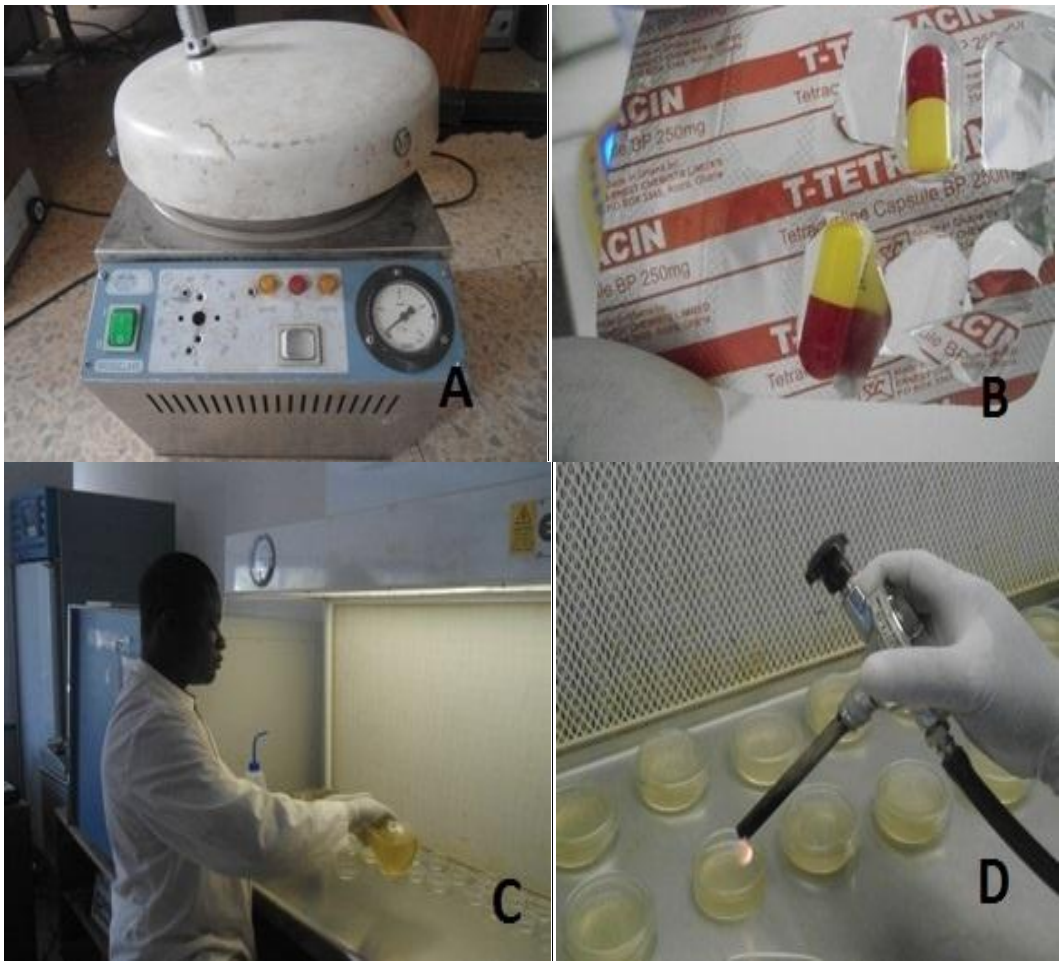


Plate 3.4: Isolating fungal species in Groundnuts samples in UDS Spanish Laboratory Complex

Plate **A** = Autoclaving prepared media and glassware **B =** Tetracycline solution added to media to stifle the growth of bacteria. **C** = Dispensing media into Petri dishes. **D** = Flaming dispensed media to eliminate bubble

3.8.3.4 Incubation and fungal species identification

Plated groundnuts were incubated at room temperature. Fungal species that grew on the media were rescued and sub-cultured to obtain pure cultures. Fungal identification done based on their cultural characteristics such as colony color, shape and nature of mycelia growth (Hocking and Pitt, 1989 ; Klich and Pitt, 1988.). After that mycelia bits and spores were fixed on slide using lactophenol blue and covered with cover

slips. The slides were then examined under a microscope for distinctive features such as the nature of the hyphae, shape, conidiophores and conidia (Mislivec, 2000).

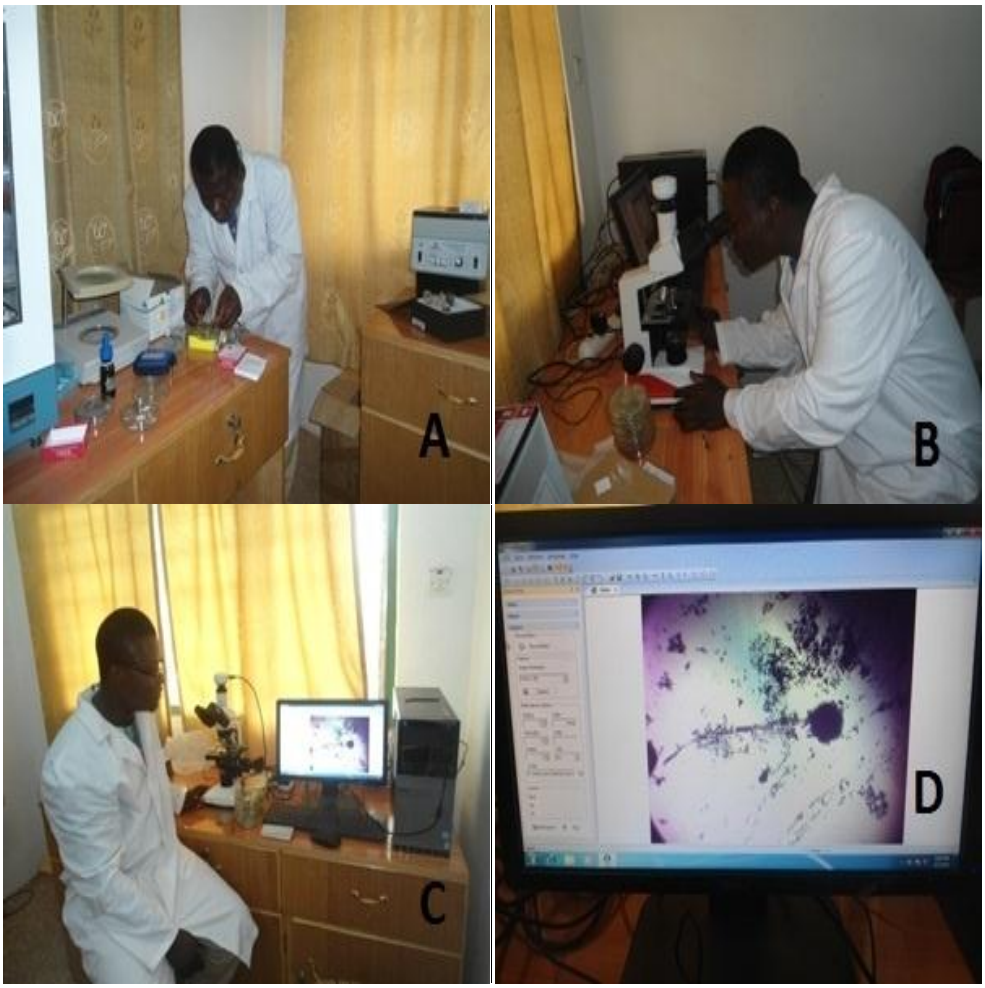


Plate 3.5: Slide preparation and microscopic identification

Plates **A** = Preparing slide to examine **B** = Viewing species under the microscope **C** = Projecting microscopic species onto the screen of a computer **D**= Image of fungal species displayed on the monitor

3.8.3.5 Disposing off cultured fungal species

To prevent cross contamination of other cultures in the laboratory, the plated fungi on media were autoclaved at 121^{o}C for fifteen (15) minutes to kill all cultures before disposing them off in dustbin.

3.9 Statistical data analysis

- ❖ Data analysis was done using Genstat discovery, 4th Edition 2011, VSN International Ltd., Hemel Hempstead, UK. The following analyses were carried out.
- ➢ Analysis of variance (ANOVA) was carried out on the data on aflatoxin levels in the groundnuts collected in the study.
- ➢ Means and Standard deviations of aflatoxin values from 20 communities were computed using summary of statistics on GenStat to determine the distribution of aflatoxin concentration in the Region.

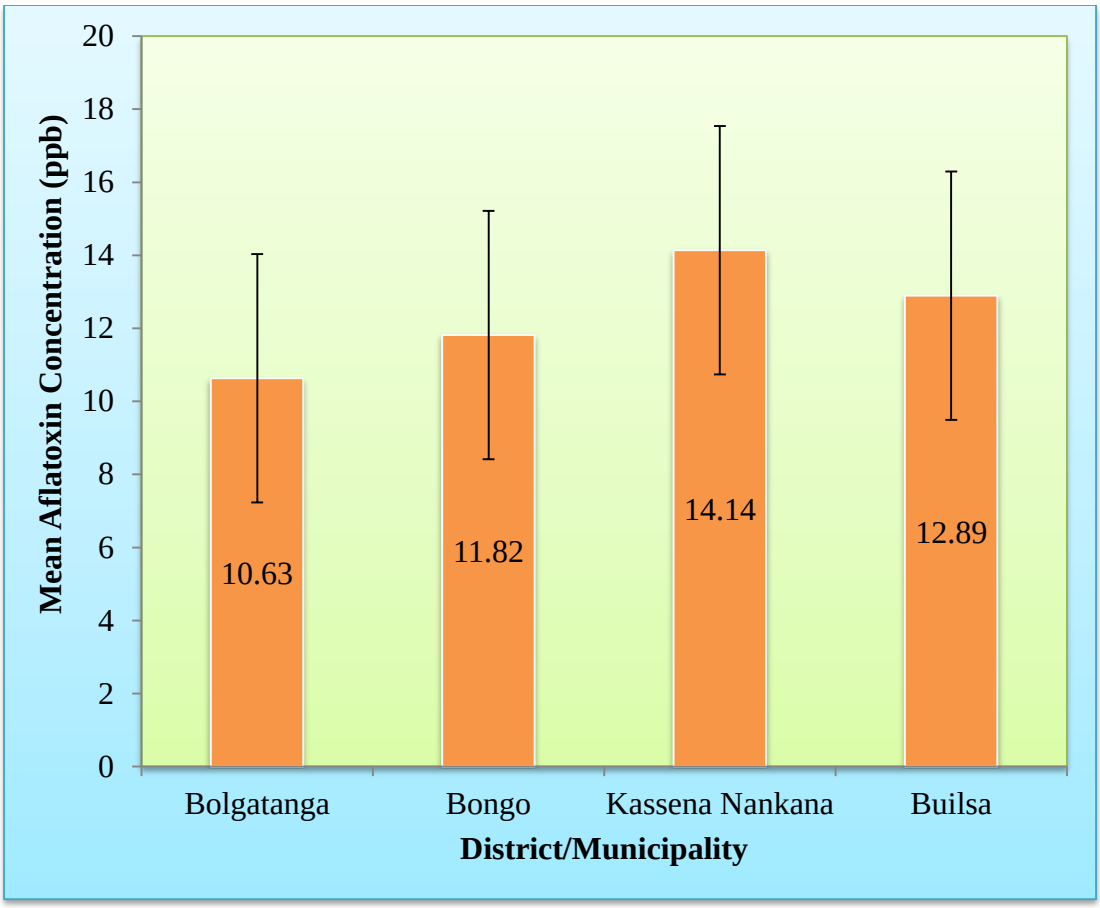
CHAPTER FOUR

4.0 RESULTS

4.1 Distribution of aflatoxin contamination in farmers stored groundnuts from the study area

Figure 4.1 shows the mean levels of aflatoxin concentration recorded on groundnut samples collected from the four districts where the study was carried. The highest aflatoxin concentration of 14.14 ppb was recorded in the Kassena Nankana district while the lowest of 10.63 ppb was recorded in the Bolgatanga municipality. Builsa district had slightly higher concentration of 12.81 ppb than the 11.82 ppb recorded in the Bongo district. The level were generally lower than the EU and Ghana permissible check of 15 ppb and the US allowable limits of 20 ppb. It was observed that, the aflatoxin distribution in the four districts in the region was significant with p <.001.

Figure 4.1: Aflatoxin levels in farmers stored groundnuts from the study area



4.1.2 Aflatoxin concentration among communities

Aflatoxin concentration in sampled groundnuts from the different communities ranged from 5.10-29.32 ppb. The lowest was recorded in Asibiga while the highest was recorded in Nyangua (Table 4.1). Though the mean aflatoxin levels were generally low across most of the studied communities, several out layers were encountered. With the exception of Bonia, Sumbrungu Kologo, Dulugu Asanorebisi, Dulugu Aginibisi, and Basiengo Amenabisi that had all their sampled groundnuts aflatoxin levels within the US permissible point, the rest of the communities recorded some higher values above the US limit.

Table 4.1: Groundnuts Aflatoxin Levels in Communities/Districts in the Region

District	Community	min	Max	Mean± SD
	Sumbrungu Kologo	5.03	17.21	8.3 ± 2.97
	Dulugu-Asanorebisi	5.57	15.34	9.58 ± 2.45
Bolgatanga	Yikene Adolibia	11.22	53.69	16.43 ±7.81
	Sumbrungu Yeobongo	4.68	35.18	11.73 ± 7.391
	Dulugu Aginibisi	3.87	11.33	6.90 ± 2.22
	Vea Gunga	5.24	47.66	14.75 ±10.40
	Asibiga	1.87	14.07	5.10 ± 2.53
Bongo	Beo-Kasingo	5.21	32.72	12.11±6.14
	Balungu Gantorisi	10.32	22.22	14.12 ± 2.72
	Feo-Asamibisi	5.60	61.79	12.54±10.79
	Bonia	8.89	13.46	9.15 ± 1.23
	Tampola	10.75	46.64	17.53 ± 6.69
Kassena Nankana	Achobisi Kasam	1.72	37.17	5.38 ± 6.52
	Nyagua	2.18	114	29.32 ± 20.75
	Basingo Amenabisi	3.04	18.27	7.81±3.28
	Chuchuliga Akpateyere	2.85	28.44	7.27 ± 5.58
	Chuchuliga Tiema	2.4	20.82	5.80 ± 3.43
Builsa	Chuculiga Yipaala	5.55	37.75	12.45 ± 8.31
	Chuchuliga Adabina	8.02	49.52	11.32 ± 7.4 9
	Wiaga Yemusa	7.67	229.9	25.07 ± 39.62

p<.001, **LSD**= 6.727

4.2 Fungal species isolated from groundnut samples used for the study.

Table 4.2 shows the type of fungi species and their cultural and morphological features isolated from groundnuts samples collected from the various districts in the region.

There was fair distribution of fungal isolates across the region. Out of the 301 fungal isolates, Bolgatanga municipality, Bongo, Kassena Nankana and Builsa districts respectively recorded 75, 73, 80 and 73 isolates. The number of aflatoxicogenic fungal isolates was higher in samples whose aflatoxin levels were above the US standard of 20 ppb. *A. flavus* was recorded 53 times in groundnut samples that had higher aflatoxin and 23 times in samples with lower aflatoxin levels than 20 ppb. *A. parasiticus* was also recorded 57 times in groundnuts with higher aflatoxin levels but 21 in groundnuts with lower aflatoxin levels. Similar observation was made in *A. fumigatus. A. niger* isolates was however a reverse of the trend as their number in low aflatoxin samples were more than in high aflatoxin infested samples (42 isolates in low and 27 isolates in high aflatoxin samples). *R. stolonifer* was recorded 25 times in both high and low aflatoxin infested samples. Generally 51.2 % of the entire isolates were aflatoxin producers (*A. flavus* and *A. parasiticus*) with the remaining being non aflatoxicogenic. Table 4.2 and 4.3 respectively shows the macroscopic/microscopic features observed on isolates and percentages of the fungal species isolated from the various districts in the region. Figure 4.2 also detailed the individual fungal species percentage isolated from the studied districts. The quantitative representation of the various isolates per community basis in the region is attached as appendix I.

Table 4.2: Cultural and morphological characteristics of isolated fungi

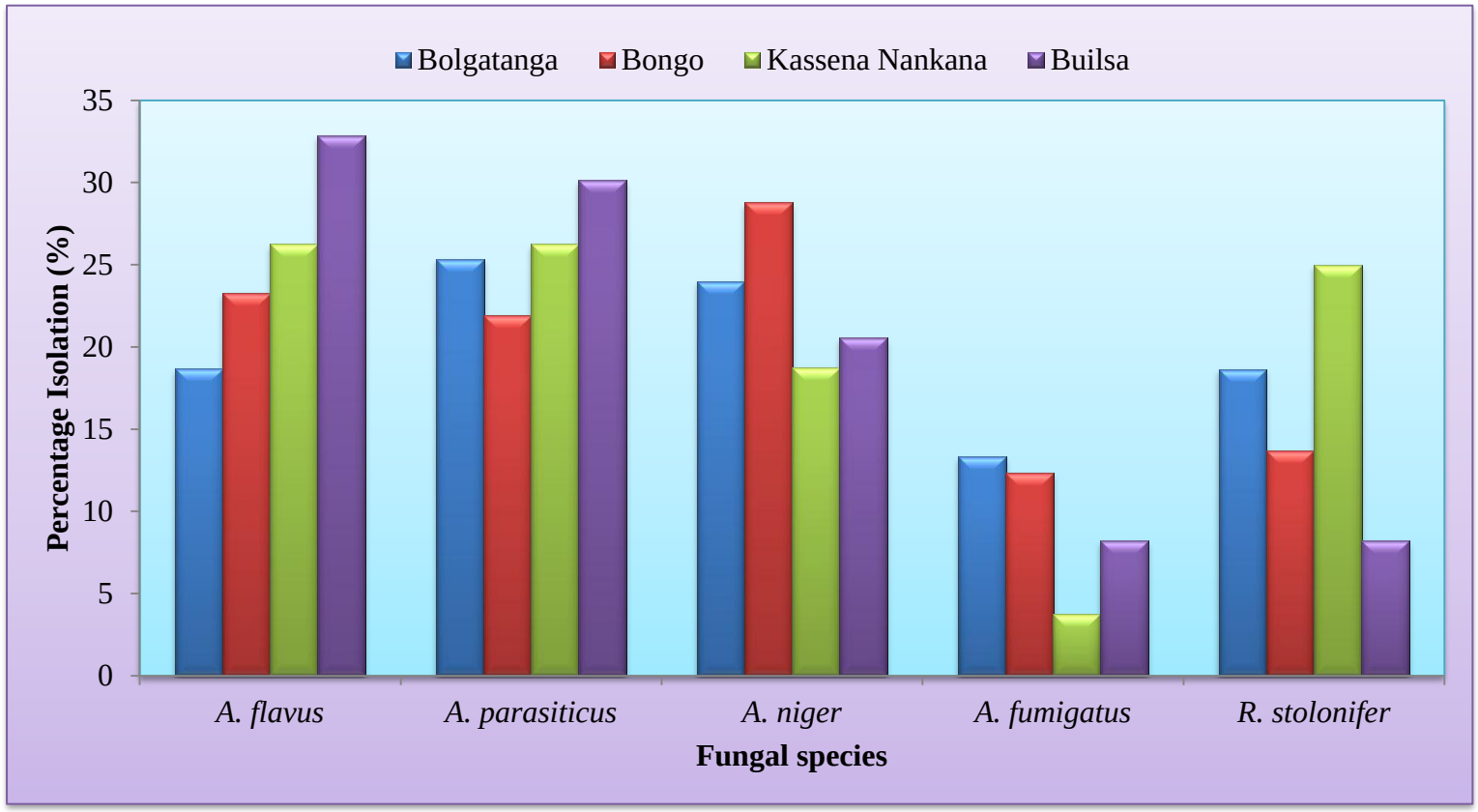
Macroscopic Feature of Fungi on PDA	**Microscopic Feature of Fungal isolates**	**suspected species**
Colony appeared Yellowish-Green in coloration with powder-like substances on the surface. Whitish mycelia formed around the edges of most colonies. The reverse side of colonies are brownish	Fungi are globose or ellipsoid and radiate head, slender conidiophores were seen terminating into swollen conidia with smooth finely hair-like sporangia. Sclerotia were a bit brownish in coloration. Vesicle serration were biseriate	*Aspergillus flavus*
Colony appeared very greenish in color and somehow pinkish on the reverse side. Colonies texture appeared rough on the media surface. Pale brownish to whitish mycelia formed around the colony	Stipes were found to be colorless, Uniserate spherical vesicles were observed, shape of the head was also glubose as in *Aspergillus flavus* with distinctly rough surface conidia held by a slender conidiophores.	*Aspergillus parasiticus*
White mycelium growth around colony and surface. Growth had black color The margins of the fungi were whitish. The back (reverse) side of the Petri dish was had pale yellowish to white color.	Conidia head was seen as glubose, rough and irregularly arranged. Conidiosphore was seen as a long, smooth, black and terminating in a swollen structure. Vesicle were observed to be biserate and large in size. Metula covered the entire Petri dish.	*Aspergillus niger*
Colony surface color was blue-green to grayish near the centre and white to tan on the reverse side of the Petri dish.	Uniserate pyriform. metula covered about two-third of the surface. Conidiosphore was glubose Smooth and spinose conidia surface	*Aspergillus fumigates*
Cotton-like colony apeared on the surface of the media which turn brownish in the third day of incubation. Rapid aerial growth was evident. The reverse side of the Petri dish was dark. Growth enormously covered the entire Petri dish.	Sporangia are elaborate, sac-like and spore bearing. Possess septate hyaline hyphae that link the sporangiophores.	*Rhizopus stolonifer*

Table 4.3: Occurrence of fungi species recorded from different districts.

	Percentages (%) of fungal Isolates in High and Low aflatoxin determined Samples										
	A. flavus		*A. parasiticus*		*A. niger*		*A. fumigatus*		*R. stolonifer*		
District/Municipality	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**TOTAL**
Bolgatanga	13.33	5.33	16.00	9.33	8.00	16.00	4.00	9.33	9.33	9.33	**100**
Bongo	17.81	5.48	13.7	8.22	12.33	16.44	5.48	6.85	6.85	6.84	**100**
Kassena Nankana	16.25	10.00	21.25	5.00	7.50	11.25	0.00	3.75	16.25	8.75	**100**
Builsa	23.29	9.59	24.65	5.48	8.22	12.33	4.11	4.11	0.00	8.22	**100**

High = Isolates from samples with Aflatoxin level >20 ppb, Low = sample Aflatoxin level < 20 ppb

Figure 4.2: Percentages of fungal species isolated from the different districts.



4.2.1 Plates of the cultural and morphological characteristics of isolated fungal species

Characterization was done with reference to Pitt and Hocking, (1996) and McClenny, (2005) fungal identification manual. Plates 4.1 – 4.4 shows specific features for the different *Aspergillus spp* isolated and identified, and 4.5, Rhizopus spp.

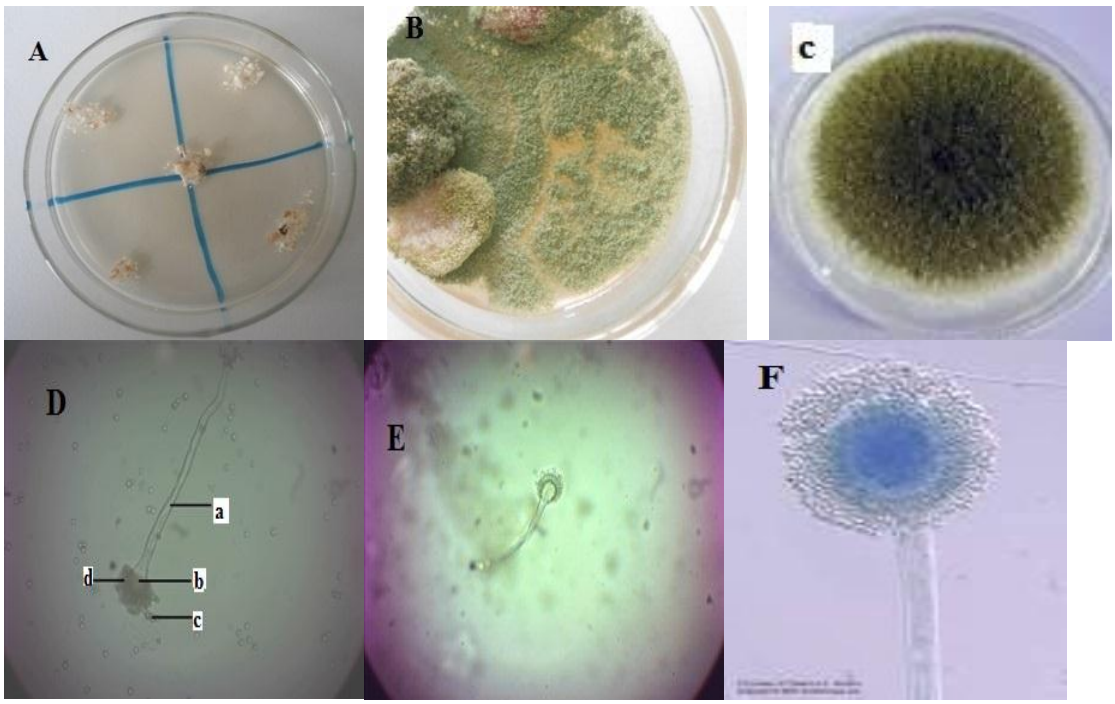


Plate 4.1: Cultural and morphological characteristics of *Aspergillus flavus*

Note: A = Inoculated sample on PDA, **B, C** = Colony growth exhibition, **D** and **E** = Microscopic view of *A. flavus* using 20X, **a** = conidiospore, **b** = vesicule **c** = conidia, d= phlalide, **F** = File image for comparative characterization (McClenny, 2005; Pitt and Hocking, 1996).

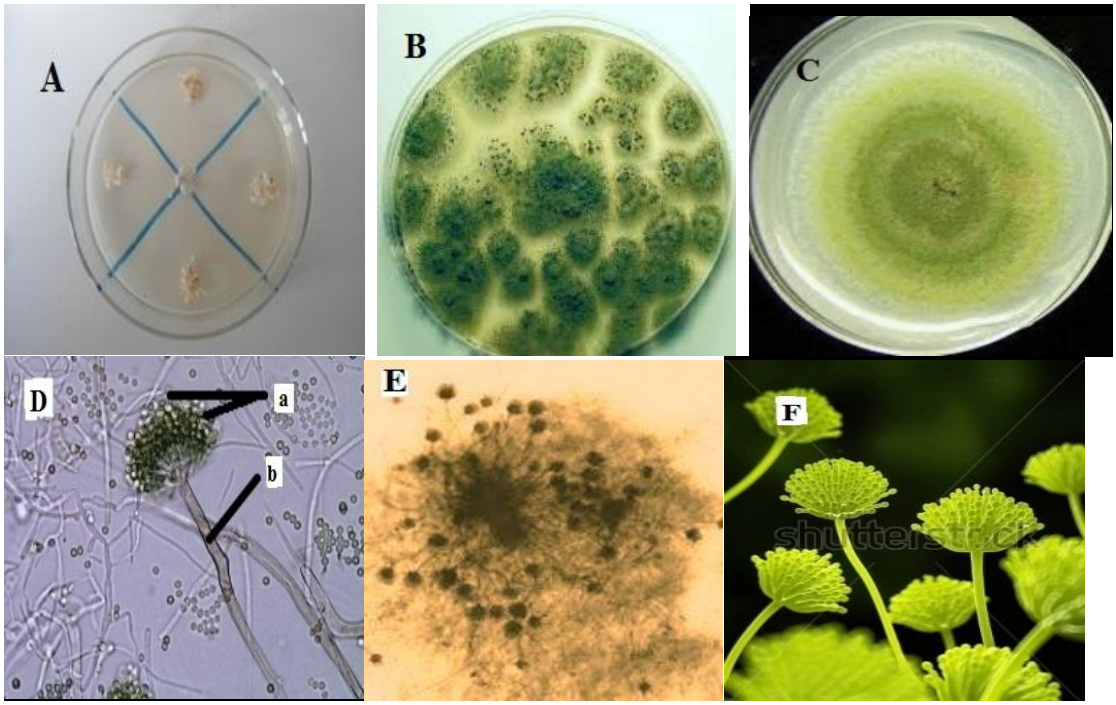


Plate 4.2 Cultural and morphological characteristics of *A. parasiticus*

A= Samples inoculated on PDF, **B =** Fungal colony growth on medium, **C** = Sub-cultured of Colony into Pure colony, **D=** Microscopic view of *A. parasiticus* using 20X a= conidia borne on phialide, b= conidiophores **E =** Microscopic view of *A. parasiticus* of whole colony on media using 10X, **F=** *Aspergillus* Identification Manual image of *A. parasiticus* for comparative studies (McClenny, 2005; Pitt and Hocking, 1996).

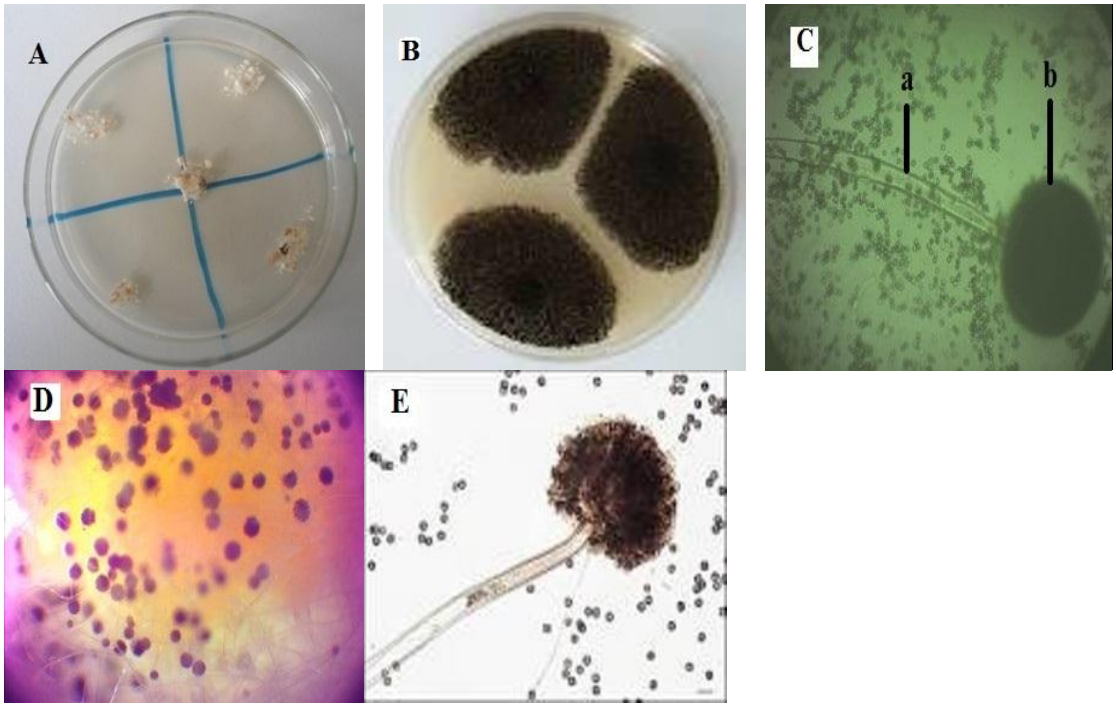


Plate 4.3: Cultural and morphological characteristics *of Aspergillus niger*

Note: **A**= Inoculated groundnut sample**, B=** Colony growth exhibition, **C=** Microscopic view of *Aspergillus niger* showing its conidiophore (a) and glubulose conidia head (b) using 20X**, E=** Whole colony view of *Aspergillus niger* using 10X**, F=** *Aspergillus* Identification Manual image of *Aspergillus niger* for comparative studies (McClenny, 2005).

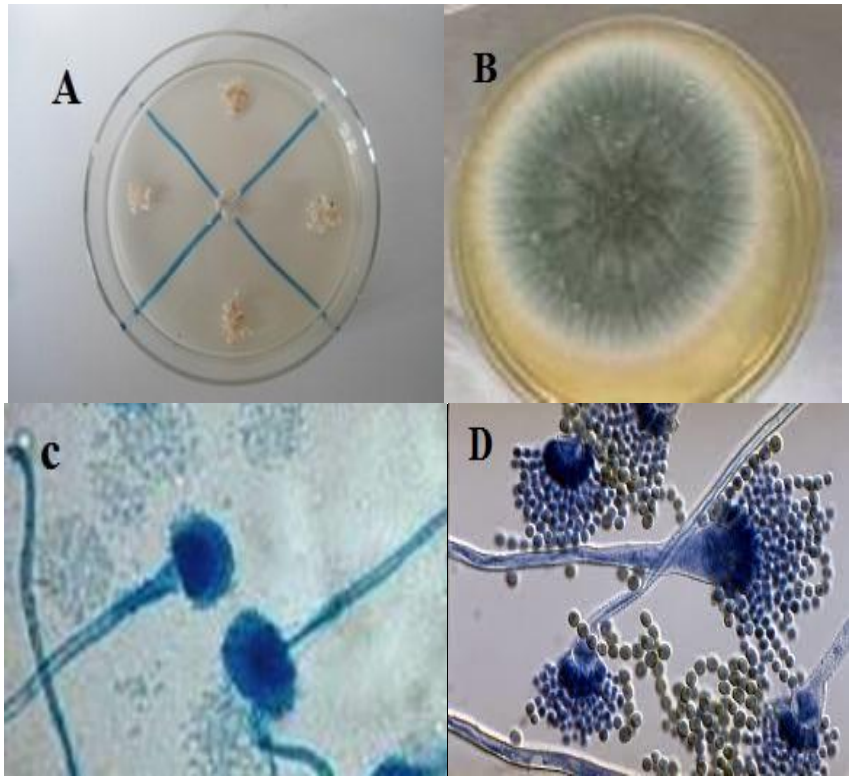


Plate 4.4: Cultural and morphological characteristics of *Aspergillus fumigates*

Note: A = Inoculated sample on PDA, **B**= Colony growth on media, **C=** Microscopic view of *Aspergillus fumigatus* using 20X showing the spinose surface of the conidia supported by the conidiophore**, D=** *Aspergillus* Identification Manual image of *Aspergillus fumigatus* for comparative studies (McClenny, 2005).

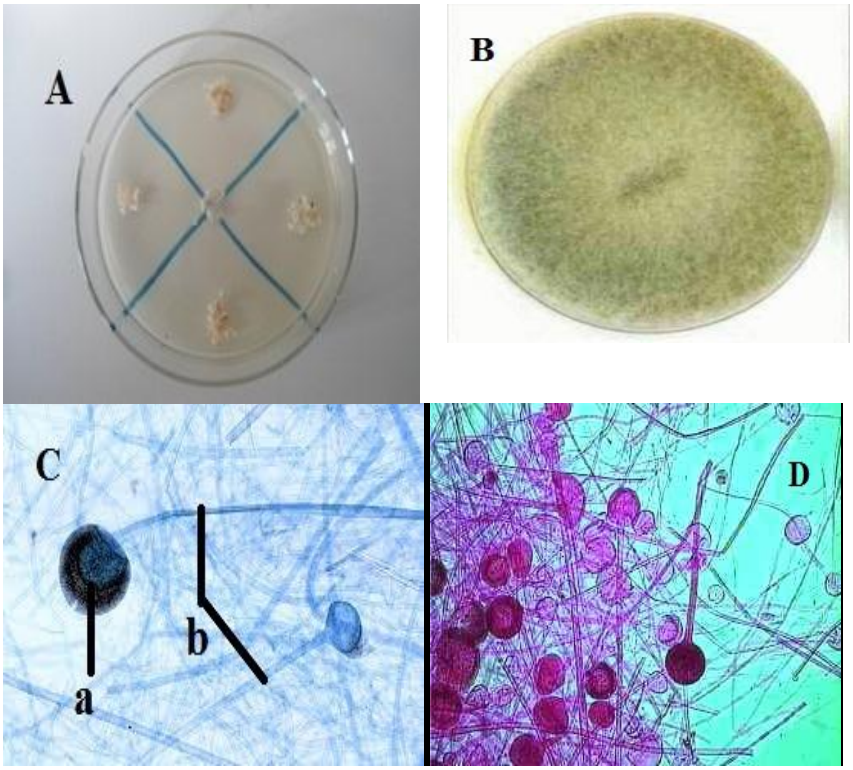


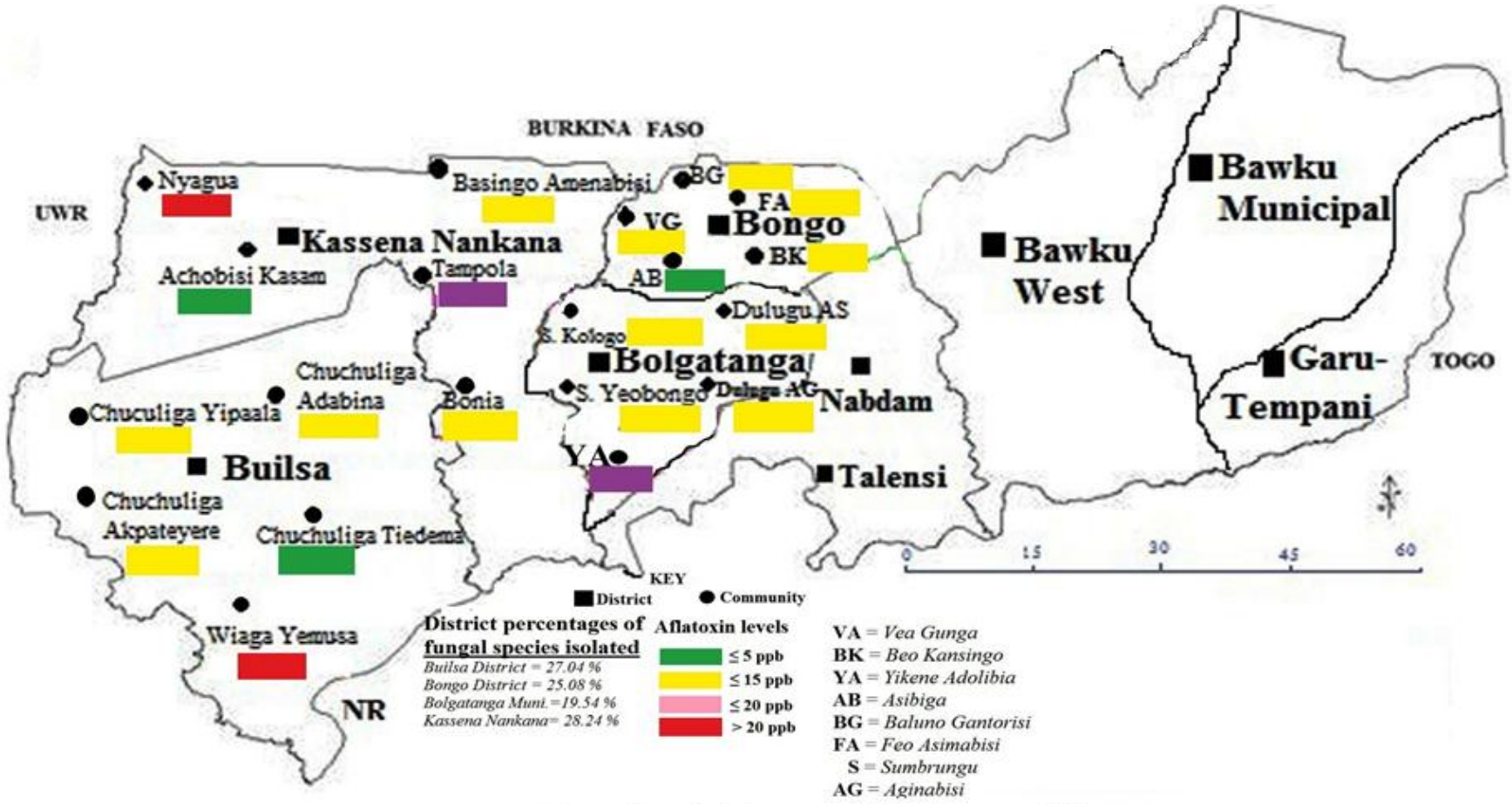
Plate 4.5: Cultural and morphological characteristics of *Rhizopus stolonifer*

Note: A = Inoculated sample on PDA, **B**= Colony growth exhibited, **C=** Microscopic view of *Rhizopus stolonifer* showing b= sporangiophore, a= sporangium containing spores showing using 20X, **D**= Rhizopus Identification manual image for comparative studies (Pitt and Hocking, 1996).

4.2.2 Fungal and Aflatoxin map of the Upper East Region

The mean Aflatoxin levels of the studied communities in the various districts are illustrated in the map below. Areas highlighted in green color indicated that the mean aflatoxin level in those communities are less or equals to 5 ppb. Yellow highlighted communities have their aflatoxin level less or equals to 15 ppb. Violet marked communities aflatoxin concentration is less or equals to the US permissible check point of 20 ppb. Nyangua and Achobisi communities highlighted red implies that their mean aflatoxin level were higher than the US allowable limit of 20 ppb. The percentages of isolated fungi species are indicated in the legend below the map.

Figure 4.3 Aflatoxin (ppb) and fungal species map of Upper East Region



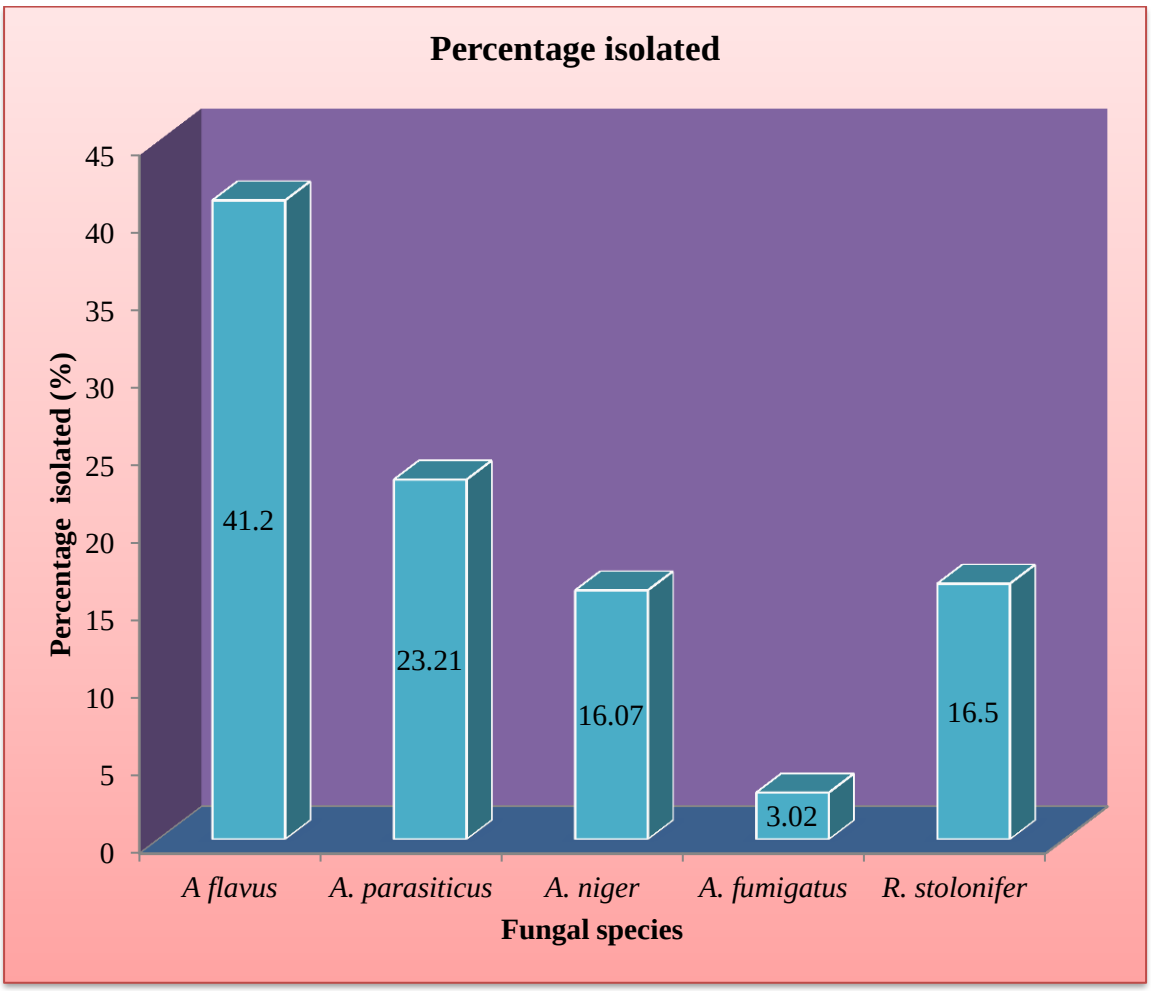
Research period: January, 2014 - January, 2015

4.3 Aflatoxin levels and fungal isolates in freshly harvested groundnuts

The mean aflatoxin level in freshly harvested groundnuts obtained was 6.707±2.107 ppb. The minimum and maximum values obtained from the analyses were 3.33 and 12.17 ppb respectively.

There was diversity of fungal species among isolates. Aflatoxicogenic species; *A. parasiticus* and *A. flavus* accounted for the highest 41.20 % and 23.21 % respectively (Figure 4.4).

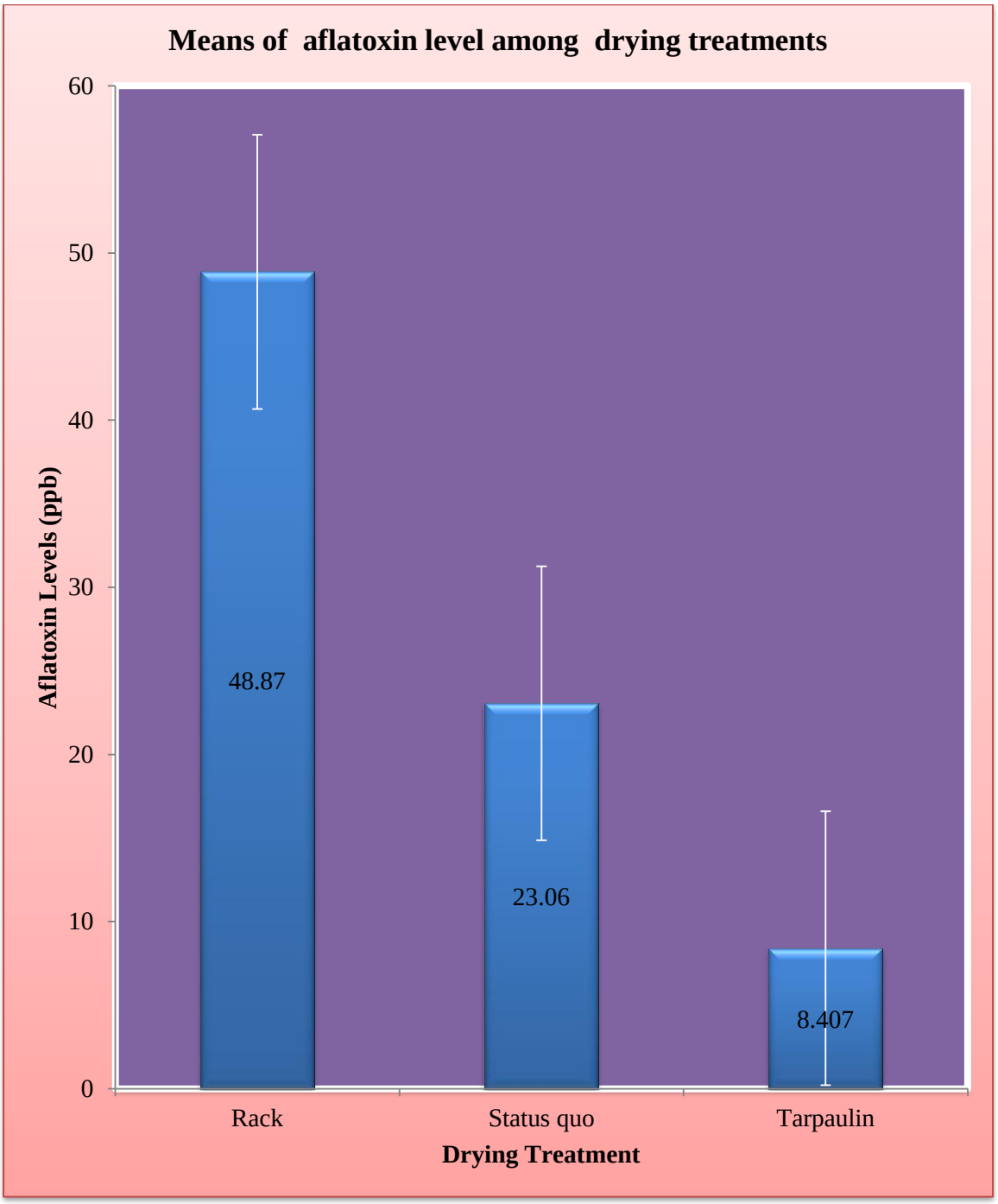
Figure 4.4: Percentage occurrence of fungal Species isolated from fresh groundnuts



4.4 Effect of different drying methods on the aflatoxin levels in groundnuts

Results from the drying treatments showed that tarpaulin dried groundnuts were successful among the three treatments in lowering aflatoxin concentration. Their aflatoxin levels were generally low (1.00- 21.97 ppb) as it was found to be 58 % lower than the US standard of 20 ppb. Status Quo drying method also presented lower mean aflatoxin level than the rack drying method. The mean aflatoxin concentration of the Status Quo drying method appreciated by 15 % per 20 ppb of the US allowable limit for human consumption. Groundnuts dried on rack were worst in aflatoxin reduction as its mean aflatoxin concentration was 48.8 ppb and 144.35 % higher than the US standard of 20 ppb. Below is a bar graph showing the mean aflatoxin concentration of the various drying protocols (Tarpaulin, rack, and farmers' method). Refer to Appendix II for raw data.

Figure 4.5: Effect of different drying methods on the aflatoxin levels in groundnuts



p = 0.034 **LSD**= 28.39 **CV%** = 53.10

4.4.1 Effect of combination of drying and storage methods on aflatoxin contamination of groundnuts

The mean aflatoxin levels of the Tarpaulin dried groundnuts stored in jute bag (T+J), Tarpaulin dried nuts stored in plastic bag (T+P) and Tarpaulin dried nuts stored in status quo bag (T+SQ) were statistically indifferent from one another. The farmers Status Quo means of drying their groundnuts proved moderate in aflatoxin reduction as compared to the rack method. Status Quo dried groundnuts stored in Jute and Plastic bags (SQ+J and SQ+P) produced aflatoxin levels that were not statistically different from the Tarpaulin drying method but numerically different from the aflatoxin concentration of nuts dried on Racks. The rack dried groundnuts performed poorly in reducing aflatoxin concentration of the nuts as its maximum value recorded was 394.6 ppb for nuts stored in Status quo bag. It was observed that, the storage bags in the various treatments did not play major role in the reducing of the aflatoxin concentrations in the region as illustrated in table 4.4. The raw data is attached as Appendix II.

Table 4.4: Aflatoxin level among drying/storage treatments

Treatment	Observations	Minimum	Maximum	Mean ± SD
R+SQ	18	1.38	394.6	71.66 ± 127.1**a**
SQ+SQ	17	2.21	451.8	39.82 ± 108.0**ab**
R+P	19	2.84	288.8	38.24 ± 63.24**ab**
R+J	19	2.39	357.5	36.70 ± 79.30**ab**
SQ+J	18	3.15	63.95	16.74 ± 15.15**b**
SQ+P	18	1.82	36.55	12.62 ± 8.88 **b**
T+P	19	1.00	21.97	9.16 ± 5.19**b**
T+J	19	3.58	18.42	8.16 ± 4.13**b**
T+SQ	17	1.56	21.46	7.90 ± 4.45**b**

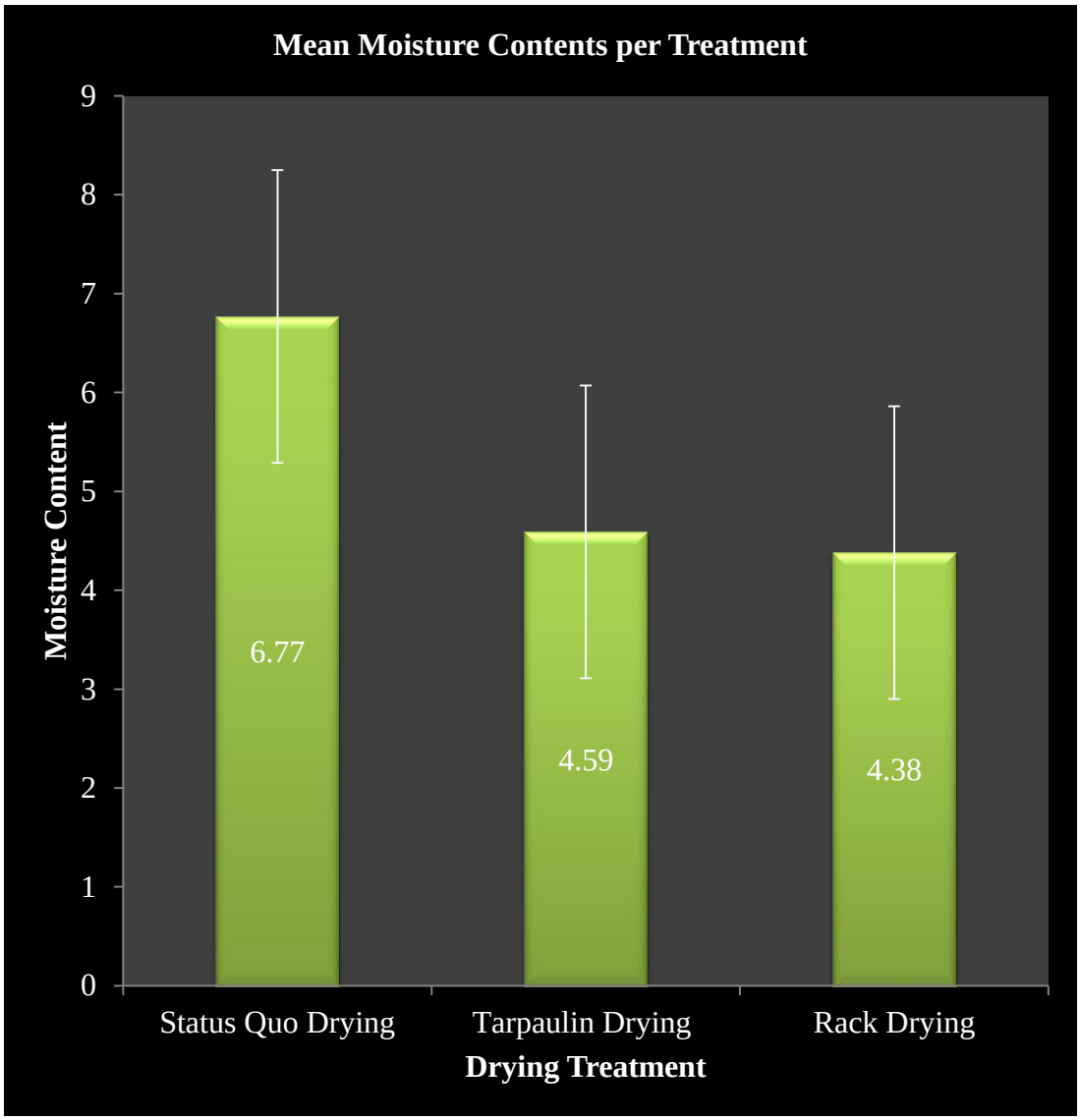
p = 0.049, **LSD**= 42.74, **CV%** = 242.50,

R= Rack, T= Tarpaulin, SQ= Status Quo, P= Plastic, J= Jute

4.5 Moisture levels among drying treatments

The minimum, maximum and mean moisture contents were respectively 2.56, 5.91 and 4.59±1.00 for nuts dried on Tarpaulin, 3.72, 13.58 and 6.77±3.10 for Status Quo drying method and 2.25, 12.2 and 4.38±2.21 for Rack dried nuts. All treatments recorded lower moisture contents than 10% (Figure 4.6).

Figure 4.6: Moisture Contents among the Drying Treatments



P= 0.003, **LSD**= 1.481, **CV%** = 43.2

CHAPTER FIVE

5.0 DISCUSSION

Generally, the Regional aflatoxin levels were low. The averages recorded per district bases were not very different from one another as all the averages were below the US and EU/Ghana regulatory limits of 20 and 15 ppb respectively. Sixteen (16) communities representing 80 % of the studied communities met the EU and Ghana aflatoxin limit of 15 ppb of human consumable groundnuts and therefore do not risk their groundnuts banned from the EU markets. 90 % of the studied communities representing 18 communities met the US aflatoxin level of 20 parts per billion. The low aflatoxin levels recorded in the Upper East Region may be as a result of good off-field handling practices of groundnuts. A close observation showed that most of the farmer's nuts in the studied communities did not contain much debris. Dirt favors aflatoxin proliferation by *Aspergillus* spp and their absence accounted for the lower aflatoxin level. Fandohan *et al*., (2005) revealed that sorting, washing, winnowing, crushing combined with de-hulling of maize grains were effective in reducing mycotoxins to a significant level.

Wiaga Yemusa community in the Builsa District and Nyagua community in the Kassena/Nankana district recorded the highest aflatoxin levels above the Ghana, US and EU acceptable limits in the region. These higher values recorded might be due to varying reasons such as improper drying technology, poor storage systems and higher pre-harvest contaminations among others. Park, (2002); Muntgei *et al*., (2012) in separate research showed that, poor post-harvest handling of crops such as bare ground drying, high moisture contents in nuts, poor storage methods among others results in high occurrence of aflatoxin in the crop. Sorting through hand picking or

winnowing out damaged and infected grains from the food commodity can result in 40-80% reduction in aflatoxins levels (Atanda *et al*., 2013).

Fungal species isolated from the high and low levels of aflatoxin infested groundnuts showed varied species consisting of aflatoxin producers. The percentage of aflatoxin producing fungal species isolated from groundnuts with higher aflatoxin level than 20 ppb (US regulatory limit) were generally found to be higher than those isolated from groundnuts samples with lower aflatoxin levels than 20 ppb across the Region. The results showed that, more aflatoxicogenic (*A. flavus* and *A. parasiticus*) species were present in groundnut samples with high concentration of aflatoxin. This suggests that these species are the main aflatoxin producers in the samples. The vice versa was also observed as fewer aflatoxicogenic species were recorded from groundnuts with lower aflatoxin levels. This finding is consistent with a research report credited to Abriba *et al*., (2013) in which aflatoxicogenic fungal species were predominantly isolated from aflatoxin contaminated foods such as groundnuts, rice among others. *A. Flavus* and *A. parasiticus* which are the known aflatoxin producers were consistently isolated from the samples. This observation was consistent with the detection of aflatoxin in the samples. This finding further agreed with research findings by The Codex Alimentarius Commission (CAC) /Representative Concentration Pathways' (RCPs), (2004); Galvez *et al*., (2003); Fiyaz *et al*., (2012) that identified *A. flavus* and *A. parasiticus* as major aflatoxin of producers. It is however worth mentioning that *A. flavus* and *A. parasiticus* occurred in all samples that tested positive for aflatoxin, a further confirmation that, the species were responsible for aflatoxin contamination in Ghana as well.

Aspergillus niger isolates presented a competitive existence with the aflatoxin producers in the samples. The quantitative representation of this fungus in the samples

was noted to be close in range with aflatoxin-producer isolates in high and low aflatoxin determined samples. This cosmopolitan fungus occurred widely in almost 90 % of the samples. Its occurrence however may have zero correlation with aflatoxin proliferation as they are not known aflatoxin producers (Azziz *et al*., 2005). *A. niger's* existence in groundnuts is both advantageous and injurious to the consumer. Its benefit arises from the fact that it stifles the development of *A. flavus* and *A. parasiticus* and offsets their effect of aflatoxin production (Muntgei *et al*., 2012). This might be the reason that samples that recorded higher occurrence of these species had lower levels of aflatoxin. The harmful aspect of its existence in groundnuts stem from the fact that, they are capacitated in the production of other mycotoxins such as Oxalic acid, Malformin which presents their own consequences on the consumer (Hedayati *et al*., 2007; Blumenthal, 2004 and Azziz *et al*., 2005).

A. fumigatus quantitatively were fever than the other isolates. The study observed that, the occurrence of aflatoxin producers may have detrimental effect on the survival of *A. fumigatus* (no scientific research data backs this assertion) hence their fewer occurrence in groundnuts that were highly infected with aflatoxin and the vice versa. The existence of *A. fumigatus* in groundnuts might not be safe for the consumer. Though they do not produce aflatoxin, they are active producers of mycotxins such as Verruculogen, Viriditoxin, fumigitin among others. This fungus eventually end up in the human systems causing various illnesses and has been isolated from sputum of diseased individuals (Fairs, 2012), This study is also in consistence with research findings reported by Frisvad and Samson, (1990) who isolated *A. fumigatus* from various foods.

Rhizopus stolonifer commonly causes postharvest diseases on many fruits and vegetables (Mari *et al*., 2002). Their presence in groundnuts across the Region was

therefore, not un-expected. However, they may not be playing any role in the aflatoxin contamination of groundnuts in the area. *R. stolonifer* have a beneficial effect on the carbon cycle as they act as decomposers and initiates spoilage in food crops (Mari *et al*., 2002).

Freshly harvested groundnuts samples were found to be positive to aflatoxin contamination. However mean contamination level recorded was within the tolerable limits as contained in the US, EU and Ghana standards for acceptable Aflatoxin level for human consumption (Allameh *et al*., 2005). The presence of *A. flavus* and *A. parasiticus* in freshly harvested groundnuts from the field is not surprising. This is because the niche of the causative fungi is the soil through which the groundnuts were borne. These species were isolated from soil samples obtained from both agricultural and non-agricultural fields (Ehrlich *et al*., 2007). Moreover, drought, plant density, fertilizer level, and insect activities were identified by Cole *et al.*, (1995) as vital determinants of pre-harvest aflatoxin contamination. Abbas *et al*., (2002) and Payne, (1992) research findings also revealed that, high temperature favors fungal growth and mycotoxin production of crops under pre-harvest conditions. These conditions could results in the pre-harvest contamination of groundnuts with aflatoxin in the region. Cotty and Cardwell, (1999); Fernandez Pinto *et al*., (2001); Geiser *et al*., (1998) revealed that *A. flavus* which is a potent aflatoxicogenic fungi is a global species as it exist in temperate regions and mainly in soils of Africa, Australia, and Argentina. This represents the entry point of aflatoxin into groundnuts under pre-harvest condition. Fungal isolation done on the freshly harvested groundnuts also agreed with these research results.

The aflatoxin concentration among the three drying protocols was significant. The mean values recorded for the Rack (R) treatments were found to be higher than the

US and EU/Ghana Standards. This may be due to torrential rainfall amidst humid weather condition that favors aflatoxin production exposed to the uncovered groundnuts during drying on raised Racks (R) on the field. The high aflatoxin level recorded for Rack dried samples is in agreement with research findings by Atanda *et al*., (2013) and Mestres *et al*., (2004) that identified humidity as coefficient in aflatoxin contamination. The mean aflatoxin level recorded for Tarpaulin dried groundnuts was lower than the US/EU/Ghana acceptable limits. The critically lower toxin level obtained could be due to good post-harvest handling that included drying on a tarpaulin which reduced the numbers of drying days hence stalling fungal activities. This is in contrast to drying on bare or loose soil which serves as the home for most fungal species including *Aspergillus* species. Sorting out bad nuts, collecting nuts into shed during rainy day and dying immediately after the downpour when the sun appeared might have contributed to the low aflatoxin levels of groundnuts in this treatment. This is collaborated in a research work by Ayodele and Edema, (2010) research findings identified these factors as good practices in aflatoxin reduction.

The moisture contents of the groundnuts among the three treatments were generally low. The mean moisture value for the Status quo nuts was the highest as compared to the Tarpaulin dried nuts. Rack dried groundnuts recorded lower moisture levels than the Tarpaulin dried nuts by 0.29 less. All treatments showed lower moisture levels 10 % that appreciably ameliorated the rate by which aflatoxin is produced since Lower moisture contents results in reduced water activity in the nuts that stifled the activities of the mycotoxin producing fungal species (Fandohan *et al*., 2005).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

It is showed by this research that drying groundnuts on a Tarpaulin can reduce aflatoxin contamination by almost 50 % as compared to other drying methods such as Rack and the traditional method (bare ground/Status quo). This drying method, when embraced could overturn threat by the European Union on banning Ghanaian groundnuts from its markets.

The research work also confirmed that *Aspergillus flavus* and *Aspergillus parasiticus* accounts for the production of aflatoxin in groundnuts produced in the Upper East Region and that the higher the aflatoxin concentration in the groundnuts, the higher the occurrence of *Aspergillus flavus* and *Aspergillus* and the vice versa. Aside the aflatoxin producing species, this research work also isolated *A. niger* and *A. fumigatus* which is a signal that mycotoxins such as Oxalic acid, Malformin produced by *A. niger* and Verruculogen, Viriditoxin, fumigitin produced by *A. fumigatus* could be present in groundnuts grown in the region. These groups of mycotoxin are mutagenic and capable of causing various degree of illness in humans. *Rhizopus stolonifer* were also found to contaminate the nuts. *Rhizopus stolonifer* presence could account for the occurrence of few rotten groundnuts encountered in the study area as they are known initiators of spoilage in food and contributes greatly to the carbon cycle.

6.2 Recommendation

It is hereby recommended that;

- Farmers adopt the tarpaulin drying method for their groundnuts to minimize aflatoxin contamination.
- Groundnuts are thoroughly dried to ameliorate the aflatoxin concentration.
- *Aspergillus niger* be used to control aflatoxin occurrence in groundnuts as they could reduce the growth of aflatoxicogenic fungi occurrence in groundnuts.
- Further research be conducted to assess the relationship between aflatoxicogenic fungi and *Aspergillus niger* in aflatoxin reduction.

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Appendix I: Fungal species isolated from groundnuts samples in study area

		A. flavus		*A. parasiticus*		*A. niger*		*R. stolonifer*	
District	**Community**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
Bolgatanga	Feo- Asamibisi	+++	+	++	++	+	++	++	++
Municipality	Beo Kasinga	++	-	++	-	++	++	+++	++
	Yikene Adolibia	-	++	+++	+++	+	-	++	-
	Sumbrungu Yeobongo	+	+	+++	++	-	+++	-	-
	Dulugu Asamabisi	++++	-	++	-	++	+++++	-	+++
Bongo	Vea Gunga	+	-	+++	+	+	+++	+++	+
District	Asibiga	++	-	+++	+	++	++	+	++
	Duligu Aginibisi	++	+	++	-	-	-	+	+++
	Sumbrungu Kologo	+++++	++	+	++	+++++	++++	-	-
	Basingo Amenabisi	+++	+	+	++	+	+++	-	-
Kassena/	Bonia	++	+++	++	+	-	+	+++++	+
Nankana	Tampola	++++	+	+++	+	-	-	-	-
Municipality	Balungu	-	++	-	+	-	-	+++++	++++
	Achobisi Kasam	++	++	++	+	-	+	+++	+
	Nyagua	+++++	-	++	-	+	-	-	+
Builsa	Chuchuliga Akpateyere	++	+++	+++	++	+	+	-	++++
District	Chuchuliga Tiema	++++	+	+++	-	++	+	-	++
	Chuchuliga Yipal	+++++	+	++++	+	-	++++	-	-
	Chuchuliga Adabina	++++	-	+++	+	+	+++	-	-
	Wiaga Yemunsa	++	++	++++	-	++	-	-	-

Note: Fungi Isolates from Samples, High= Isolates from samples with Aflatoxin Level > 20 ppb, low= Aflatoxin level < 20 ppb, + means isolated, (-) means no species was isolated in that community.

Appendix II: Raw data of Aflatoxin (ppb) Concentration among Treatments

FARMER	T+SQ	T+P	T+J	R+SQ	R+P	R+J	SQ+SQ	SQ+P	SQ+J
Norbert Adumbisa	11.2	7.47	7.31	14.73	45.83	9.08	3.56	5.42	20.33
Georgina Adawina	1.56	6.39	7.39	394.65	50.02	9.67	2.21	1.82	23.98
Achelisiwine Adagiwe	1.64	1	4.45	3.1	3.78	16.59	4.59	10.51	3.15
Anyoka Agangkoliba	8.81	5.79	5.17	39.76	17.42	4.01	14.02	14.02	20.01
Amiriba Euthant	8.5	8.05	5.05	23.6	36.23	16.44	10.25	14.22	12.29
Achelisiwine Atibiru	8.13	5.01	3.58	13.77	22.39	23.6	8.83	9.72	7.22
Peter Atongu	8.17	9.72	7.67	234.94	59.73	5.39	6.48	17.37	16.47
Ben Akamuriba	9.33	7.46	7.36	6.42	36.13	357.53	35.01	3.73	16.23
Oldman Anabila	9.5	12.1	6.97	1.38	2.84	27.59	2.27	26.53	63.95
Emmanuel Nchor	7.33	21.97	11.2	19.59	37.51	2.39	7.44	12.81	8.27
Isaac Amongba	8.63	14.01	10.27	39.26	14.01	45.22	83.07	11.82	44.04
Benjamin Ayeliya	4.39	14.42	15.47	18.36	49.09	44.79	4.43	19.9	6.3
Kojo Agumata	8.66	4.75	5.06	4.78	9.74	11.89	5.19	6.68	8.66
Justin Alugu	7.62	7.72	4.71	11.92	288.79	13.31	3.42	36.55	14.61
Ataaba Albert	21.46	6.39	18.42	52.74	18.79	8.15	27.17	3.92	7.64
Nchorbire Awinebisa	5.83	3.98	4.7	19.14	8.05	35.03	451.77	19.14	15.53
Norbert Aburiga	3.56	18.88	13.26	384.43	8.71	4.99	7.25	8.05	7.46
Aburanyaga Atubiga		10.11	11.42	7.25	8.33	56.08		4.95	5.17
Janet Adoba		8.79	5.5		9.08	5.5			

Appendix III: Aflatoxin concentration versus Moisture levels of dried groundnut samples from the study area

Tarp Aflatoxin	Tarp Moisture	Rack Aflatoxin	Rack Moisture	SQ Aflatoxin	SQ Moisture
8.66	4.48	23.21	6.25	9.77	5.69
5.11	3.56	151.45	12.2	9.34	5.48
2.36	2.65	7.82	4.89	6.08	4.48
6.59	4.49	20.4	5.45	16.02	6.54
7.2	4.96	25.42	3.96	12.25	6
5.57	3.76	19.92	4.8	8.59	4.84
8.52	5.29	100.02	3.81	13.44	6.89
8.05	5.15	133.36	2.65	18.32	6.54
9.52	5.55	10.6	2.65	30.92	12.53
13.5	3.86	19.83	4.59	9.51	5.53
10.97	5.88	32.83	4.19	46.31	13.58
11.42	5.91	37.41	2.37	10.21	5.72
6.16	4.32	8.8	4.82	6.84	3.93
6.68	4.58	104.67	4.61	18.19	6.65
15.42	5.67	26.56	4.37	12.91	6.03
4.84	4.06	20.74	2.94	162.15	13.46
11.9	2.56	132.71	2.32	7.59	4.32
10.77	5.6	23.89	2.25	5.06	3.72
7.15	4.89	7.29	4.03		

Key: T+SQ = Tarpaulin dried groundnut stored in Status Quo bag, **T+P** = Tarpaulin dried groundnut stored in Plastic bag, **T+J** = Tarpaulin dried groundnut stored in Jute bag, **R+SQ** = Rack dried groundnut stored in Status Quo bag, **R+P** = Rack dried groundnut stored in Plastic bag, **R+J** =Rack dried groundnut stored in Jute bag, **SQ+SQ** = Status Quo dried groundnut stored in Status Quo bag, **SQ+P** = Status Quo dried groundnut stored in Plastic bag and **SQ+J** = Status Quo dried groundnut stored in Jute bag

Appendix IV: GenStat Output of Analysis

GenStat Release 10.3DE (PC/Windows 7) 20 June 2015 16:30:45

GenStat Discovery Edition 4

GenStat Procedure Library Release PL18.2

```
 1  %CD 'C:/Users/VINCENT/Documents'
 2  "Data taken from unsaved spreadsheet: New Data;1"
 3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
 4  READ [PRINT=*; SETNVALUES=yes] _stitle_
 7  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard

on: 20-Jun-2015 16:31:08

```
 8  DELETE [REDEFINE=yes] Treatment,Aflatoxin_level_ppb
 9  UNITS [NVALUES=*]
 10  FACTOR [MODIFY=yes; NVALUES=163; LEVELS=9;
LABELS=!t('R+J','R+P','R+SQ',\
 11  'SQ+J','SQ+P','SQ+SQ','T+J','T+P','T+SQ'); REFERENCE=1] Treatment
```

```
 12  READ Treatment; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Treatment	163	0	9

```
 18  VARIATE [NVALUES=163] Aflatoxin_level_ppb
 19  READ Aflatoxin_level_ppb
```

Identifier	Minimum	Mean	Maximum	Values	Missing	Skew
Aflatoxin_level_ppb	1.000	26.83	451.8	163	0	

```
 32
 33  "One-way design"
 34  DELETE [REDEFINE=yes] _ibalance
 35  A2WAY [PRINT=aovtable,information,means; TREATMENTS=Treatment;
FPROB=yes; PSE=diff,\
 36  lsd,means,alldiff,alllsd; LSDLEVEL=5; PLOT=*; EXIT=_ibalance]
Aflatoxin_level_ppb;\
 37   SAVE=_a2save
```

Analysis of variance

Variate: Aflatoxin_level_ppb

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	8	67968.	8496.	2.01	0.049
Residual	154	651898.	4233.		
Total	162	719866.			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

units 57	323.0 approx. s.e.	63.2
units 72	312.8 approx. s.e.	63.2
units 87	250.6 approx. s.e.	63.2
units 99	319.3 approx. s.e.	63.2
units 126	411.9 approx. s.e.	63.2

Tables of means

Grand mean 26.83

Treatment	R+J	R+P	R+SQ	SQ+J	SQ+P
mean	38.23	38.24	71.66	16.74	12.62
rep.	18	19	18	18	18
s.e.	15.34	14.93	15.34	15.34	15.34
Treatment	SQ+SQ	T+J	T+P	T+SQ	
mean	39.82	8.16	9.16	7.90	
rep.	17	19	19	17	
s.e.	15.78	14.93	14.93	15.78	

Standard errors of differences of means

Treatment R+J	1	*			
Treatment R+P	2 21.40	*			
Treatment R+SQ	3	21.69	21.40	*	
Treatment SQ+J	4	21.69	21.40	21.69	*

Treatment SQ+P	5	21.69	21.40	21.69	21.69	*
Treatment SQ+SQ	6	22.00	21.72	22.00	22.00	22.00
Treatment T+J	7	21.40	21.11	21.40	21.40	21.40
Treatment T+P	8	21.40	21.11	21.40	21.40	21.40
Treatment T+SQ	9	22.00	21.72	22.00	22.00	22.00
		1	2	3	4	5
Treatment SQ+SQ	6	*				
Treatment T+J	7	21.72	*			
Treatment T+P	8	21.72	21.11	*		
Treatment T+SQ	9	22.32	21.72	21.72	*	
		6	7	8	9	

Minimum standard error of difference	21.11
Average standard error of difference	21.64
Maximum standard error of difference	22.32

Least significant differences (at 5%)

1	*				
2	42.28	*			
3	42.84	42.28	*		
4	42.84	42.28	42.84	*	
5	42.84	42.28	42.84	42.84	*
6	43.47	42.91	43.47	43.47	43.47
7	42.28	41.70	42.28	42.28	42.28
8	42.28	41.70	42.28	42.28	42.28

9	43.47	42.91	43.47	43.47	43.47
	1	2	3	4	5
6	*				
7	42.91	*			
8	42.91	41.70	*		
9	44.09	42.91	42.91	*	
	6	7	8	9	

Minimum least significant difference 41.70

Average least significant difference 42.74

Maximum least significant difference 44.09

Identifier	Minimum	Mean	Maximum	Values	Missing
T_SQ	1.560	7.901	21.46	19	2

14 VARIATE [NVALUES=19] T_P

15 READ T_P

Identifier	Minimum	Mean	Maximum	Values	Missing
T_P	1.000	9.158	21.97	19	0

18 VARIATE [NVALUES=19] T_J

19 READ T_J

Identifier	Minimum	Mean	Maximum	Values	Missing
T_J	3.580	8.156	18.42	19	0

22 VARIATE [NVALUES=19] R_SQ

23 READ R_SQ

Identifier	Minimum	Mean	Maximum	Values	Missing
R_SQ	1.380	71.66	394.6	19	1

Skew

26 VARIATE [NVALUES=19] R_P

27 READ R_P

Identifier	Minimum	Mean	Maximum	Values	Missing
R_P	2.840	38.24	288.8	19	0

Skew

30 VARIATE [NVALUES=19] R_J

31 READ R_J

Identifier	Minimum	Mean	Maximum	Values	Missing
R_J	2.390	36.70	357.5	19	0

Skew

34 VARIATE [NVALUES=19] SQ_SQ

35 READ SQ_SQ

Identifier	Minimum	Mean	Maximum	Values	Missing
SQ_SQ	2.210	39.82	451.8	19	2

Skew

38 VARIATE [NVALUES=19] SQ_P

39 READ SQ_P

Identifier	Minimum	Mean	Maximum	Values	Missing
SQ_P	1.820	12.62	36.55	19	1

```
 42  VARIATE [NVALUES=19] SQ_J
 43  READ SQ_J
```

Identifier	Minimum	Mean	Maximum	Values	Missing
SQ_J	3.150	16.74	63.95	19	1

Skew

```
 46
 47  DESCRIBE [SELECTION=nobs,nmv,mean,median,min,max,q1,q3,var,sd]
R_J,R_P,R_SQ,SQ_J,SQ_P,\
 48  SQ_SQ,T_J,T_P,T_SQ
```

Summary statistics for R_J

Number of observations =	19
Number of missing values =	0
Mean =	36.70
Median =	13.31
Minimum =	2.39
Maximum =	357.5
Lower quartile =	6.162
Upper quartile =	33.17
Standard deviation =	79.30
Variance =	6289

Summary statistics for R_P

Number of observations =	19
Number of missing values =	0

Mean =	38.24
Median =	18.79
Minimum =	2.84
Maximum =	288.8
Lower quartile =	8.803
Upper quartile =	43.75
Standard deviation =	63.24
Variance =	4000

Summary statistics for R_SQ

Number of observations =	18
Number of missing values =	1
Mean =	71.66
Median =	18.75
Minimum =	1.38
Maximum =	394.6
Lower quartile =	7.25
Upper quartile =	39.76
Standard deviation =	127.1
Variance =	16149

Summary statistics for SQ_J

Number of observations =	18
Number of missing values =	1
Mean =	16.74

Median =	13.45
Minimum =	3.15
Maximum =	63.95
Lower quartile =	7.46
Upper quartile =	20.0
Standard deviation =	15.15
Variance =	229.4

Summary statistics for SQ_P

Number of observations =	18
Number of missing values =	1
Mean =	12.62
Median =	11.16
Minimum =	1.82
Maximum =	36.55
Lower quartile =	5.42
Upper quartile =	17.37
Standard deviation =	8.880
Variance =	78.85

Summary statistics for SQ_SQ

Number of observations =	17
Number of missing values =	2
Mean =	39.82
Median =	7.25

Minimum =	2.21
Maximum =	451.8
Lower quartile =	4.212
Upper quartile =	17.31
Standard deviation =	108.0
Variance =	11665

Summary statistics for T_J

Number of observations =	19
Number of missing values =	0
Mean =	8.156
Median =	7.31
Minimum =	3.58
Maximum =	18.42
Lower quartile =	5.052
Upper quartile =	10.97
Standard deviation =	4.127
Variance =	17.03

Summary statistics for T_P

Number of observations =	19
Number of missing values =	0
Mean =	9.158
Median =	7.72
Minimum =	1

Maximum =	21.97
Lower quartile =	5.94
Upper quartile =	11.60
Standard deviation =	5.194
Variance =	26.98

Summary statistics for T_SQ

Number of observations =	17
Number of missing values =	2
Mean =	7.901
Median =	8.17
Minimum =	1.56
Maximum =	21.46
Lower quartile =	5.47
Upper quartile =	8.94
Standard deviation =	4.451
Variance =	19.81

GenStat Discovery Edition 4

GenStat Procedure Library Release PL18.2

```
 1  %CD 'C:/Users/VINCENT/Documents'
 2  "Data taken from unsaved spreadsheet: New Data;1"
 3  DELETE [REDEFINE=yes] _stitle_: TEXT _stitle_
 4  READ [PRINT=*; SETNVALUES=yes] _stitle_
 7  PRINT [IPRINT=*] _stitle_; JUST=left
```

Data imported from Clipboard

on: 1-Sep-2013 9:58:54

```
 8  DELETE [REDEFINE=yes]
District_Municipality,Community,Aflatoxin_Level_ppb
 9  UNITS [NVALUES=*]
 10  FACTOR [MODIFY=yes; NVALUES=538; LEVELS=4; LABELS=!t(\
 11  'BOLGANTANGA MUNICIPALITY','BONGO DISTRICT','BUILSA
DISTRICT',\
 12  'KASSENA NANKANA MUNICIPALITY'); REFERENCE=1]
District_Municipality
 13  READ District_Municipality; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
District_Municipality	538	0	4

```
29  FACTOR [MODIFY=yes; NVALUES=538; LEVELS=20;
LABELS=!t('ACHOBISI KASAM',\
 30  'ASIBIGA','BALUNGU','BASINGO AMENABISI','BEO
KASINGA','BONIA',\
 31  'CHUCHULIGA ADABINA','CHUCHULIGA
AKPATEYERE','CHUCHULIGA TIEMA',\
 32  'CHUCHULIGA YIPAALA','DULIGU AGINIBISI','DULUGU
ASAMABISI','FEO- ASAMIBISI',\
 33  'NYAGUA','SUMBRUNGU KOLOGO','SUMBRUNGU
YEOBONGO','TAMPOLA','VEA GUNGA',\
 34  'WIAGA YEMUNSA','YIKENE ADOLIBIA'); REFERENCE=1] Community
 35  READ Community; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
Community	538	0	20

```
 55  VARIATE [NVALUES=538] Aflatoxin_Level_ppb
 56  READ Aflatoxin_Level_ppb
```

Identifier	Minimum	Mean	Maximum	Values	Missing	
Aflatoxin_Level_ppb	1.720	12.40	229.9	538	0	Skew

```
 96
 97  "One-way design in randomized blocks"
 98  DELETE [REDEFINE=yes] _ibalance
 99  A2WAY [PRINT=aovtable,information,means; TREATMENTS=Community;
BLOCKS=District_Municipality;\
 100   FPROB=yes; PSE=diff,lsd,means; LSDLEVEL=5; PLOT=*;
EXIT=_ibalance] Aflatoxin_Level_ppb;\
 101   SAVE=_a2save
```

Analysis of variance

Variate: Aflatoxin_Level_ppb

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
District_Municipality stratum					
Community	19	890.6	46.9		
Residual	-16	0.0			
District_Municipality.*Units* stratum					
Community	19	20773.5	1093.3	6.97	<.001
Residual	515	80795.5	156.9		
Total	537	102459.7			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

District_Municipality BONGO DISTRICT *units* 55

48.69 approx. s.e. 12.25

District_Municipality BUILSA DISTRICT *units* 97

38.18 approx. s.e. 12.25

District_Municipality BUILSA DISTRICT *units* 134

65.55 approx. s.e. 12.25

District_Municipality BUILSA DISTRICT *units* 144

204.80 approx. s.e. 12.25

District_Municipality KASSENA NANKANA MUNICIPALITY *units* 111

38.79 approx. s.e. 12.25

District_Municipality KASSENA NANKANA MUNICIPALITY *units* 128

84.70 approx. s.e. 12.25

Tables of means

Grand mean 12.397

CommunityACHOBISI KASAMASIBIGABALUNGUBASINGO AMENABISI BEO KASINGA

mean	3.632	5.672	14.693	6.063	12.681
rep.	27	24	26	25	27
s.e.	2.411	2.557	2.456	2.505	2.411

Community BONIACHUCHULIGA ADABINACHUCHULIGA AKPATEYERECHUCHULIGA TIEMACHUCHULIGA YIPAALA

mean	7.407	10.848	6.781	5.307	11.959
rep.	25	29	27	29	28
s.e.	2.505	2.326	2.411	2.326	2.367

CommunityDULIGU AGINIBISIDULUGU ASAMABISIFEO- ASAMIBISI

	NYAGUASUMBRUNGU KOLOGO				
mean	8.711	11.347	13.114	27.570	10.065
rep.	24	27	25	28	27
s.e.	2.557	2.411	2.505	2.367	2.411

	CommunitySUMBRUNGU YEOBONGOTAMPOLAVEA GUNGA WIAGA YEMUNSAYIKENE ADOLIBIA				
mean	13.504	15.785	15.323	24.585	18.204
rep.	26	28	26	34	26
s.e.	2.456	2.367	2.456	2.148	2.456

Minimum standard error of difference	3.166
Average standard error of difference	3.424
Maximum standard error of difference	3.616
Minimum least significant difference	6.220
Average least significant difference	6.727
Maximum least significant difference	7.103

102 SET [IN=*]

108 DESCRIBE [SELECTION=mean,min,max,sd; GROUPS=Community]

Aflatoxin_Level_ppb

Summary statistics for Aflatoxin_Level_ppb: Community ACHOBISI KASAM

Mean =	5.377
Minimum =	1.72
Maximum =	37.17
Standard deviation =	6.518

Summary statistics for Aflatoxin_Level_ppb: Community ASIBIGA

Mean = 5.097

Minimum = 1.87

Maximum = 14.07

Standard deviation = 2.533

Summary statistics for Aflatoxin_Level_ppb: Community BALUNGU

Mean = 14.12

Minimum = 10.32

Maximum = 22.22

Standard deviation = 2.717

Summary statistics for Aflatoxin_Level_ppb: Community BASINGO AMENABISI

Mean = 7.808

Minimum = 3.04

Maximum = 18.27

Standard deviation = 3.275

Summary statistics for Aflatoxin_Level_ppb: Community BEO KASINGA

Mean = 12.11

Minimum = 5.21

Maximum = 32.72

Standard deviation = 6.141

Summary statistics for Aflatoxin_Level_ppb: Community BONIA

Mean = 9.152

Minimum = 7.42

Maximum = 13.46

Standard deviation = 1.225

Summary statistics for Aflatoxin_Level_ppb: Community CHUCHULIGA ADABINA

Mean = 11.34

Minimum = 8.02

Maximum = 49.52

Standard deviation = 7.488

Summary statistics for Aflatoxin_Level_ppb: Community CHUCHULIGA AKPATEYERE

Mean = 7.270

Minimum = 2.85

Maximum = 28.44

Standard deviation = 5.584

Summary statistics for Aflatoxin_Level_ppb: Community CHUCHULIGA TIEMA

Mean = 5.796

Minimum = 2.4

Maximum = 20.82

Standard deviation = 3.433

Summary statistics for Aflatoxin_Level_ppb: Community CHUCHULIGA YIPAALA

Mean = 12.45

Minimum = 5.55

Maximum = 37.75

Standard deviation = 8.306

Summary statistics for Aflatoxin_Level_ppb: Community DULIGU AGINIBISI

Mean = 6.940

Minimum = 3.87

Maximum = 11.33

Standard deviation = 2.222

Summary statistics for Aflatoxin_Level_ppb: Community DULUGU ASAMABISI

Mean = 9.576

Minimum = 5.57

Maximum = 15.34

Standard deviation = 2.449

Summary statistics for Aflatoxin_Level_ppb: Community FEO- ASAMIBISI

Mean = 12.54

Minimum = 5.6

Maximum = 61.23

Standard deviation = 10.79

Summary statistics for Aflatoxin_Level_ppb: Community NYAGUA

Mean = 29.32

Minimum = 2.18

Maximum = 114.0

Standard deviation = 20.75

Summary statistics for Aflatoxin_Level_ppb: Community SUMBRUNGU KOLOGO

Mean = 8.293

Minimum = 5.03

Maximum = 17.21

Standard deviation = 2.963

Summary statistics for Aflatoxin_Level_ppb: Community SUMBRUNGU YEOBONGO

Mean = 11.73

Minimum = 4.68

Maximum = 35.18

Standard deviation = 7.391

Summary statistics for Aflatoxin_Level_ppb: Community TAMPOLA

Mean = 17.53

Minimum = 10.73

Maximum = 46.64

Standard deviation = 6.683

Summary statistics for Aflatoxin_Level_ppb: Community VEA GUNGA

Mean = 14.75

Minimum = 5.24

Maximum = 47.66

Standard deviation = 10.40

Summary statistics for Aflatoxin_Level_ppb: Community WIAGA YEMUNSA

Mean = 25.07

Minimum = 7.67

Maximum = 229.9

Standard deviation = 39.6

Summary statistics for Aflatoxin_Level_ppb: Community YIKENE ADOLIBIA

Mean = 16.43

Minimum = 11.22

Maximum = 53.69

Standard deviation = 7.808

109 DESCRIBE [SELECTION=mean,min,max,sd;

GROUPS=District_Municipality] Aflatoxin_Level_ppb

Summary statistics for Aflatoxin_Level_ppb: District_Municipality

BOLGANTANGA MUNICIPALITY

Mean = 10.63

Minimum = 3.87

Maximum = 53.69

Standard deviation = 6.099

Summary statistics for Aflatoxin_Level_ppb: District_Municipality BONGO

DISTRICT

Mean = 11.82

Minimum = 1.87

Maximum = 61.23

Standard deviation = 8.069

Summary statistics for Aflatoxin_Level_ppb: District_Municipality BUILSA DISTRICT

Mean = 12.89

Minimum = 2.4

Maximum = 229.9

Standard deviation = 20.91

Summary statistics for Aflatoxin_Level_ppb: District_Municipality KASSENA NANKANA MUNICIPALITY

Mean = 14.14

Minimum = 1.72

Maximum = 114.0

Standard deviation = 13.68