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EFFECTS OF DIFFERENT DRYING AND STORAGE METHODS ON THE AFLATOXIN CONCENTRATION OF GROUNDNUTS (*Arachis hypogaea Linn)* IN THE NORTHERN REGION OF GHANA

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BY

SABULI NOAH

(UDS/MBT/0015/13)

2017

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THIS THESIS IS SUBMITTED TO THE DEPARTMENT OF BIOTECHNOLOGY, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF PHILOSOPHY IN BIOTECHNOLOGY

2017

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DECLARATION

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 the university or elsewhere. Works that were consulted have been duly acknowledged by way of references.

Name: SABULI NOAH

Candidate's signature: __________________________ Date:_________________

Supervisor's Declaration

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

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ABSTRACT

Arachis hypogaea Linn is a very important cash crop and of great nutritional value but has a high tendency to be infested with aflatoxin by *Aspergillus flavus*. A survey was conducted to find out the general levels of aflatoxin contamination in dried groundnuts in storage over a period of three months in Northern Region. Groundnuts samples were taken from a total of five hundred households in twenty communities using purposive sampling. Aflatoxin analysis on the samples was carried out using Flouro Quant AflaPlus machine. Aflatoxin concentrations obtained were in the range of 11.08ppb to 367.5ppb for the samples in storage over a period of three month whilst those that were tested freshly from the field were in the ranges of 4.35ppb to 13.06ppb with one abnormality recorded at 110ppb in a community called Tarikpaar. Different drying treatments were used on the fresh samples after which they were tested for aflatoxin after being in storage over a period of three (3) months in different storage bags. The results obtained showed that the best method for the reduction of aflatoxin levels in peanuts was to dry them on tarpaulin and store them in platic bags and placed on pallets. This treatment gave the lowest reading of 21.03±27.91 ppb in the aflatoxin concentration. Isolations of fungi species were done using dilution and direct plating method from groundnut samples with aflatoxin concentrations above 30ppb and those below 10ppb to identify the fungi species responsible for the high and low aflatoxin values recorded. The percentage occurrence of these species were found to be 60% for *Asperghillus flavus , 20% Aspergillus niger,* 15% *Aspergillus parasiticus* and the last two species *Rhizopus stolonifer* and *Penicillium chrysogenum* had 5% occurrence respectively. The findings from this works recommend that tarpaulin should be used by farmers for drying their nuts and storage should be done in a plastic bag stored on a raised structure. It also recommends that plugging of nuts should be done right immediately after harvest.

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DEDICATION

I dedicate this work to God Almighty and my entire family.

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LIST OF ACRONYMS AND ABBREVIATIONS

SDA= Saboroud Dextrose Agar

PPB= Parts per billion

ANOVA…..Analysis of Variance

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CHAPTER ONE

INTRODUCTION

1.0 Background study

Groundnuts (*Aracheas hypogaea L.*) are one of the most crucial and strategic crops in Africa and the developing world in general (IITA, 2000). It is produced in different parts of the continent under diverse climatic and ecological conditions. Due to their increasing importance, groundnuts have become a major staple and cash crop for smallholder farmers (IITA, 2000). Increased productivity in staple food, such as peanuts and maize, is critical to raising rural incomes and stimulating broad-based economic growth (Eicher & Byerlee, 1997).

Peanut is one of the most important staple crops in Ghana and accounts for more than 40 percent of total cereal production in the country (MiDA 2010; MoFA 2012). A survey conducted by IFRI in 2015 showed that the bulk of peanuts produced go into food consumption and it is arguably the most important crop for food security.

In the Northern part of Ghana groundnut is one of the most important grains, not only on the basis of the number of farmers that are engaged in their cultivation, but also in their economic value (IFRI, 2014). Peanuts have been in the diet of many Ghanaians for centuries. It started as subsistence crop and has gradually become an important commercial crop on which many agro-based industries depend for raw materials (Iken and Amusa, 2004).

The prime focus of this research which is to investigate the best post-harvest treatment intervention to critically reduce to an acceptable level and possibly stifled

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the growth and deleterious effect of these carcinogenic fungal species on *Arachis hypogaea (*peanut) will not only add its quota of knowledge to literature but eradicate poverty in northern Ghana

1.1 Problem statement

Aflatoxins are potent carcinogens and immuno-suppressants; and produce several other effects collectively referred to as aflatoxicosis **(**Peers *et al*., 1987; Ellis *et al*., 1991**)**. They have a negative impact on human and animal health, and consequently, on national **economies** (Bhat & Vashanti, 1999). According to James *et al*., (2004), poor knowledge of the aflatoxins and its health risks causes consumers to be exposed to the toxins through consumption of contaminated groundnuts. The International Institute of Tropical Agriculture (IITA) in 2003 conducted an aflatoxin information campaign in some West African countries, including Ghana, concluded that there is poor baseline information on aflatoxins cure among groundnut, millet and maize consumers (James *et al.,* 2004).

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1.2 Objective of the study

In view of the paucity of information on the aflatoxin contamination of groundnuts in Ghana, this research work was carried out to determine the distribution of aflatoxin contamination in groundnut, isolate and identify the major fungi species associated with aflatoxin production and evaluate different interventions to help reduce the levels of aflatoxin contamination in groundnuts in the Northern Region of Ghana.

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1.3 Specific objectives

The specific objectives are as follows

1. To determine aflatoxin levels in farmer-stored shelled groundnuts and freshly harvested groundnut from farmer fields.

2. To isolate and identify the aflatoxin producing fungi in the stored groundnuts.

3. To determine the effect of different drying methods (tarpaulin, rack and status quo thus farmers own method of drying) on moisture content and aflatoxin levels in groundnuts.

4. To determine the effect of different types of storage bags (jute, plastic and status quo bags) on aflatoxin concentration of dried groundnuts in storage.

1.4 Research questions

The questions to be answered by the research are as follows:

1 What is the level of aflatoxin contamination of freshly harvested groundnuts cultivated in the Northern Region of Ghana?

2 What is the level of aflatoxin contamination in stored groundnuts in Northern Region of Ghana

3 What is the major route of entry of these fungi species into the groundnut (on-field, off-field)

4 Which drying treatment (bare ground, rack or tarpaulin) reduces the levels of aflatoxin contamination in groundnuts?

5 What are the dominant species of *Aspergillus* responsible for aflatoxin levels in groundnuts in Northern Region?

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6 What are the conditions that favor the contamination of groundnuts by aflatoxin.

1.5 Significance of study

This study is intended to look at effects of storage and drying treatments on the aflatoxin levels in groundnuts. The study is also intended to make new recommendation so as to effectively reduce levels of aflatoxin and enhance quality groundnut production which will ultimately improve profit margin of farmers. Furthermore, findings of the study will also be useful for governments and other actors in the agricultural sector in the formulation of policies and strategies to ensure sustainable quality groundnut production as well as higher profit margin of farmers. Finally, the study will contribute to knowledge by creating awareness and filling existing gaps on the effects of aflatoxin levels on our health. It will also contribute to knowledge by providing alternative strategies in the management of factors that cause aflatoxin contamination in groundnut production.

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1.6 Organization of study

This study shall contain five chapters. Chapter one shall contain background information of the study, problem statement, general and specific objectives, research questions, significance of the study as well as the scope of the study. Chapter two shall review literatures relevant to the study and also establish the theoretical and conceptual frame work for the study. Chapter three which is the research methodology shall outline the research design, the study area, the study population, the sample size determination, sources and methods of data collection, data collection tools and instruments, methods of data analysis and presentation.

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Chapter four shall present findings and discussion of the research. Chapter five will show summary of the findings of the study, draw conclusions and provide recommendation for appropriate stakeholders.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Food safety

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Food and Agricultural Organization (1996) defined food safety as providing assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use. FAO (2001) also asserted that food safety is becoming a dominant theme among the agricultural research and development community due to its linkage with national food security, agricultural and rural development and international agricultural trade. Conway and Toeniessen, (2003) asserted that the safety of food and feed for human and animal consumption should be of topmost priority with regards to agricultural and food industries. Those involved in farming in sub-Saharan Africa constitute about 70% of the population, and food commodities are the major items of international trade for many West African countries (WHO, 2001). According to Bhat and Vashanti (1999), the quality and safety of food is important so that domestic and foreign markets are not compromised by the sale of low quality or unsafe food.

In 2005, both FAO and WHO argued that it is imperative that food safety remains a concern in all situations in order to derive maximum benefit from even the little available food. Hence relevant food safety systems are essential from production to consumption. Resolution AFR/RC53/R5 of the WHO Regional Committee for Africa, urging countries to strengthen food safety programs, was endorsed in 2003. Since then, many countries have initiated activities to improve food safety. This

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strategy on food safety consolidates past gains and provides a framework for protecting public health and economic development through reduction of the burden of food-borne diseases. They also pointed out that persons suffering from diseases such as HIV/AIDS, tuberculosis, malaria, and other ailments which affect the African region are at a greater risk of being debilitated by unsafe food. This is because their immune systems are already compromised. FAO and WHO emphasized that the assurance of safe food is essential to improving the quality of life for those already affected by disease. Similarly, persons suffering from food-borne illness are more likely to contract other communicable diseases. Furthermore, food-borne diseases are one of the most important underlying factors for malnutrition.

In 1991, World Health Organization (WHO) explained that food-borne diseases create an enormous burden on the economies of developing countries and consumer costs include medical, legal, and other expenses, as well as absenteeism at work and school. For many consumers who live at a subsistence level, the loss of income due to food-borne illness can perpetuate the cycle of poverty. Costs to national governments, as claimed, stem from increased demands on already overburdened and poorly funded healthcare systems in developing countries. According to the Food and Agricultural Organization (FAO) (1996), food security is defined as physical and economic access to sufficient, safe and nutritious food to meet dietary needs. Food safety is an integral part of food security and is defined as protecting the food supply from microbial, chemical and physical hazards that may occur during all stages of food production (i.e growing, harvesting, processing,

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transporting, retailing, distributing, preparing, storing and consumption) in order to prevent food-borne illnesses. Because of insufficient food to meet demand on the African continent, the majority of people are only concerned with satisfying hunger and do not give due attention to the safety of food.

2.2 History of groundnuts

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Groundnut or peanut is commonly called the poor man's nut (FOA, 2002) but today it's an important oilseed and food crop. This plant is native to South America and has never been found uncultivated (FOA and ICAR, 2002). The botanical name for groundnut, *Arachis hypogaea Linn.,* is derived from two Greek words, *Arachis* meaning a legume and *hypogaea* meaning below ground, referring to the formation of pods in the soil (AGSI/FOA, 2002). Groundnut is an upright or prostrate annual plant. It is generally distributed in the tropical, sub-tropical and warm temperate zones. Ethnological studies of the major Indian tribes of South America document the widespread culture of groundnut and provide indirect evidence for its domestication long before the Spanish Conquest. When the Spaniards returned to Europe they took groundnuts with them. Later traders were responsible for spreading groundnuts to Asia and Africa where it is now grown between latitudes 40°N and 40°S (Pattee and Young, 1982).

2.3 Taxonomy of groundnuts

Arachis hypogaea belongs to the family Leguminosae. Its subfamily, tribe and subtribe are *Fabaceae, Aeschynomeneae* and *Stylosanthenae* respectively. The type that is cultivated is a member of the genus *Arachis*, which has 69 diploid and tetraploid species of South American origin (Krapovickas & Gregory, 1994). The

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Arachis is categorised into nine sections depending 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 & Simpson, 1995). The tetraploids (2n = 40) are more evolutionarily advanced and have evolved independently only in sections *Rhizomatosae* and *Arachis* (Smartt & Stalker, 1982). Due to the branching pattern difference and the occurrence of reproductive nodes on the main stem, *A. hypogaea* is sub-grouped into *hypogaea* and *fastigiata* subspecies (Krapovickas & Rigoni, 1960). Hypogaea (subspecies) 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. This was the basis of the species being subdivided into subspecies based on their botanical varieties. Virginia and Runner U.S. market types which are mostly grown in Africa including Ghana is classified into *hypogaea* subspecies whilst *hirsuta* is the Peruvian humpback or Chinese dragon type. Subspecies *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 & Stalker, 1982).

2.4 Economic and social impact of groundnut

China and India together are the world's leading groundnut producers accounting for nearly 60 percent of the production and 52 percent of the crop area (FOA, 2002). India cultivates about 7.74 million hectares and produces 7.61 million tonnes of groundnut with the productivity level of 991.8 kg ha-1. South Africa is the major producer in Africa, while in Latin America almost one half of the total

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groundnut produced in that region may be credited to Argentina. Among the developing countries Egypt has the highest productivity and capacity to produce groundnuts (FOA, 2002)

In most of the developing countries, the productivity levels are lower than in the United States of America. This is mainly due to a number of production constraints such as the cultivation of the crop on marginal lands under rain fed conditions, occurrence of frequent drought stress due to vagaries of monsoon and higher incidence of disease and pest attacks. It also include low input-use and factors related to socio-economic infrastructure (FOA and NRCFG, 2002).

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2.5 Groundnuts and World trade

Over half of the groundnut harvested worldwide is crushed for oil and a substantial quantity of groundnut produced in developing countries is traded in domestic markets (FOA, 2002). International trade of groundnuts is mainly in the form of in shell (pods), shelled (kernels) and meal (cake) (FOA, 2002). A large trade of confectionery groundnut is also booming in the international market. The major exporter of groundnut in shell and shelled is shown in table Table 2. Developed countries like UK, Holland, Germany, France, Canada and Japan accounted for 65 percent of world groundnut demand. However, the major suppliers of groundnut are the United States of America, China and Argentina (FOA, 2002). The international price of groundnuts is generally decided by the crop size and quality in United States of America (FOA, 2002). Except for India and United States of America, the price ratio is above 40 percent in the leading exporting countries (Rama Rao *et al.*, 2000). Though India is the largest producer of groundnut in the

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world, its share in the worldwide edible groundnut market is insignificant (FOA, 2002).

Table 2.0 Major exporting countries of groundnut in-shell, shelled, cake and their values.

COUNTRIES	Groundnuts in shell		Groundnuts shelled	
	Export-Mt	Value(1000 $)	Export-Mt	Value(1000 $)
CHINA	49078	30849	289213	202412
INDIA	4394	2303	86494	50276
ARGENTINA	7539	16068	115	541
SOUTH AFRICA	4378	3370	25406	16722
NETHERLANDS	6089	5564	81335	79868
INDONESIA	1992	1874	206	110
BRAZIL	2100	1679	558	440
SUDAN	144	737	170	3666
SENEGAL	120	799	823	5324
MYANMAR	55	20	130	132
NIGERIA	18	15	1277	624

Source: FAOSTAT, database 1990 to 1998. Values are the average for the year 1990 to 1999.

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2.6 Products obtained from groundnut

2.6.1 Primary Products

Groundnut oil has traditionally been a significant dietary component in several countries in Western Africa. In some countries like Nigeria, Gambia and Senegal, oil extraction has been important rural cottage industry for many years (ICAR, 2002). Industrial processing of oil from groundnuts exists in many countries like, India, Sudan, Senegal, Nigeria and Gambia. Oil extraction at the village level is still quite common throughout the developing countries. The major groundnut oil and cake producing countries are shown in Table 2.1. Groundnut oil is generally used as a cooking medium according to the United Nations FOA and it may be processed into different products. For instance, it is hydrogenated to make *vanaspati* or vegetable cheese (FOA, 1998). After oil extraction, groundnut cake is obtained as a by-product (what is generally used in making "kulikuli") in Ghana. In general, the resultant cake contains about 43 to 65 percent protein and 6 to 20 percent fat plus some B-group vitamins depending upon the method of extraction (FOA, 2002). Incidentally, NASA of the United States of America has selected groundnut as a possible food for the Advanced Life Support system for extended space missions.

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Table 2.1 Five major developing countries producing oil (Mt 000) and groundnut cake (Mt 000).

Table 2.1a

COUNTRY	YEAR					
	1995	1996	1997	1998	1999	2000
	GROUNDNUT OIL					
CHINA	1585	1646	1592	1750	1740	1644
INDIA	1859	2123	1725	1675	1050	1175
ARGENTINA	48	60	52	85	72	60
BRAZIL	21	23	19	26	24	24
INDONESIA	33	32	31	26	27	27

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Table 2.2b

COUNTRY	YEAR					
	1995	1996	1997	1998	1999	2000
	GROUNDNUT CAKE					
CHINA	2078	2158	2087	2293	2281	2154
INDIA	2216	2511	2065	2110	1286	1449
ARGENTINA	71	90	93	139	123	84
BRAZIL	29	30	25	34	32	32
INDONESIA	29	29	28	24	24	24

Source: FAOSTAT database 1990 to 1998.

Roasting groundnut with 1 to 4 percent salt is a very common practice throughout the world. This crop is utilized in various forms including roasted, boiled, raw, ground or paste. Out of the several million tonnes of groundnut produced in the world each year, hulls represent about 25 percent of the total mass produced. This is utilized mainly in cattle and poultry feed (FOA, 2002). The hauls are important as livestock fodder, especially in dry season in the semi-arid tropics. (FAO, 2012)

2.6.2 Secondary Products

Among prominent cultivated crops in the developing countries, groundnut is unique. The plant and its products have a wide range of use in the daily lives of the people as well as in the various industries. The roots of the plant help to enrich the soil and the vines serve as excellent fodder for cattle (NRCFG, 2002). The nuts, in addition to being the most consequential source of edible oil, are useful in

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numerous other ways. When the cake is powdered and extracted in solvent, it yields defatted groundnut meal. Thus the crop has gained great popularity, based on its all-round usefulness and the financial returns it brings to the grower (FOA, 2002)

Slightly over half of groundnut produced is crushed into oil for human consumption or industrial uses. Protein meal, a by-product of crushing is an ingredient in livestock feeds. Groundnut is also consumed directly and is used in processed food and snacks. Approximately one-third of world production is used in the confectionery products. Utilization of oil, meal and confectionery groundnuts are all increasing concurrently with a gradual shift away from oil and meal into confectionery use. In many groundnut-producing countries, several products and by-products are processed and consumed locally as a few are exported. Among the by-products traded in the international market is peanut butter and roasted groundnuts. Today, technologies exist for several value-added products from groundnut with very simple locally available materials. Their products are quite easy to follow. The groundnut-based products derived from these technologies may be consumed by the farming family or sold in the domestic market. These products may add value to groundnut and enable the farming family to earn additional income. (FAO, 2012)

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2.7 Importance of Groundnuts

2.7.1 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). Results of proximate analysis conducted in groundnuts by Ayoola *et al*. (2012) are shown in table 2.2 and 2.3 below.

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Table 2.2 Proximate composition of groundnuts on percentage dry weight basis

	Types of nuts/percentage dry weight		
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).

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Table 2.3 Mineral composition of the groundnut on dry weight basis

	Types of nuts /percentage dry weight		
Mineral	Raw	Sun-dried	Roasted
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).

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2.7.2 Oil Composition of Groundnuts

Common cooking and salad oil can be produced from groundnuts. The nuts are 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 & 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).

2.7.3 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; 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 groundnut has some beneficial effects to cancer, hypertension, and inflammation patients (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 of groundnuts on inflammation, oxidative stress, and vascular reactivity patients (King *et al*., 2008). Blood pressure, visceral adiposity and the metabolic syndrome also appear

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to be positively impacted by intake of the 15 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).

2.7.3.1 Groundnuts Phytochemicals

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

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2.8 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 (FAQF, 2012). Groundnuts are not excluded since it presents its own medical issues.

2.8.1 Allergies

(FAQF, (2012) reports that approximately 0.6 % of the entire population in the United States has experienced mild to severe allergic reactions to the exposure of groundnuts. Symptoms of these allergies come in varying forms, some individuals experience watery eyes to anaphylactic shock. This can result in fatality if remained untreated for an extended period of time (FAQF, 2012). The allergenic effect of groundnuts correlates with how they are processed in countries where consumption

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is high, such as 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 trigger 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 individuals lifetime, Fleischer *et al*.2003 report that 23.3% of children will outgrow an allergy.

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2.9 Groundnuts Diseases

Many diseases affect groundnuts leading to loss of yield. Some of these diseases 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 toxins (mycotoxins). Thouvenel *et al*. 1976 identified groundnuts as one of the most severely infected tropical plants in terms of fungal diseases.

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2.9.1 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.

2.9.2 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).

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2.9.3 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.

2.9.4 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 Ghana and most part of Africa.. Some fungal diseases, their symptoms and causative fungi are tabulated in table 2.4;

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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.

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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)

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Global food production especially in 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).

2.10 Aflatoxin

In rescent years attention has been given to aflatoxin due to growing human population against food insecurity, lack of dietary diversity and facilities in monitoring the presence of the toxin's occurrence in food. Chronically, aflatoxin may be ingested through food especially groundnut. International standards for aflatoxin loads for peanuts differ in accordance to its usage; raw form and cooked. (Mbaye & Aly, 2005). Aflatoxin levels are generally regulated and recorded as parts per billion (ppb). United States Aflatoxin standards permits 20 ppb level on a

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lot-by-lot basis of food meant for human and animals consumption. Australian and European Union (EU) on the other hand set the aflatoxin acceptance level at 15 ppb for peanuts meant for direct human and animals consumption and 4 ppb for those nuts that are to be processed (GIPSA, 2009). The standards for the Europeans Union and Australia were harmonized in the late 1990s among member states after an analysis jointly carried out by FAO- WHO Expert Committee on Food Additives (JECFA). investigated the harmful nature and related health implications of the aflatoxin levels for peanuts targeted for further processing and for consumption by humans(Otsuki & Tsunehiro, 2001)

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Plate 2.0 Fungi growth on groundnuts**:** Debtanu *et al* .(2014).

The maximum acceptable level of aflatoxin B1 is established at 2 ppb for edible groundnuts while that for groundnut cakes is 20 ppb (Mbaye & Aly, 2005).

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According to (ICRISAT 2012) and (FAO, 2013), peanuts are a vital crop grown throughout Sub-Saharan Africa (SSA) which is made up of 40% of the world's peanut harvested regions, but minimally contributes only 26% of the world's peanut production. This is mainly due to poor groundnut yield in most African countries against the world's average as result of the prevailing abiotic, biotic and socio-economic factors Pande & Pandey. (2003); Upadhyaya *et al.*, 2006), Caliskan *et al*. (2008). Aflatoxin infection might account for the 14% loss of the product either through poor on-farm practices or ineffective post-harvest handling such as drying and storage. This research seeks to contribute its quota to bridge the cap of the losses to ensure revenue for farmers and also boost the Gross Domestic Product (GDP) of the nation.

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Aflatoxin infection of cash crop like groundnuts has adversely affected food availability, access, utilization, and stability which are the four pillars of food security. This indirectly reduces food availability and income. Products rejection, market value, or ability to gain access to international market is an impediment for farmers and has virtually stalled the economic development of most peanut farmers in Northern Ghana.

Aflatoxins are toxic products which are group of related difuranocoumarin, produced by the toxigenic strains of *Aspergillus flavus*, and *Aspergillus parasiticus* during their growth on crops such as corn, peanuts, wheat, vegetables and other tree nuts according to the U. S. department of Agriculture 1990 and Cary *et al*., 1998. It was also said to be the most hepatotoxic and carcinogenic toxin of fungal origin as

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well as the most widely and intensively studied group of mycotoxins and the second most abundant fungal toxin in 1990 by the U. S. Department of Agriculture. There are about ten types of aflatoxins. Aflatoxin B (AFB) is the most abundant and toxic member of the group. Aflatoxin BI is found to be toxic to all animals tested and it is the most hepatocarcinogen known (Squire, 1981). The toxicology of aflatoxin has been well documented for several animal species and humans. The signs of aflatoxin are diverse and numerous and often depend on species, sex, age, stress, reproduction and health status of the animal and humans. They include cancer, reduced immune function with compromised resistance to infections and diseases Charmley and Trenholm. (2000).

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Aflatoxin producing strains of *Aspergillums* usually contaminate crops both in the field and when they have been harvested and stored Hudler (1998); Far *et al*. (1989; Norton (1995).

In general, it appears that in those areas of the world where humidity is high or moderate and temperature are moderately high and where harvesting, storage and marketing facilities are primitive, there is considerable frequency of contamination of foods and feeds by aflatoxin producing species of *Aspergillus* Hunter (1989); Charmley and Prelusky. (1995).

Aflatoxin are said to be hepatocarcinogen, and naturally occurring mixtures of aflatoxins have been claasified as a class1 human carcinogen IARC (1993). The IARC in 1993 also concluded that there was inadequate evidence for the carcinogenicity of aflatoxin M1. EHSO (2005) explained that aflatoxin is a toxic product of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. The name

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aflatoxin was derived from a toxin producing fungus which caused a disease popularly referred to as "Turkey X disease" in England in 1960. It killed 100,000 young turkeys at the time. The fungus was later identified as *Aspergillus flavus* in 1961 and the toxin named as aflatoxin due to its origin (*A.flavis-Afla*). The EHSO indicated further that *Aspergillus flavus* is common and widespread and the fungi is mostly found in the soil, decaying vegetation and grains undergoing microbial deterioration.

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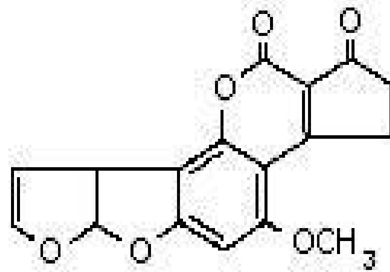
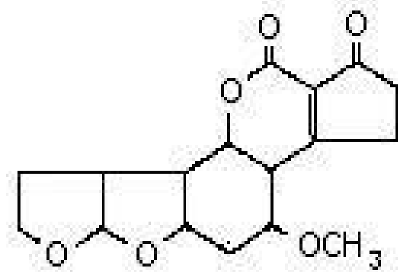
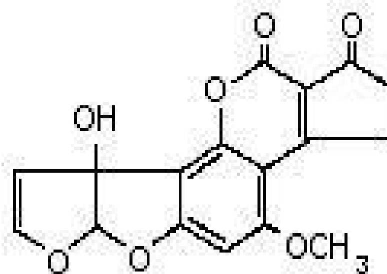
2.10.1 Chemical structures of the various types of aflatoxins.

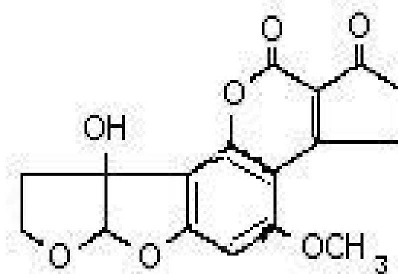
Figure 2.1 below shows the chemical structure of the different types of aflatoxin

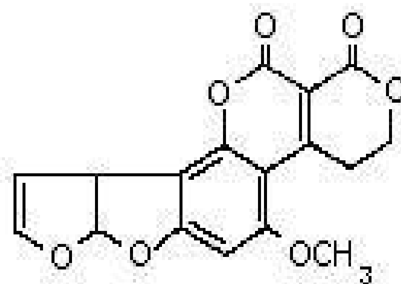
Fig. 2.1: Chemical structure of aflatoxins

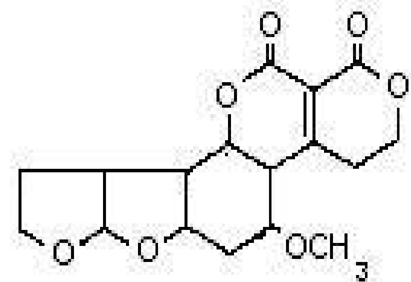












(Cole and Cox, 1981)

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2.10.2 Properties of aflatoxins

Hell (1997) stated that basically four major groups of aflatoxins are identified: B1, B2, G1 and G2 (Table 2.5). These abbreviations are indicative of the colours they exhibit/fluorescence under the ultraviolet light (385 nm); thus B is for blue and G is for yellow-green, The M is a hydroxylated metabolic product of B Bankole and Adebanjo. (2003).

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Table 2.5: The properties of aflatoxins

Property	Aflatoxins				
Chemical	B1	B2	G1	G2	M1
Formular	C17H12O8	C17H14O8	C17H12O7	C17H14O7	C17H12O7
Mole Weight	312	314	328	330	328
Meltingpt(°C)	268-269 (D)1	287-289 (D)	244-249 (D)	230(D)	299(D)
Sorbent+Pentane	Chloroform	chloroform	Chloroform	Ethyl acetate	Methanol
Flourescence	425 nm	425 nm	450 nm	425 nm	425 nm

D1= Decomposition, Source: Cole and Cox (1981).

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2.11 Biology of some toxin producing-fungi in groundnuts

2.11.1 *Aspergillus flavus*

Aspergillus flavus is a haploid filamentous fungus. It is a saprophyte and obtains its nutrient from decaying materials. On organisms such as plants and animals including human beings, *A. flavus* could be a pathogen (Saori and Keller, 2011) most often when the immune system of the organism is compromised or suppressed. Like other moulds, *Aspergillus flavus* grows by producing hyphae (Forbes *et al.,* 2007). The hyphae are known to be the basic structural units of the moulds and are tubelike projections (Forbes *et al.,* 2007). The hyphae are responsible for secreting catabolic enzymes which break down complex food molecules into smaller molecules. The small molecules are readily absorbed by the mycelium to produce asexual spores called conidia (Wilson *et al*., 1981). The fungus produces extracellular enzymes such as proteases.

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2.11.2 *Rhizopus* species

The *Rhizopus* species are grouped under the class Zygomycetes. This class is characterized by the production of large, ribbon-like hyphae that are irregular in diameter and contain occasional septa (Forbes *et al*., 2007). The identification of organism in this class is confirmed by observing the sporangia which produce internally spherical, yellow or brown spores as shown in Figure 4. Rhizopus species have been used in solid state fermentation due to its high protease producing ability (Kranthi *et al*., 2012). *Rhizopus oligosporus* and *Rhizopus nigrican* have been used for solid state fermentation (Belewu *et al*., 2010). According to Schipper, (1984), malt agar is the best growth medium for *R.*

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stolonifer, while in many other studies potato-dextrose-agar is commonly used but it may not be the best medium to grow this fungus (Mari *et al*., 2002; Nishijima *et al.,* 1990; Northover and Zhou, 2002)

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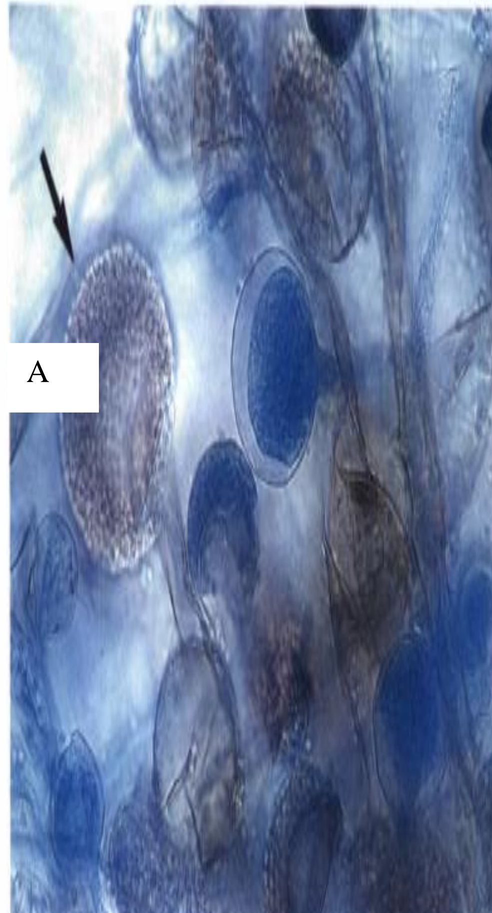

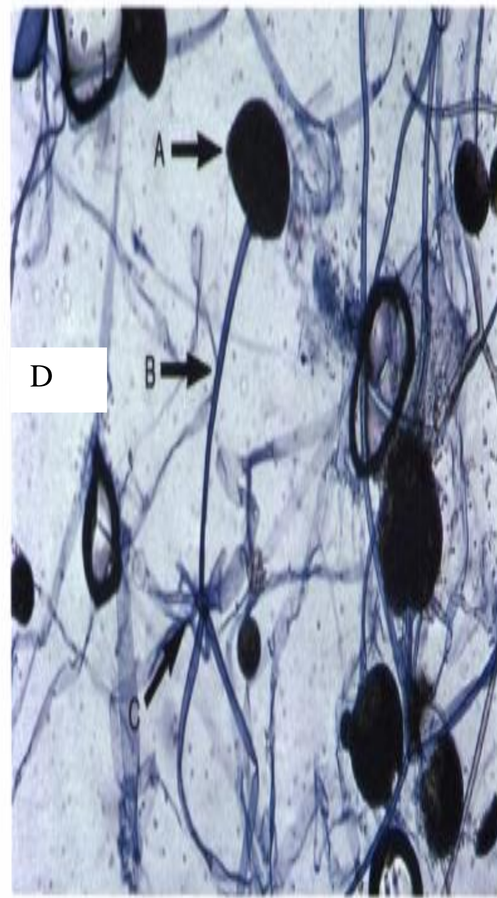

(Forbes *et al., 2007*)

Plate 2.1 Large saclike sporangi that contain sporangiospores characteristic of the zygomycetes

Note: A= sporangiophore **B**= pauciseptate hyphae **C** = rhizoids **D**= sporangium

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2.11.3 *Penicillium* species

Species of *Penicillium* are often found in soils Samson *et al.* (2004), foods and grains among others. Colonies of *Penicillium* are often characterized by shades of green or blue-green, but pink, white, or other colours may be found. The surface of the colonies may be velvety to powdery because of the presence of conidia (Forbes *et al.,* 2007). Some *Penicillium* species have been reported to possess enzymes of high levels of activity Abdel-Fattah *et al. (*1972). Currently, image analysis is a fast quantitative method commonly used to analyze image features giving a more precise and accurate data in identification of fungi Hernández-Lauzardo. (2005).

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2.12 Peanut infection by aflatoxin

Peanuts have been documented by several authors as one of the excellent substrates for fungal growth and aflatoxin contamination in some West African countries Dick. (2008). According to Setamou *et al*. (1997) pre-harvested peanuts samples infested with aflatoxin in Benin was 42.5% in 1994 and 30% in 1995. Bouraima *et al.* (1993) also found that aflatoxin levels in stored kernels in Benin were 14 ppb for B1 and 58 ppb for G1. According to Udoh *et al*. (2000) 33% of maize samples from different ecological zones in Nigeria were contaminated with aflatoxin. Hell *et al*. (2000a) reported that 9.9 to 32.2% of maize samples in different ecozones in Benin prepared for storage had aflatoxin levels more than 5 ppb and the levels increased to 15% and 32.2% after six months of storage. Kpodo (1996) reported that maize samples from silos and warehouses in Ghana contained aflatoxin levels in the range of 20 to 355 ppb; while fermented maize dough collected from major processing sites contained aflatoxin levels of 0.7 to 313 ppb. Insects have also been

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reported as playing a role in the spread of *A. flavus* and increase in aflatoxin contamination in peanuts. Setamou *et al*. (1998) found that the percentage of grains infected with *A. flavus* and samples contaminated with aflatoxin including the mean aflatoxin content of samples increased correspondingly with increased insect damage in pre-harvested peanuts in Benin. According to Hell *et al*. (2000b) maize free of insect damage had no aflatoxin contamination, but maize with 70% of the cobs damaged by insects had 30.3% of the cobs contaminated with aflatoxin. Payne. (1992) indicated that pre-harvest aflatoxin production in maize depended on weather conditions during crop maturations. In addition, the risk of aflatoxin contamination before harvest is highest when there is moisture stress coupled with elevated temperatures (Payne, 1992).

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2.13 Factors influencing fungi infection and aflatoxin development in peanuts

Diener *et al*. (1987) reported that the factors that influence the growth of *A. flavus* and the formation of aflatoxins can be classified into three categories: climatic factors, agronomic factors and biotic factors. Each fungus require special conditions like substrate, moisture, temperature for its growth and other conditions for its toxin production Diener *et al*. (1987). However, the main factors affecting toxin production are genetic which are related to the fungus, its strain and its genetic capability. It also includes environmental factors, the substrate (on which fungus will grow) and its nutritional content. Toxin production depends on a lot of factors such as water content of substrate and ambient relative humidity, temperature, oxygen content, carbon dioxide, mechanical damage (enabling fungal invasion and mycotoxin production) and insect invasion (enable fungal invasion

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and mycotoxin production) results in toxin production (www.aquafind.com/index.php)

2.13.1 Climatic factors

Studies in Thailand revealed that moisture content of maize samples varied from 16% to 30.7% depending on the time of harvest (Dick, 2008). Maize that was harvested early (113days) had the highest levels of aflatoxins due to the moisture level (Kawasugi *et al.,* 1988). Moisture content of less than 17% showed no infection with *A. flavus* (Dick, 2008). Increase in relative humidity above 85%, results in the growth of *A. flavus*. In this range, a small increase in moisture can be very influential in terms of increasing the risk of aflatoxin infection (Christensen and Mirocha, 1976). Aflatoxins can be synthesized at a relative humidity of 88, 90 and 99% (Lillehoj, 1983), which are common in the Southern parts of West Africa (Hell, 1997). Furthermore, Hell (1997) indicated that commodities stored at humidity between 75% and 85% are susceptible to fungal attack within the normal storage time.

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The other factors that influence the aflatoxin contamination of groundnuts are agronomic and biotic factors. When groundnut is grown with a crop such as maize that is also susceptible to aflatoxin development there is an increased risk of toxin metabolism Cotty. (1994). Cole *et al*. (1982) investigated the effect of peanut, maize, soyabean crop rotation on aflatoxin development. They found more aflatoxin if the groundnuts was planted after maize. Griffin *et al*. (1981) also observed that when maize was cropped in rotation with groundnut there was *A. flavus* population build – up in the soil over the years. Barry *et al*. (1992) showed

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that maize cultivars that had a resistance to ear- infesting insects also produced less aflatoxin in pre- harvest grains. The incidence of *A. flavus* fungi and aflatoxin contamination was comparatively higher in insect-damaged groundnuts samples from different localities in India than in insect free samples Sinha and Sinha. (1992). Zuber *et al.*. (1986) reported that insects that feed on groundnut pods in the field predispose kernels to *A. flavus* infection through the physical damage caused by their feeding.

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2.14 Relationship between Storage structures, climatic conditions and aflatoxin contamination

Hell (1997) studied stored maize and aflatoxin contamination in four agro-ecological zones in Benin observed that 20% of the maize samples tested positive for aflatoxin. Maize stored in jute bags for six months had the highest aflatoxin content with a mean of 250 ppb. The “Ago” and “Ebliva” storage structures recorded aflatoxin in maize at means of 71.1ppb and 32.0 ppb respectively. Hell (1997) also stated further that high level of aflatoxin contamination (40%) in maize was recorded in clay storage structures (in a zone with annual rainfall range of 1000-1300 mm). The highest aflatoxin concentration was recorded in crib with mean of 394.7 ppb. Baskets, floor and platform stores showed low mean aflatoxin levels. There was more aflatoxin in maize stored in jute bags than baskets, conical stores and the “Ago “storage structure.

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Zones with annual rainfall range of 900-1300 mm had aflatoxin contents of maize stored in baskets being 133.9 ppb, on platform and plastic bags being 75.2 ppb and 67.7ppb respectively. The mean contamination levels were lower than the zone with annual rainfall range of 1000-1300 mm. However, 52.1% of the sample tested positive for aflatoxin. The last zone with annual rainfall less than 1000 mm and with a relative humidity of 40% - 60% had the highest contamination level in the clay stores (116.4 ppb) and the "Secco" (98.8 ppb). In this zone more than 35.5% of the stores were contaminated Hell. (1997). In Uganda with an annual rainfall range of 500-2000 mm and an average temperature of 25°C, conditions are conducive for the growth of *A. flavus* and the subsequent production of aflatoxin Kaaya and Warren. (2005). Kayaa and Warren (2005) in a review on aflatoxin contamination of maize in Uganda indicated that maize stored by 17 traders (majority in woven polypropylene bags) for six to seven months had mean aflatoxin levels of 107 ppb which is an indication that the grains were not suitable for export and local markets. They also reported that in an earlier study of fungi and aflatoxin in maize grains in five districts of Uganda (Kampala, Mpigi, Mubende, Luwero and Mukono), maize samples monitored from shops and markets for five months gave aflatoxin levels in the range of 0-50 ppb; with seven out of the eight samples contaminated by the B-group. Coupled with this, over 30% of the samples had aflatoxin levels above 20 ppb; while 50% contained up to 10 ppb. They explained further that the high aflatoxin levels resulted from high moisture content as 48% of the maize samples had moisture content favourable for mould growth. Kaaya and Warren (2005) observed that one of the methods that

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protected maize against aflatoxin contamination was storage above fire racks which was unsuitable for larger quantities of grains.

2.15 Aflatoxins and their effects on human and animal health

There is a widespread exposure to aflatoxin in West Africa probably starting in the utero. Blood tests have shown that very high percentage of West Africans are exposed to aflatoxins (Bankole *et al*., 2003; Wild, 1996). Studies carried in Gambia, Guinea, Nigeria and Senegal indicated that over 98% of respondents tested positive to aflatoxin markers (Bankole *et al*., 2003; Wild, 1996). The economic and health importance of aflatoxins need much attention because of their ability to contaminate human foods and animal feeds, especially cereals (Keller *et al*., 1994). Losses in livestock and poultry production resulting from direct aflatoxin contamination of feeds include death, immune system suppression, reduced growth rates and losses in feed efficiency. In humans the effects are acute aflatoxicosis, liver cell cancer and Hepatitis B. Li *et al*. (2001) in a study in China found that the levels of aflatoxins B1, B2, and G1 were quite high in maize from the high incidence area for human hepatocellular carcinoma. They also indicated that the average daily intake of aflatoxin B1 from the high risk area was 184.1µg/ml. Hepatocellular carcinoma is the fifth leading cause of cancer mortality in the world and it accounts for about 70% of cancer deaths in certain parts of Asia and Africa Farombi. (2006). Aflatoxin positive "kwashiorkor" children in Togo and Benin showed much greater severity of edema, increased number of infections, lower haemoglobin levels and longer duration of hospital stay than aflatoxin-negative "kwashiorkor" children Adhikari *et al*. (1994); Ramjee. (1996).

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Presumably, the protein deficiency characteristic symptom of "kwashiorkor" reduces the capacity of the liver to detoxify aflatoxins. It is speculated that aflatoxin may be a contributory factor in increasing the morbidity of children suffering from the disease Ramjee. (1996). Uriah *et al*. (2001) in their study in Nigeria found that blood and semen aflatoxin levels ranged from 700 to 1393 µg/ml and 60 to148 µg/ml respectively in infertile men and were significantly higher than that in fertile men. Gong *et al*. (2002) reported that children in Togo and Benin who ate foods contaminated with aflatoxins had the syndrome of stunted growth and underweight, which are normally associated with malnutrition. Aflatoxins have also been shown to be immunotoxic to both livestock and humans. Turner *et al*. (2003) detected aflatoxin albumin adducts in 93% of sampled children (6-9years) in Gambia and provided evidence that immunoglobin A (IgA) in saliva may be reduced because of high dietary levels of aflatoxin exposure. The study confirmed that children in rural areas of Gambia were frequently exposed to high levels of aflatoxin. In the US, the FDA uses an action level of 20 ppb as the maximum residue limit allowed in food for human consumption, except for milk (FAO, 1996). The European Union, as a sanitary precaution measure enacted in 1998 very severe aflatoxin tolerance standards of 2ppb aflatoxin B1 and 4ppb total aflatoxins for nuts and cereals for human consumption (CEC, 1998), which came into effect from January 2001 Dimanchie. (2001).

(Lewis *et al*., (2005) reported that one of the largest aflatoxicosis outbreaks in Kenya in April 2004 with 317 cases claimed 125 lives (39.4%). They also indicated that home grown maize was the source of the outbreak. In their findings

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55% of maize products had aflatoxin levels greater than the Kenyan regulatory limits of 20 ppb, 35% had levels exceeding 100 ppb and 7% had levels above 1000 ppb. Furthermore, they stated that the outbreak was one of the largest and the most severe of acute aflatoxicosis documented worldwide. Nyamong and Okioma (2005) reported that in April to September 2004 and April 2005 the largest outbreaks of aflatoxins occurred in 15 geographical areas (40,149km^2) and claimed over 123 lives in Eastern Kenya. They also indicated that descriptive epidemiological studies showed a relationship between the outbreak and the local methods of harvesting, storing and processing of maize. In another development, Sharif (2004) reported that 40 people in Makueni and Kitui Districts of Kenya died out of aflatoxin poisoning resulting from maize meal. Others on admission due to the consumption of the meal suffered from jaundice, leg edema and hepatomegaly. (Turner *et al*., 2000) stated that aflatoxin synergies other agents such as Hepatitis B in the causation of liver cancer. Ankrah *et al*. (1994a) stated that there is a relationship between aflatoxin contamination in diet and liver inflammation where serum, urine and faecal samples were monitored for aflatoxins or their metabolites. The results showed that 35% of the subjects studied had B1 or its metabolites which is indicative of contamination of the food items by *A. parasiticus* not *A. flavus*. They also concluded that results were suggestive of liver inflammation, not liver cancer. Other studies reported on significant correlation between the primary liver mortality rates and aflatoxin intake in the local foods in five villages in China Yu *et a*l. (1989).

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2.16 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, Powell *et al*., 1994). Mould that produce characteristic asexual spore-heads belong to the genus *Aspergillus.* These spores are the most important microscopic character used in *Aspergillus* taxonomy (Samson & Varga, 2009). Approximately, 250 species of this genus are known (Geiser *et al*., 2008). This number is however likely to see significant increase in future due to rising application of the phylogenetic species based concept on DNA sequence data instead of visual morphological characters. Benneth, 2010 classified Aspergillus as follows:

Table 2.6: Classification of *Aspergillus*

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

(Bennett, 2010)

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

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2.16.1 Aspergillus as a mycotoxin producing fungi

Aspergillus fungus is a saprophytic mould Madunagu and Umana (2000). It is often referred to as filamentous fungus Disalvo (2000; Woods (2002). It was originally/formally classified as Deuteromycotina (Fungi imperfecto) but further research reassigned it as Ascomycotina (sac fungus) because of its ability to form asci within closed ascocarps Porter, (2001). However, the genus *Aspergillus* has not yet been subdivided into classes or orders because of the fact that *Ascomycotina* classification was not complete. But today the genus *Aspergillus* has been given complete classification Dixon and Fromtting, (1991).

Morphologically, *Aspergillus* fungi are monomorphic but display a complex regulated development. They have septate hyphae and conidia. They form fruiting bodies that are spherical or flask or cup shaped and whose sexually formed spores are borne in sacs called asci. They are the largest group of fungi that produce both sexual and asexual spores and conidia Balbach and Bliss. (1982).

There are over 200 different species of *Aspergillus.* Some of the more common ones include: *Aspergillus niger, A. flavus, A. carbonaceous, A. terreus, A. parasiticus* and *A. tamari*. *Aspergillus* can be found in various habitats under

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different environmental conditions. Some strains of *Aspergillus* are used to produce antibiotics and some beneficial genetic manipulation mechanisms.

They can also be used in food fermentations, production of citric acid, used as preservation in soft drinks and canned foods, also as sources of enzymes Balbach and Bliss (1982), Porter. (2001).

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In spite of these merits, there are some demerits associated with this fungus. *Aspergillus* tends to cause the spoilage of foodstuffs and has the ability to decompose other materials such as woods, textiles, paints Gupeta. (1981) and while some species severely damage agricultural crops by producing secondary metabolites called aflatoxin.

2.16.2 Infection of peanuts by *Aspergillus flavus*

Aspergillus flavus infects peanuts in the field in three stages. Initially, air-borne or insect transmitted conidia infest the pod and grow into the developing kernel. Subsequently, the nuts in the pod, presumably damaged by insects or birds become infested with *A. flavus* and infested with aflatoxins Dick. (2008). Stress to the plant resulting from climatic and cultural factors such as drought and nutrient deficiency make the plant more susceptible to fungal infection Wicklow. (1998). Marsh and Payne (1984) also observed that nuts infection was possible through the scar left by endosperm detachment. Related literature by Fennel et al. (1993) show that records on *A. flavus* infection of maize planted indicated 68% for the kernels, 48% at the tip and 12% through the endosperm. (Mycock *et al.*, 1992) stated that maize grains internally infected with *A. flavus*, when left to germinate amongst other grains could develop and infect others with the same fungus. It has been concluded that

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the fungi may be seed-borne according to Cotty, *et al*. (1994). Saito *et al*. (1989) suggested that *A. flavus* was soil-borne and it survived for long periods in the soil.

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CHAPTER THREE

3.0 Methodology

3.1 General Overview

This section of the thesis provides information on the study area, research design, study population and sampling procedures used. Information about the sample size, instrumentation, pilot study and data collection as well as the types of data analyses conducted will also be provided in this chapter. The rationales behind the analyses conducted will also be explained.

3.2 Research Design and Methods

This examines how the research was carried out. It discusses the subject matter, the various tools that were used, condition under which the study was carried out as well as how it was analyzed. The research adopted a survey research design to obtain varying opinions from the farmers on the effects of aflatoxin concentration in peanuts as well as the effect on yields.

3.3 Research Data Source

The research sourced both quantitative and qualitative data from the farmers. Primary data was sourced directly from farmers and their household. Data on groundnuts output, prices, annual rainfall and temperature from the district were used. Secondary data was obtained from statistical abstracts, Tamale district development plans, and from annual agricultural reports in the Ministry of Agriculture offices in Tamale Metropolis. This was to obtain information on those factors that affect good yields of groundnuts from farmers and ultimately their income. Secondary data was also taken from the office of Ministry of Food and

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Agriculture (MoFA), libraries, internet, Ghana Statistical Service and other relevant stakeholders so as to aid in this research.

3.4 Field study

3.4.1 The study area

The research was conducted in thirty (30) communities within five Districts: Tolong, Kumbungu, Savelugu, Yendi and Nanton in the Northern region of Ghana. In each community two farmers were chosen. The areas that were chosen were purposively done because, the natives were found to be dominantly farmers and produce groundnuts every season. The mean annual rainfall varies from between 900 mm and 1200 mm (MoFA, 2013). The vegetation of the area is mainly savannah and deciduous forests which favours the growth of trees and cash crops such as mango, dawadawa trees and subsistence crops like groundnut, yam, cassava, maize and guinea corn (MoFA, 2013).

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3.4.2 Sampling of farmer-stored groundnuts for aflatoxin analysis and isolation of fungi species

3.4.2.1 Selection of farmers for the study

Purposive and random sampling technique was employed to select groundnut farming households (HH) in the Northern Region for the study. Farmers that had obtained a minimum of twelve (12) bags/sacks of groundnuts after harvest in the previous season and equally willing to be part of the study, were included in the study. In all, 500 groundnut HH farmers were selected in thirty (30) typical groundnut farming communities in the Tolon, Kumbungu, Savelugu, Yendi and Nanton Districts. Selection of farmers was done in the month of February, 2014.

Questionnaires designed to obtain quantitative information on the frequent usage of groundnuts in their diet, the health status of their household members and their reactions to consumption of groundnuts were administered to the farmers.

3.4.2.2 Sampling of groundnuts from the farmer-stored grains

Sampling protocols adopted for the study was consistent with Schuller *et al.*, (1976); Whitaker & Dickens, (1983). The groundnut samples were collected at three different levels of the storage bags to give a reasonable representation of the sample. These samples were put in sampling bags, labelled and transported to the laboratory for aflatoxin analysis and isolation of fungi species.

3.4.3 Evaluation of some drying and storage methods on the aflatoxin concentrations of groundnuts

3.4.3.1 Selection of farmers based on drying and storage methods evaluated

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A sub-set of twenty (20) HH farmers were selected randomly from the initial 500HH used in the survey. Three drying protocols were evaluated in the study. These were drying on tarpaulins; drying on racks and the farmers' method of drying that is, drying on bare floor. Since the trials were carried out on the farmers' farms houses, each of them were provided with the tarpaulin and racks were raised on their field. Also the storage bags (jute sack, plastic bags) evaluated in the study were acquired and distributed to each of the participating farmers. These were evaluated alongside the sacks provided by farmers (status quo).

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3.4.4. Sampling, Plugging, Drying and storage of harvested groundnuts

3.4.4.1. On-field Sampling

After the maturity of groundnuts has been established by each farmer on their field, samples of the groundnuts were taken for aflatoxin and microbial contamination, analysis followed by drying and storage experiments. The plants were uprooted from four quadrants of the field and the middle portion on the days of harvesting. The nuts were detached and one (1) kilogram of peanuts was obtained from the field of each farmer. Sub-sampling of 50 grams was taken from the lots for aflatoxin analysis. *Aspergillus* species which are the producers of aflatoxin and other mycotoxin producing species were also isolated from the samples. Plate 3.0 shows on-field sampling whilst plates 3.1 and 3.2 shows on-field plugging of groundnuts.

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Plate 3.0 Sampling directly from field

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3.4.4.2 On-field plugging of groundnuts

Plate 3.1 Plugging nuts from the field

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Plate 3.2 On-fields plugging by farmer

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The rest of the harvested peanuts from each famer were divided into three parts. First part was dried on the bare ground (status quo drying), second part dried on a tarpaulin and the third part dried on raised racks on the field. Groundnuts from each of the three drying methods were divided into three (3) portions and stored in different types of bags; new jute bag, new plastic bag and old bag (status quo bag) and kept on a wooden pallet. Drying and storage treatments are illustrated in table 3.0 below.

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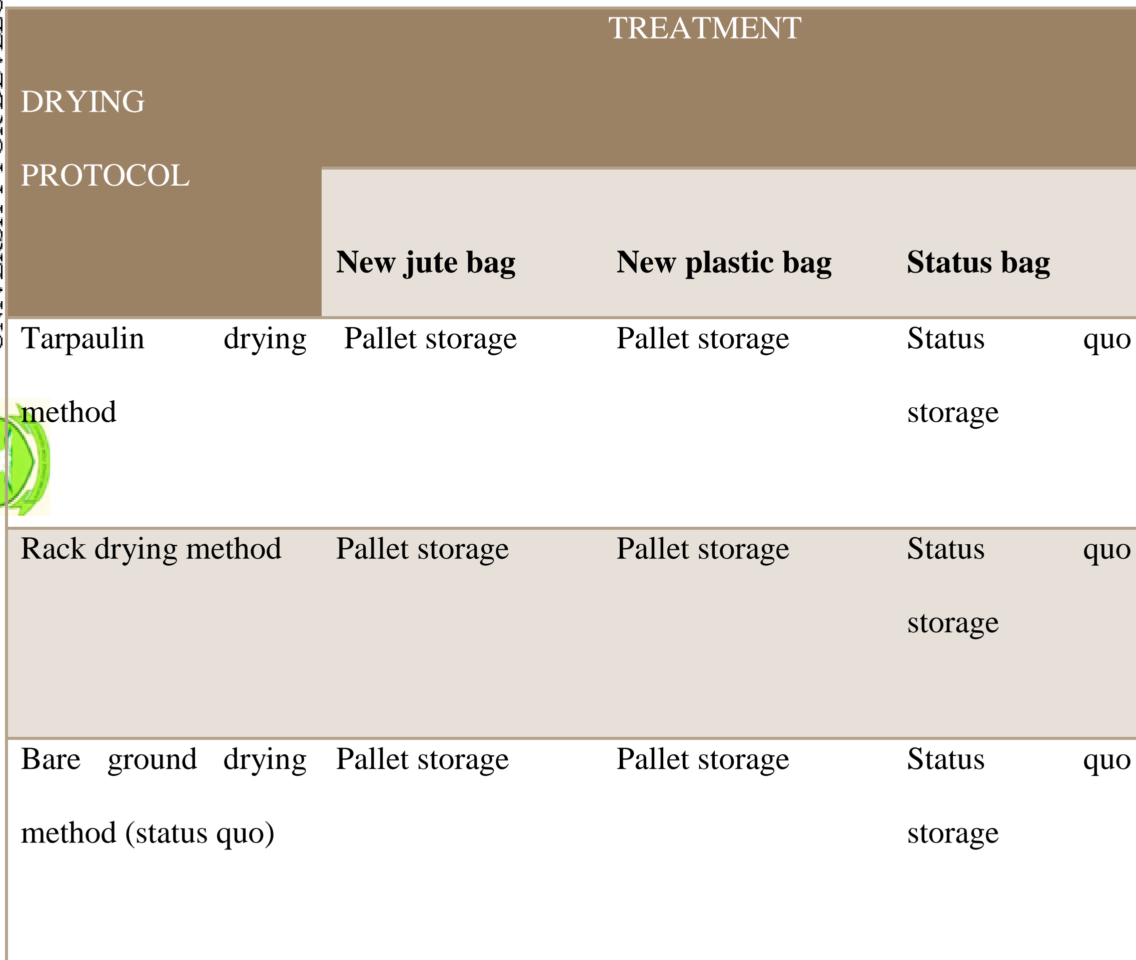
Table 3.0 Drying and Storage Treatment Protocol



DRYING PROTOCOL	TREATMENT		
	New jute bag	**New plastic bag**	**Status bag**
Tarpaulin drying method	Pallet storage	Pallet storage	Status quo storage
Rack drying method	Pallet storage	Pallet storage	Status quo storage
Bare ground drying method (status quo)	Pallet storage	Pallet storage	Status quo storage

Field Survey, 2014

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3.4.4.3. Drying of groundnuts on Tarpaulins

After harvesting of groundnuts by farmers, three medium plastic sacks full of the groundnuts were spread uniformly on the tarpaulin for a maximum of seven (7) days. Days that had rains disrupting drying process were recorded. At the end of each day, the nuts were collected back into the plastic sack and placed on top of a pallet to prevent contact of the grains with the bare floor. This continued until the nuts were well- dried. Plate 3.3 shows an illustration of tarpaulin drying.

Plate 3.3 Tarpaulin drying method

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3.4.4.4 Use of Rack

Racks were built on the farms for the farmers as one of the drying interventions. Groundnut plants with the pods attached were harvested at a quantity of three mini plastic sacks. These were put on top of the rack, raised at a height of about 5ft from the ground to create space for air circulation through the nuts. These were turned intermittently to avoid accumulation of heat and improper drying of the nuts. The nuts were then plugged after seven days and equally stored in clean sacks placed on a pallet to prevent direct contact with the floor as illustrated by plate 3.4.

Plate 3.4 Rack drying method

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3.4.4.5 Farmers method of drying

Each participating farmer was made to use their own method of drying. In all these cases, these nuts were spread uniformly in the open yard of the house or outside on the bare floor. The dried nuts were then stored in the farmers' own storage sacks and placed in the room where they normally keep their produce. Plate 3.5 illustrates how the farmer dries his groundnuts.

Plate 3.5 Status Quo drying method

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Plate 3.6 Types of storage bags labeled A and B

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3.4.5 Sampling of groundnuts for aflatoxin analysis after drying.

A representative sample from the dried nuts was randomly obtained from each of the three drying protocols (Tarpaulin, Rack and Status Quo drying methods) from eighteen (18) farmers out of the twenty. A total of sixty-seven (67) samples were taken from the selected household groundnuts producers in late November and early December after drying was completed for aflatoxin analysis

3.4.6 Sampling of groundnuts after three months of storage

Representative peanut samples were randomly taken from top, middle, bottom and sides of each storage bag. Nine (9) samples of 200g each were taken from eighteen groundnut farming households out of the twenty farmers. A total of one hundred and seventy (170) samples were obtained from farmers after three months of storage in the month of February, 2015. These samples were analyzed for aflatoxin contamination for comparative studies.

3.4.7 Sample Coding

Unique codes were assigned to the Districts, Communities and Households samples were taken. 004/SV/YG means sample from household number four (4) in the Savelugu District (SV) and Yong Community.

3.4.8 Sample Compositing/Preparation/Storage

3.4.8.1 Compositing and Re-sampling

Moist and dried peanut samples from the lots were composited and analytical sample of 50g required for the FQ reader analysis was taken. They were then relabeled and put in storage.

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3.4.8.2 Storing by Refrigeration

Prior to the analysis, unshelled freshly harvested and dried peanuts samples were stored in a freezer at -4 ^{0}C and thawed for two weeks before analysis was carried out on them. This was done to stop the activities of the *Aspergillus* and other fungal species.

3.5 Laboratory studies

3.5.1Material/Equipment/Tools/Chemical

The list of apparatus used in testing the aflatoxin contamination levels in the peanuts samples collected were obtained from the Food Technology Laboratory of the University.

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7 Flouro Quant Afla*Plus* Reader was used to read aflatoxin levels in the groundnuts.

8 Waring Commercial Blender was used for dry and wet/mixed blending groundnut samples.

9 Filter paper and funnel for filtering grounded nuts

10 Syringe plunger for extracting groundnut filtrate.

11 Pipette (Fixed 1000 µL)

12 Test tube rack

13 Extraction jars/containers

14 Curvets and covers

15 100Ml cylinder

16 Timer

17 Vortex mixer

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18 Scale

19 Developer concentrate that serves as diluents of the filtrate for testing

3.5.2 Safety Precautions

Since the extracting chemicals are very potent in rendering humans infertile, inhalation/swallowing could also course death. Safety laboratory coats, hand gloves and nose masks were worn.

3.5.3 Aflatoxin Determination

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Aflatoxin analysis was carried out using the FQ reader, following the manufacturer's instructions. The samples of groundnut selected for aflatoxin analysis were shelled manually in the laboratory. To prevent cross contamination, gloves were won and hands were washed intermittently and disinfected with 80/20 ethanol between shelling of sample. After that, a Warings' commercial blender was used to blend the samples to obtain fine grounded sample that can pass through one millimeter ($1mm^2$) square mesh. The analytical sample of fifty grams (50g) was then obtained from the lots of the blended samples and 100ml of 86/14 acetonitril/deionised water added and blended again for one (1) minute after which the blended samples were then put into an extraction container. In some cases, a methanol/water (80/20) solution was used. In such a situation, the sample was blended for three (3) minutes. The sample was grounded in such a way that 70% passed through a twenty (20) mesh sieve and mixed well to ensure homogeneity.

The FQ Reader was calibrated using the low and high standards calibers as well as the control to ensure that the values obtained are within the limits of the protocol of

testing. The high caliber was 18.6; the low caliber was 0.8 while the control was 7.7-10.7

3.5.4. Analysis of the extracted samples by using the SolSep® 2001 column.

Two thousand microlitres of the extracted sample was then put into columns using a pipette. The columns were then placed in a 12×75mm cuvette. A syringe plunger and stopper were assembled on top of the column. It was then pushed to extract completely through the column. 1000µl of the extract was then transferred into a clean scratch-free cuvette, (Romer lab, 2011). One developer concentrate content vial was transferred into 25ml deionized/distilled water using a disposable transfer pipette. The developer concentrate was discarded when it's pulled into a disposable pipette and the colour does not turn yellow/brown. The empty vial was then rinsed three times with the prepared developer, returning the rinse to the 50ml bottle each time. 1000µl of prepared developer was then added to the purified extract. The cuvette was capped and vortex for five (5) seconds. The vortex sample was then wiped using a lint-free paper and inserted into a calibrated fluorometer. The samples in the cuvette after a programmed 30 seconds the result were then displayed and recorded in parts per billion (Romer lab, 2011)

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3.5.5 Isolation of fungal species from samples.

Fungi species were isolated from the samples collected from the farmer-stored groundnuts using the method of dilution and direct plating proposed by Warcup, (1960) and Misiivec (1984). Single analysis of shelled peanuts samples were respectively carried out to isolate the aflatoxin producing *Aspergillus* and other fungal *sp* from the groundnuts samples. Samples from communities where aflatoxin

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concentration were higher than 30pbb and lower than 10pbb were selected for the study.

3.5.6 Media preparation and dispensing

The isolation of fungal species was carried out on potato dextrose agar (PDA). The media was prepared at a rate of 39 g/l and was autoclaved at 121°C for 15 minutes after which the molten mixture was allowed to cool and dispensed into sterile petri dishes and allowed to set under the sterile conditions of lamella flow chamber to avoid contamination. Bubbles were eliminated from the poured media by flaming using bursen burner.

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Pieces of the tissues of the groundnut samples were washed with deionized water, sterilized with alcohol and then placed in bleach for a minute before it was then placed on a tissue paper to dry. The sterilized pieces were then placed on the set media. Flames of fire were used over the set media to prevent contamination and bubbles in the media. Loops to be used for inoculation groundnut samples were dipped in alcohol and then passed over a flame to prevent any environmental agent from being cultured along the main sample. Plated peanuts were incubated at room temperature of about 25^0C for five (5) days for all species of fungi present to grow. Fungal species that grew on the media were sub-cultured after the 5th day of plating to obtain pure cultures. Plate 3.7 and 3.8 shows the culturing process and incubation at room temperature respectively.

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Plate 3.7 Culturing process in the laminar flow hood

Plate 3.8 culturing of Aspergillus species at room temperature

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3.5.7 Identification of Aflatoxin producing isolates

Aflatoxin producing *Aspergillus* species were identified by two techniques; macroscopic and microscopic analysis.

3.5.7.1 Macroscopic technique

This identification technique involved observation of the morphological and cultural characteristics of pure colonies of culture, e.g. colonies color, fruiting bodies etc.

3.5.7.2 Microscopic technique

Wet mount slides were prepared from the cultured plates using lactophenol blue and covered with cover slide. The slide was then examined using both the low and high power magnification of the compound microscope. The distinctive features of each species including the nature of the hyphae, nature of conidiophore and the nature of the conidia were observed and used for the identification of species.

3.6 Moisture analysis of dried sampled groundnuts

The moisture levels of the dried sampled groundnuts from storage were determined using the oven drying method. Calculations of the moisture levels were done using the following equation;

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

%W = Percentage of moisture in the sample

A = Weight of wet sample (grams)

B = Weight of dry sample (grams)

W% were reported to nearest tenth

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3.7 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. Summary of contents variants of aflatoxin values for storage treatments were performed for comparative studies using One-way ANOVA and summary of statistics on GenStat. Means and Standard deviations of aflatoxin values from 20 communities were analyzed using summary of statistics on GenStat.

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CHAPTER FOUR

4.0 Results

4.1. Harvesting and post-harvest practices carried out by farmers on harvested groundnuts in the study area.

4.1.1 Number of days taken for harvesting of mature groundnuts

The number of days taken for farmers to harvest their mature groundnuts ranged from one (1) day in the Yong community to four (4) days in the Tarikpaar community. In Denyokpaligu, Nanton-kurugu and Nyerikiyiili, farmers took approximately one and half days to harvest while those in Nyetua took three (3) days.

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Table 4.0 Number of days used to uproot fresh groundnuts

Communities	Minimum	Maximum	Mean
Denyokpaligu	1	2	1.5
Moglaa	1	1	1.0
Nanton-kurugu	2	3	1.5
Nyerikiyilli	1	2	1.5
Nyetua	3	3	3.0
Sandu	1	1	1.0
Tarikpaar	1	7	4.0
Yilkpani	2	3	2.5
Yong	1	1	1.0

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4.1.2 Number of day's nuts stayed on-filed after uprooting of plants

The number of days taken to plug the nuts after uprooting of the groundnut plants ranged from 1 to 4.5 days after harvesting. Farmers in the Kyirikyilli and Nanton-Kurugu leave nuts for at least a day before plugging while those in the other communities took more than a day.

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Table 4.1 Number of day's fresh groundnuts stayed on field.

Communities	Minimum	Maximum	Mean
Denyokpaligu	1	2	1.5
Kyirikiyilli	1	1	1.0
Moglaa	3	4	3.5
Nanton-Kurugu	0	2	1.0
Nyetua	4	4	4.0
Sandu	1	2	1.5
Tarikpaar	2	7	4.5
Yilkpani	2	3	2.5
Yong	1	2	1.5

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4.1.3 Number of days taken to plug nuts from uprooted plants

Averagely each community used a day to plug the groundnuts. Sandu was the only community with a maximum of two days (Table 4.2)

Table 4.2 Number of days used to plug fresh groundnuts

Communities	Minimum	Maximum	Mean
Denyokpaligu	1	1	1.0
Moglaa	1	1	1.0
Nanton-kurugu	1	1	1.0
Nyerikiyilli	1	1	1.0
Nyetua	1	1	1.0
Sandu	1	2	1.5
Tarikpaar	1	1	1.0
Yilkpani	1	1	1.0
Yong	1	1	1.0

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4.2 Distribution of aflatoxin contaminations in farmer stored-groundnuts from the different communities

Results from the survey of the aflatoxin concentration of farmer-stored groundnuts in the various communities of the Northern Region are shown in Table 4.3. The minimum values obtained ranged from 0.92ppb to 8.89ppb. The lowest of the groundnuts concentration of 0.92ppb was recorded in Gbrimani while the highest of 8.89ppb was recorded in groundnuts from Machiyilli community. However, there were no detectable minimum concentrations of aflatoxin for the Zoggu community. The highest concentration of 6977ppb was found in the Machiyilli community, while the lowest of 133.1 was recorded in the Zoggu community (Table 4.3).

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On the average, the aflatoxin concentrations ranged from a minimum of 11.08ppb to 367.5ppb among the various communities. The highest concentration was recorded in Digbila community while the lowest was recorded in the Satani community. The concentrations recorded in Satani, Mion, Naadundu and Tamalgu were almost the same and were much lower than what were recorded in Machiyili, Digbila and Sambu-Yesu. Communities such as Kpisinga, Kplung and Bachelbado recorded higher concentrations of aflatoxin than in Satani and others but lower than what was recorded in Machiyilli and Digbila (Table 4.3).

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Table 4.3 Aflatoxin levels in stored groundnuts collected from farmers in the Northern Region in 2016

	Aflatoxin concentrations (ppb)		
Communities	**Minimum**	**Maximum**	**Mean±SE**
Machiyilli	8.89	6977	341.90 ± 1224.00
Satani	1.96	47.45	11.08 ± 8.59
Bachelbado	4.11	2580	144.60 ± 491.10
Zion	2.51	65.71	11.40 ± 13.70
Digbila	9.53	6597	367.50 ± 1279.00
Galuwei	2.08	3002	332.00 ± 782.30
Gbrimani	0.92	123.5	14.54 ± 29.75
Kpakyiyilli	6.01	644.2	49.17 ±132.10
Kpaliga	8.29	522.6	45.64 ±104.50
Kpisinga	5.99	3618	263.80 ± 791.30
Kplung	5.85	4693	216.50 ± 865.10
Nadundu	2.45	141.1	15.28 ± 25.24
Nyong	3.18	58.96	16.02 ± 9.97
Sambu-Yesu	5.89	4034	349.30 ± 882.90
Tamalegu	2.43	73.23	11.17 ± 13.82
Yepilgu	7.15	223.2	23.24 ± 42.09
Ying	3.09	1510	182.40 ± 397.00
Nyong	5.59	275.8	33.37 ± 59.16
Zoggu	0	133.1	14.35 ± 26.28

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4.3 Fungi species isolated from farmer-saved groundnuts

Five different fungi species were isolated from the groundnut samples collected from farmers. These were *Aspergillus flavus*, *A. niger*, *Aspergillus parasiticus, Rhisopus stolonifer and Penicillium chrysogenum.* These fungal species were identified as follows:

4.3.1 *Aspergillus niger*

It produced black colony with yellow edges or hallow region with aerial growth. Hyphae were septate, conidiophores formed terminated in a swollen vesicle. This is illustrated in plate 4.0 below.

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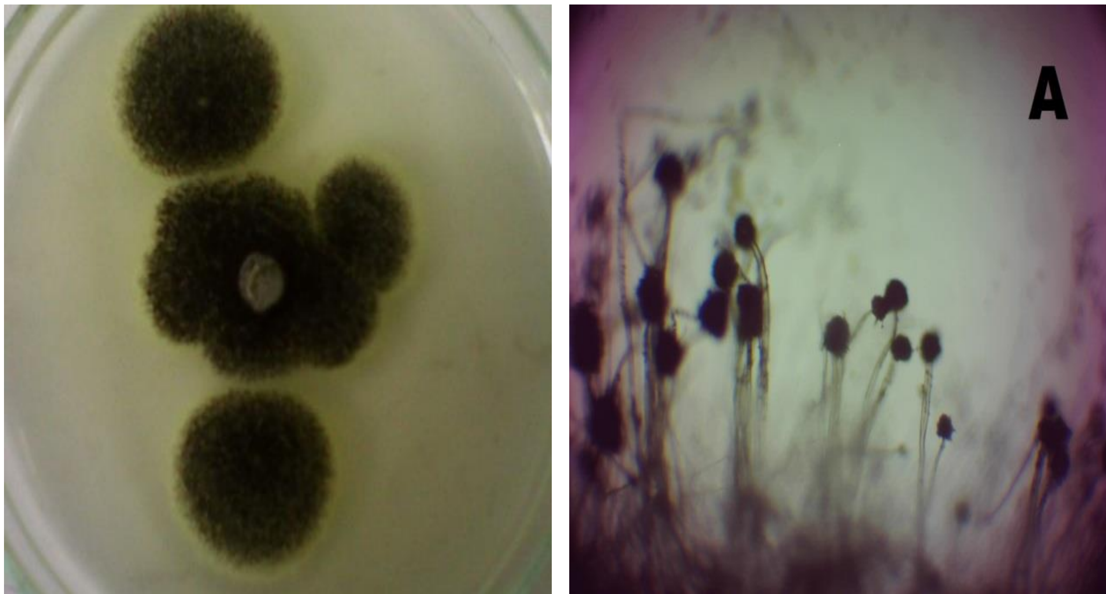

Plate 4.0 Morphological characteristics of *Aspergillus niger*.

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4.3.2 *Aspergillus flavus*

The fungus produces yellow-green colony with whitish powdery edge on PDA. Vesicles produced were globose and phialides were produced directly from the vesicle surface. Conidiophores terminated in swollen vesicles. This is shown below in plate 4.1

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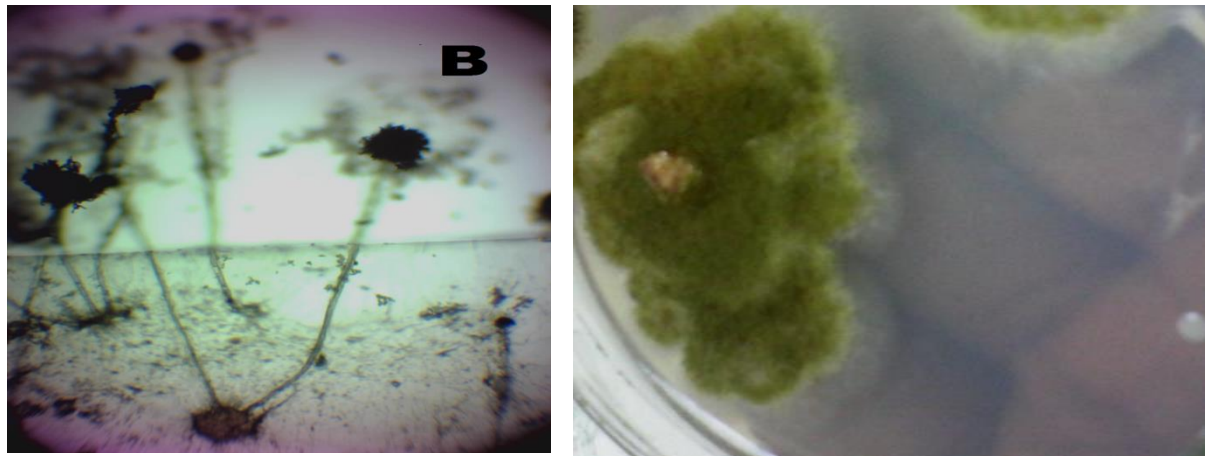

Plate 4.1 Cultural and morphological characteristics of *A. flavus*

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4.3.3 *Aspergillus parasiticus*

Colonies on PDA are ivy- green or deep green in appearance with rough edges. Vesicles were not globose and only phialides which were uniseriate were produced. Colony contained metulae and phialide. This is illustrated below in plate 4.2.

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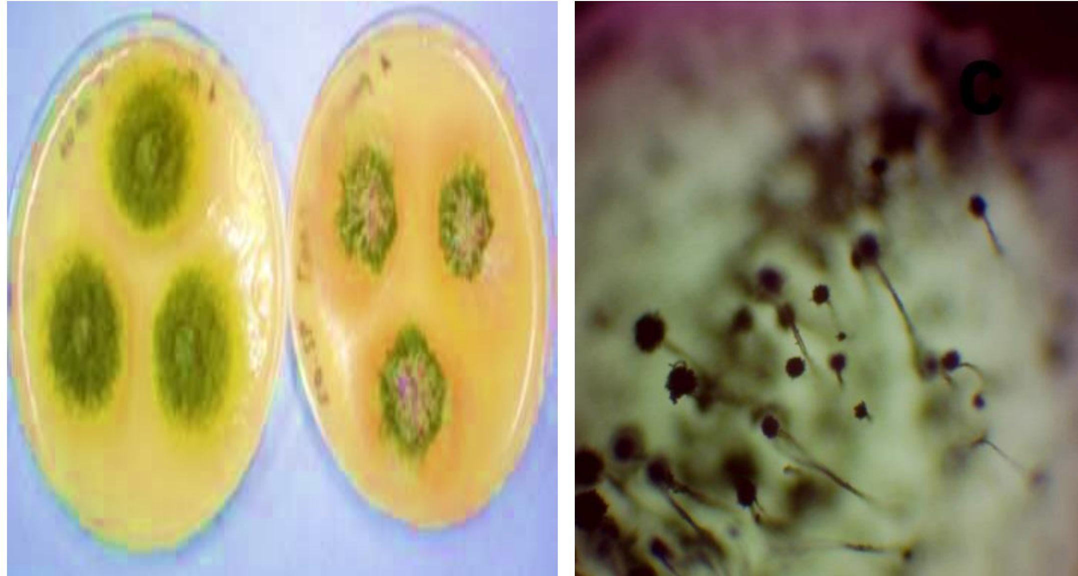

Plate 4.2 Cultural and morphological characteristics of A. parasiticus

4.3.4 *Rhizopus stolonifer*

Colony on PDA was initially white and cotton like but turned gray in three days The fungus produced aerial growth on media. The reverse of the plate showed black colouration. The fungus grew very fast covering the entire petri dish (90 mm diameter) in 3 days. It produced large saclike sporangia that contained sporangio-spores on long sporangiophore. Sporangiophores were connected to one another by septate hyphae (stolons). Plate 4.3 below show its appearance

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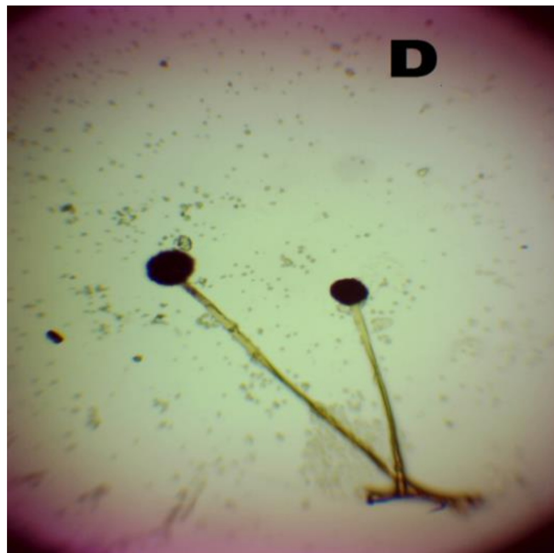

Plate 4.3 Cultural and morphological characteristics of *Rhizopus stolonifer*

4.3.5 *Penicillium chrysogenum*

The fungus produces greenish colonies with white edges. The surface of colonies appeared powdery. The mycelium grew slowly. The hyphae were hyaline and septated. Conidiophores produce a brush-like structure. This is illustrated in plate 4.4 below

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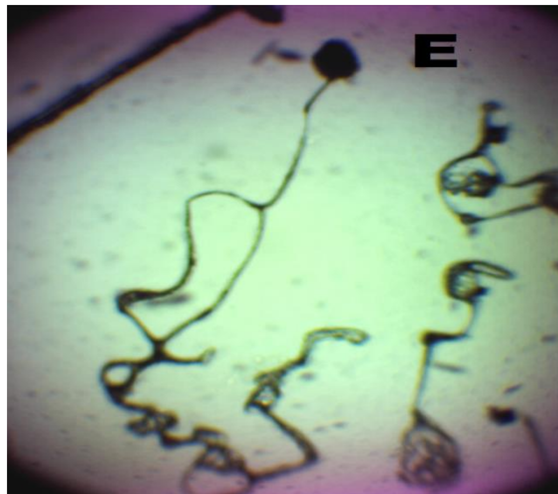

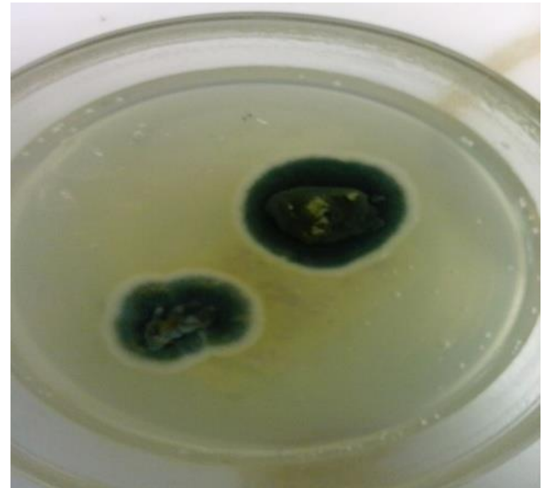

Plate 4.4 Cultural and morphological characteristics of Penicillium chrysogenum

Table 4.4 Types and population of fungal spp isolated from groundnuts

	Fungi Isolates									
Community	***A. flavus***		***A. niger***		***A. paraciticus***		***Rhizopus stolonifer***		***Penicillium chrysogenum***	
	H	**L**	**H**	**L**	**H**	**L**	**H**	**L**	**H**	**L**
MAN(n=20)	5	0	5	0	5	0	0	5	0	0
YIP(n=20)	10	0	0	5	0	5	0	0	0	0
SA(n=20)	5	5	5	0	0	0	0	0	5	0
DIG(n=20)	5	5	0	5	5	0	0	0	0	0
GEL(n=20)	5	0	1	0	5	5	0	2	0	2
BAC(n=20)	10	5	0	0	0	3	0	0	0	2
KPL(n=20)	5	5	3	2	0	3	2	0	0	0

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Continuation: - Table 4.4 Types and population of fungal spp isolated from groundnuts

KPI(n=20)	0	5	5	0	0	2	0	3	3	0
GBR(n=20)	0	5	5	5	3	0	2	0	0	0
YIN(n=20)	10	5	0	5	0	0	0	0	0	0
NYO(n=20)	5	5	5	5	4	1	0	0	0	0
SAM(n=20)	5	0	10	0	2	2	1	0	0	0
KPA(n=20)	5	5	5	5	0	0	0	0	0	0
ZIO(n=20)	10	5	5	0	0	0	0	0	0	0
KPK(n=20)	5	0	5	0	5	0	5	0	0	0
ZIG(n=20)	5	5	5	5	0	0	0	0	0	0
TAM(n=20)	5	5	5	5	0	0	0	0	0	0
ZOG(n=20)	5	5	5	0	3	2	0	0	0	0

Note: High means samples with aflatoxin levels >20ppb and low means samples with aflatoxin levels ≤20ppb

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Table 4.5 Percentage occurrence of fungi species on sampled farmer-stored groundnuts

Communities	Fungi species/percentage occurrence (%)				
	A. flavus	*A. niger*	*A. parasiticus*	*R. stolonifer*	*P. chrysogenum*
YIP	25.0	25.0	25.0	25.0	0.0
SAB	50.0	25.0	25.0	0.0	0.0
DIG	50.0	25.0	0.0	0.0	25.0
GEL	25.0	5.0	50.0	10.0	10.0
BAC	75.0	0.0	15.0	0.0	10.0
KPL	50.0	25.0	15.0	10.0	0.0
KPI	25.0	50.0	15.0	10.0	0.0
GBR	25.0	50.0	15.0	10.0	0.0
YIN	75.0	25.0	0.0	0.0	0.0
NYO	50.0	50.0	0.0	0.0	0.0
SAM	25.0	50.0	20.0	5.0	0.0
KPA	50.0	50.0	0.0	0.0	0.0
ZIO	75.0	25.0	0.0	0.0	0.0
KPK	25.0	25.0	25.0	25.0	0.0
ZIG	50.0	50.0	0.0	0.0	0.0
TAM	50.0	50.0	0.0	0.0	0.0
ZOG	50.0	25.0	25.0	0.0	0.0

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Table 4.6 Mycoflora isolated of fungi species from groundnuts by agar plate methods

The isolation revealed six different fungi species that were in the groundnut samples which was taken from the communities.

Fungi	Morphology on PDA	Microscopy of fungi	Suspected fungi
A	Black colony with yellow edges or hallow region. Aerial growth.	Hyphae are septate. Conidiophores formed terminate in a swollen vesicle.	*Aspergillus niger*
B	Yellow-green colony with whitish powdery edge. As shown below	Vesicles are globose and phialides are produced directly from the vesicle surface. Conidiophores terminating in swollen vesicles.	*Aspergillus flavus*
C	Ivy- green colony/ deep green in appearance with rough edges	Vesicles are not globose and only phialides which are (uniseriate conidial head) is produced. Colony contain metulae and phialide	*Aspergillus parasiticus*
D	Initially white and cotton like but turns gray in three days on ward with black dots present all over (sporangia). Aerial growth on media. The reverse plate has a black colour. Fast growth rate. Covers the entire petri dish (90 mm diameter) in 3 days.	Large saclike sporangia that contain sporangio-spores on long sporangiophore. Sporangiophores are connected to one another by septate hyphae (Stolons).	*Rhisopus stolonifer*
E	Greenish with white edges. Powdery surface. Slow growth rate.	The presence of hyphae which are hyaline and septate. Conidiophores produces a brush like structure	*Penicillium chrysogenum*

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4.4 Effect of different drying and storage methods on moisture content and aflatoxin concentrations in groundnuts

4.4.1 Aflatoxin concentration of freshly harvested groundnuts.

The mean aflatoxin contaminations in groundnuts harvested fresh and before storage are stated in Table 4.7. It ranged from 4.9 pbb in Nanton-Kurugu community to 110 in the Tarikpaar community. The lower concentrations of less than 10 were recorded in Nanton-Kurugu (4.90), Sandu (4.35), Nyetua (7.8), Yikpani (8.44) and Kyirikyilli (9.63). The aflatoxin concentrations from the other communities were more than 10, ranging from 10.06 to 110 (Table 4.7)

Table 4.7 Aflatoxin concentration of freshly harvested groundnuts in some Northern Regional communities.

Communities	Minimum	Maximum	Mean±SE
Denyokpaligu	8.10	14.20	11.15 ± 4.31
Kyirikiyilli	8.96	10.30	9.63 ± 0.95
Moglaa	7.49	18.63	13.06 ± 7.88
Nanton-Kurugu	4.80	5.00	4.90 ± 0.14
Nyetua	5.70	9.90	7.80 ± 2.97
Sandu	4.30	4.40	4.35 ± 0.07
Tarikpaar	40.20	179.8	110 ± 98.68
Yilkpani	7.72	9.17	8.44 ± 1.03
Yong	8.40	11.72	10.06 ± 2.35

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4.4.2 Moisture content of dried groundnuts

Groundnuts samples of the different treatments when it was said to be completely dried according to the farmers' discretion were tested for moisture. Analytically it was realized that the moisture value recorded were less than 9.00% (Table 4.8)

Table 4.8 Moisture content of dried groundnuts

	Moisture analysis of dried sampled groundnuts (%)		
Communities	Tarpaulin	Status Q.	Rack
Dinyokpaligu	6.4	6.4	6.4
Tarikpaar	8.7	8.7	6.4
Nyarikiyilli	6.4	6.4	6.4
Moglaa	6.4	4.2	4.2
Nyetua	6.4	6.4	2
Sandu	6.4	6.4	6.4
Yilkpani	4.2	4.2	4.2
Yong	8.7	6.4	6.4
Nanton-kurugu	6.4	8.7	6.4

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4.5 Aflatoxin concentrations in groundnuts stored in different types of bags after drying on tarpaulin.

The aflatoxin concentration in groundnuts showed a wide range of variation among different storage bags after drying on tarpaulin. Groundnuts stored in plastic bags had the lowest concentration for aflatoxin whilst storage in jute bags resulted in the highest value for aflatoxin concentration (figure 4.0). These differences showed some significant difference statistically.

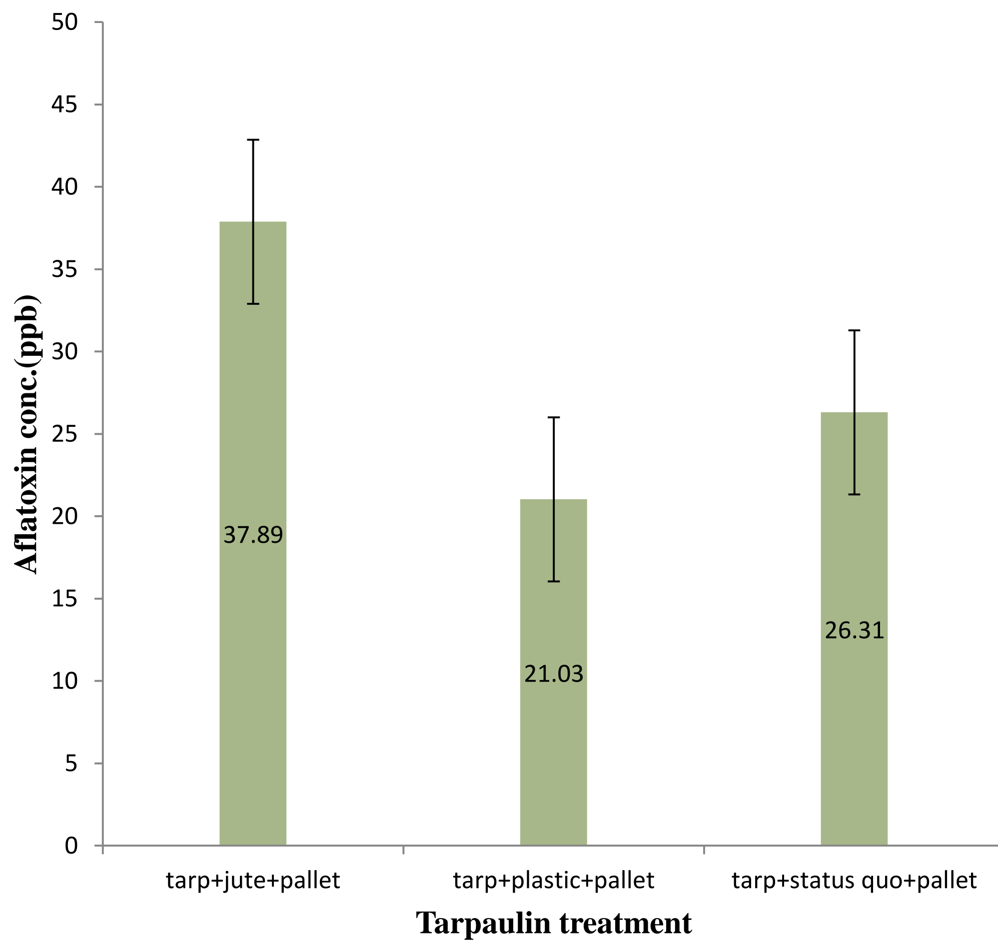

Figure 4.0 Aflatoxin concentrations in tarpaulin treatment in different storage bag

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4.6 Aflatoxin concentrations in groundnuts stored different bags after drying on Rack.

Figure 4.1 showed the concentration of aflatoxin in groundnuts stored in different bags after drying on racks. The highest was found in groundnuts stored in jute and plastic while the lowest was found in status quo. Storage in status quo showed some significant difference with the other types of storage.

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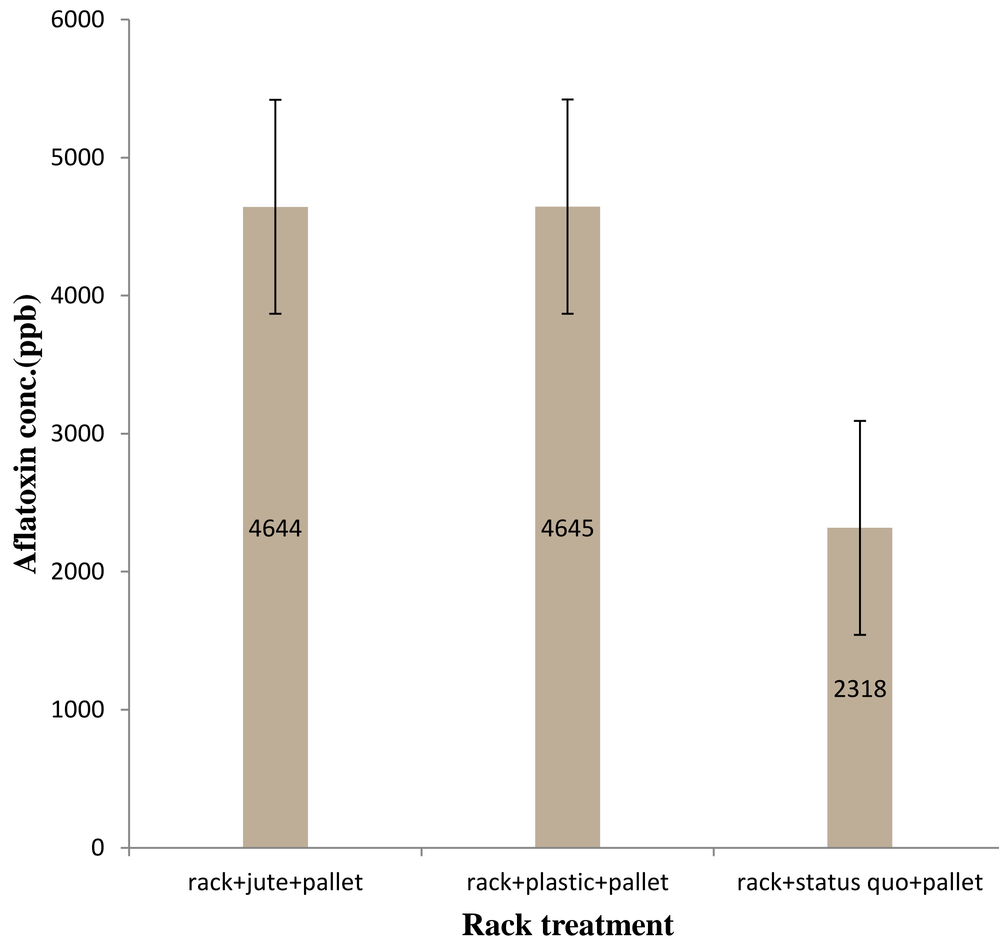

Figure 4.1 Aflatoxin concentrations in rack treatment with different storage bag

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4.7 Aflatoxin concentrations in groundnuts stored in different bags after drying on bare ground.

Figure 4.2 shows aflatoxin concentrations in groundnuts stored in different bags after drying on the bare ground. The highest concentration was found in groundnuts stored in jute and farmers own bag, while the lowest was found in plastic bags.

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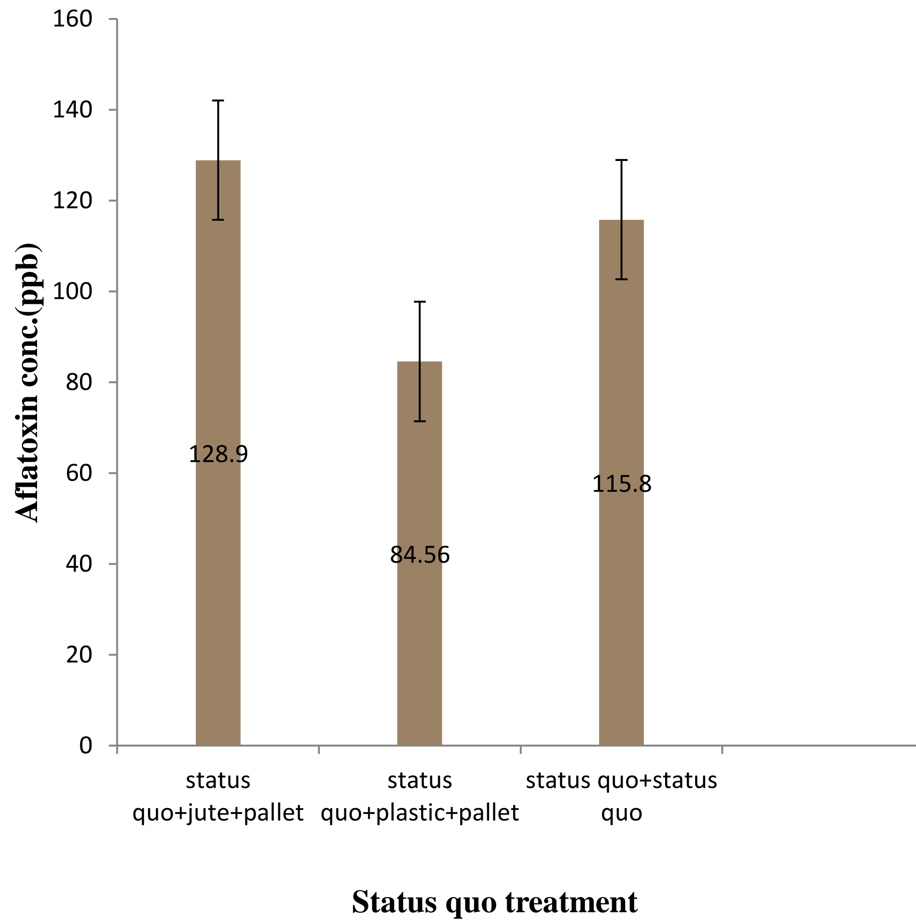

Figure 4.2 Aflatoxin concentration in status quo treatment with different storage bags

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4.8 Effect of different drying methods and storage bags on aflatoxin concentration in groundnuts in storage.

Figure 4.3 shows the concentration of aflatoxin in groundnuts stored after drying using different methods. Significant difference in the aflatoxin concentration existed among the different treatment combinations. The lowest concentration of 21.03ppb was recorded on groundnuts stored in plastic bags after drying on tarpaulin while the highest concentration of 4644ppb was recorded on groundnuts stored in jute sacks after drying on rack. The data also showed that groundnuts that were dried on racks recorded the highest concentration of aflatoxin when stored in any of the three bags compared to the when they were dried on bare ground or on tarpaulin. Also, the lowest concentration recorded on groundnuts stored in jute sacks (37.89ppb) was recorded on nuts that were previously dried on tarpaulin, while the highest in jute sack (4644ppb) was on nuts previously dried on racks. The concentrations of aflatoxin in groundnuts stored in jute sacks (128.9ppb), plastic bags (84.56ppb) and farmers' own bag (115.80ppb) after drying on bare ground were significantly lower than what was recorded in similar bags after drying of nuts on racks i.e, 4644ppb (jute bags), 4645ppb (plastic bag) and 2318 (farmers bag) (fig 4.3). However, these figures were significantly higher compared to what were recorded in groundnuts stored in similar bags after drying on tarpaulin.

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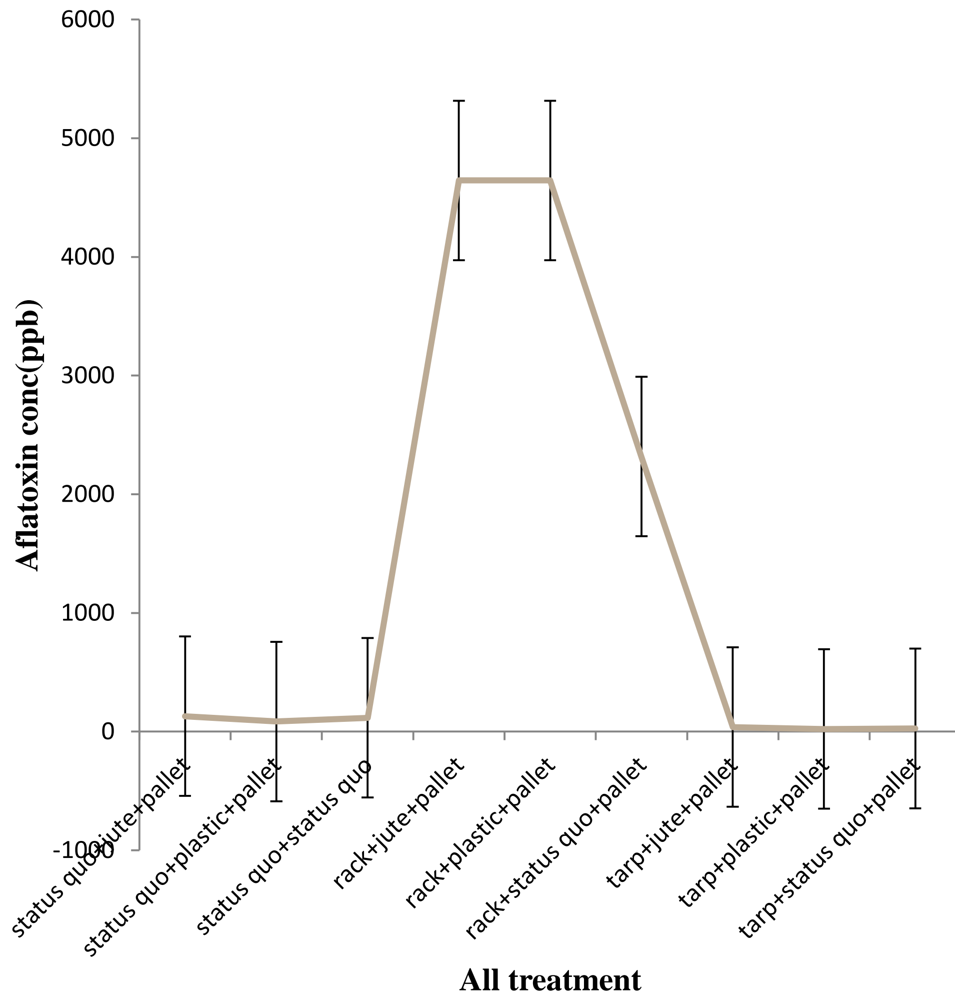

Figure 4.3 Aflatoxin concentration in groundnuts stored in different storage bags after drying on bare ground (status quo), racks or tarpaulins

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CHAPTER FIVE

5.0 DISCUSSION

The aflatoxin concentration in the groundnut sampled from farmers-saved nuts showed a high concentration of the mycotoxin within the study area (Table 4.3). Out of the 19 communities surveyed, only 7 had aflatoxin concentrations below 20ppb while some were in excess of 100ppb. This means that more than 60% of the communities sampled had their groundnuts contaminated well beyond the American food standard board limit of 20ppb. This implies that, most of the groundnuts from the Northern region do not meet the necessary requirement for exportation. This finding conforms to findings by Kayaa and Warren (2005). To be able to understand this phenomenon, fresh samples of groundnuts were analyzed to determine the extent of contamination prior to storage. Table 4.7 showed the levels of contamination of the nuts prior to storage.

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Groundnuts from most communities had their aflatoxin readings between the ranges of 4.4ppb and 14.00ppb which fall within the acceptable limit of 20ppb per the USA safety board in food regulation with the exception of one abnormality which fell in the range of 110ppb from Tarikpaar community. The comparatively lower concentrations of aflatoxin in freshly harvested nuts compared to the nuts collected from storage were in agreement with what was reported by Wild and Hall. (2000) and Turner *et al*. (2005) where they stated that improper management practices and adverse climatic conditions at harvest and after harvest are predisposing factors for aflatoxin contamination of groundnuts. According to Klich

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et al. (2009), post-harvest deterioration in groundnut is largely due to development of molds especially *Aspergillus spp*. It was observed in this study that due to labour constrains farmers would often take so long to harvest even when the groundnuts samples are ready for harvesting. In table 4.0, the minimum number of days used to uproot groundnuts was two (2) days whilst the maximum was four (4) days in the communities. This exposed the groundnuts to infections by molds in the field.

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Five fungi species were isolated and identified in this study. They include *Aspergillus flavus*, *A. niger*, *Aspergillus parasiticus, Rhizopus stolonifer and Penicillium chrysogenum*. In general, *A. flavus* was the commonly isolated fungi among the others, occurring about 60% in all samples analyzed from the region. *A. flavus* has been reported as a major producer of aflatoxins in most cereals and leguminous grains (Dick, 2008) and this is corroborated by the findings in this study. The high occurrence of the fungus and *A. parasiticus*, another aflatoxin producer could be a major reason why the groundnuts collected from the Northern region were highly contaminated by aflatoxins. Therefore, the presence of both *A. flavus* and *A. parasiticus* in the aflatoxin contaminated nuts was an indication and a further confirmation that these fungi were aflatoxin producers. In most of the communities studied, it was observed that an inverse relationship existed between *A. flavus* and *A. niger*. In cases where *A. niger* occurred at a higher frequency, the percentage of occurrence of *A. flavus* is reduced. This observation is similar to what was reported by (Vincent; Unpublished). This gives credence to suggestions that

that *A. niger* , which is not an aflatoxin producer could be used to control *A. flavus* and hence the levels of aflatoxin concentration in stored groundnuts.

It was generally observed that good drying and storage practices could effectively reduce the levels of aflatoxin contamination in groundnuts. One important factor that promotes the growth and development of fungi and hence the production of harmful metabolites on stored produce is the high moisture content Christensen and Mirocha, (1976). Results from this study showed that all the methods employed for drying, namely the tarpaulin, rack and bare ground resulted in less than 9% moisture content, an indication that all drying methods evaluated were able to perform satisfactorily. However, it also implied that the drying of groundnuts on the bare ground by farmers was effective in reducing moisture content of groundnuts to appreciable level before storage. This means the farmers' method effectively reduces growth of mold on groundnut in storage as reports suggest that moisture levels below 15% do not encourage microbial activities (Hamilton, 2000). Therefore other factors may account for the high aflatoxin levels in the groundnuts in storage.

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In the evaluation of drying and storage methods, the highest concentrations of aflatoxin were found in nuts that were dried on racks while still attached to the plants and then stored in the different storage bags. On the other hands, the least aflatoxin concentrations were found in nuts stored in the different bags after drying on the Tarpaulin. The variations could be traced to the mode of drying. It could be that the nuts dried on the racks were infected severally by the aflatoxin causing species since they were attached to the main plants whose different parts such as

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leaves and stem may be harboring the fungi. It is an indication that rapid detaching of nuts from the plants could be an important way of reducing aflatoxin concentration. This assertion is further supported by the fact that when nuts were detached and dried on the bare ground, they contained less aflatoxin compared to the rack drying, while they contained for less aflatoxin when dried on tarpaulin. Therefore, among the three drying methods evaluated, the Tarpaulin is recommended while the rack method is highly discouraged.

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CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

From the results of the study, the following conclusions could be made:

- Groundnuts stored by farmers in the Northern Region were mostly contaminated with aflatoxin beyond the U.S allowable limits of 20pbb.
- Concentrations of aflatoxin in groundnuts in storage were higher than in freshly harvested groundnuts.
- Post-harvest handling practices were highly suspected to be responsible for increase concentration in aflatoxins in groundnuts in storage.
- Drying of groundnuts on tarpaulin before storage drastically reduces aflatoxin concentration in stored groundnuts.
- The dominant species of aflatoxin producing fungi was found to be *Aspergillus flavus*

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6.2 Recommendation

The following recommendations are needed if we must reduce aflation concentrations.

- Prompt harvesting and plugging of nuts must be carried out by farmers to reduce aflatoxin concentration in groundnuts.
- Harvested groundnuts must not be dried on bare grounds but rather on tarpaulins
- Storage of dried groundnuts should be put in plastic bags and placed on a raised structure like the pallet to avoid direct contact with the floor.

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APPENDIX A1

Summary statistics for AFLATOXIN_CONC: COMMUNITY MACHIYILLI

Number of observations = 33

Number of missing values = 0

Mean = 341.9

Median = 22.24

Minimum = 8.89

Maximum = 6977

Lower quartile = 12.43

Upper quartile = 84.84

Standard deviation = 1224

Variance = 1499078

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Summary statistics for AFLATOXIN_CONC: COMMUNITY SATANI

Number of observations = 34

Number of missing values = 0

Mean = 11.08

Median = 8.285

Minimum = 1.96

Maximum = 47.45

Lower quartile = 6.09

Upper quartile = 14.44

Standard deviation = 8.585

Variance = 73.70

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Summary statistics for AFLATOXIN_CONC: COMMUNITY BACHALBADO

Number of observations =29

Number of missing values = 0

Mean = 144.6

Median = 12.99

Minimum = 4.11

Maximum = 2580

Lower quartile = 7.68

Upper quartile = 25.52

Standard deviation = 491.1

Variance = 241212

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Summary statistics for AFLATOXIN_CONC: COMMUNITY MION

Number of observations = 31

Number of missing values = 0

Mean = 11.40

Median = 7.36

Minimum = 2.51

Maximum = 65.71

Lower quartile = 4.853

Upper quartile = 11.23

Standard deviation = 13.70

Variance = 187.6

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Summary statistics for AFLATOXIN_CONC: COMMUNITY DIGBILA

Number of observations =	27
Number of missing values =	0
Mean =	367.5
Median =	20.54
Minimum =	9.53
Maximum =	6597
Lower quartile =	14.35
Upper quartile =	97.59
Standard deviation =	1279
Variance =	1636990

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Summary statistics for AFLATOXIN_CONC: COMMUNITY GALUWIE

Number of observations =	30
Number of missing values =	0
Mean =	332.0
Median =	11.91
Minimum =	2.08
Maximum =	3002
Lower quartile =	7.36
Upper quartile =	54.28
Standard deviation =	782.3
Variance =	611948

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Summary statistics for AFLATOXIN_CONC: COMMUNITY GBRIMANI

Number of observations = 19

Number of missing values = 0

Mean = 14.54

Median = 5.12

Minimum = 0.92

Maximum = 123.5

Lower quartile = 2.78

Upper quartile = 7.883

Standard deviation = 29.75

Variance = 885.2

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Summary statistics for AFLATOXIN_CONC: COMMUNITY KPAKYIYILLI

Number of observations = 25

Number of missing values = 0

Mean = 49.17

Median = 15.22

Minimum = 6.01

Maximum = 644.2

Lower quartile = 11.18

Upper quartile = 18.94

Standard deviation = 132.1

Variance = 17463

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Summary statistics for AFLATOXIN_CONC: COMMUNITY KPALIGA

Number of observations = 24

Number of missing values = 0

Mean = 45.64

Median = 20.15

Minimum = 8.29

Maximum = 522.6

Lower quartile = 13.88

Upper quartile = 28.66

Standard deviation = 104.5

Variance = 10921

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Summary statistics for AFLATOXIN_CONC: COMMUNITY KPISINGA

Number of observations = 25

Number of missing values = 0

Mean = 263.8

Median = 15.24

Minimum = 5.99

Maximum = 3618

Lower quartile = 8.75

Upper quartile = 18.79

Standard deviation = 791.3

Variance = 626079

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Summary statistics for AFLATOXIN_CONC: COMMUNITY KPLUNG

Number of observations = 29

Number of missing values = 0

Mean = 216.5

Median = 26.22

Minimum = 5.85

Maximum = 4693

Lower quartile = 11.54

Upper quartile = 57.53

Standard deviation = 865.1

Variance = 748472

Summary statistics for AFLATOXIN_CONC: COMMUNITY NADUNDU

Number of observations = 29

Number of missing values = 0

Mean = 15.28

Median = 8.42

Minimum = 2.45

Maximum = 141.1

Lower quartile = 5.803

Upper quartile = 14.68

Standard deviation = 25.24

Variance = 636.9

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Summary statistics for AFLATOXIN_CONC: COMMUNITY NYONG

Number of observations = 53

Number of missing values = 0

Mean = 16.02

Median = 14.16

Minimum = 3.18

Maximum = 58.96

Lower quartile = 8.5

Upper quartile = 20.92

Standard deviation = 9.997

Variance = 99.95

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Summary statistics for AFLATOXIN_CONC: COMMUNITY SAMBU-YESU

Number of observations = 24

Number of missing values = 0

Mean = 349.3

Median = 27.51

Minimum = 5.89

Maximum = 4034

Lower quartile = 14.62

Upper quartile = 150.1

Standard deviation = 882.9

Variance = 779589

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Summary statistics for AFLATOXIN_CONC: COMMUNITY TAMALGU

Number of observations = 33

Number of missing values = 0

Mean = 11.17

Median = 7.59

Minimum = 2.43

Maximum = 73.23

Lower quartile = 5.148

Upper quartile = 10.45

Standard deviation = 13.82

Variance = 190.9

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Summary statistics for AFLATOXIN_CONC: COMMUNITY YEPILGU

Number of observations = 25

Number of missing values = 0

Mean = 23.24

Median = 13.03

Minimum = 7.15

Maximum = 223.2

Lower quartile = 11.02

Upper quartile = 18.32

Standard deviation = 42.09

Variance = 1772

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Summary statistics for AFLATOXIN_CONC: COMMUNITY YING

Number of observations = 27

Number of missing values = 0

Mean = 182.4

Median = 20.71

Minimum = 3.09

Maximum = 1510

Lower quartile = 8.933

Upper quartile = 119.7

Standard deviation = 397.0

Variance = 157629

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Summary statistics for AFLATOXIN_CONC: COMMUNITY ZIONG

Number of observations = 29

Number of missing values = 0

Mean = 33.37

Median = 16.79

Minimum = 5.59

Maximum = 275.8

Lower quartile = 9.543

Upper quartile = 23.25

Standard deviation = 59.16

Variance = 3499

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Summary statistics for AFLATOXIN_CONC: COMMUNITY ZOGGU

Number of observations =	25
Number of missing values =	0
Mean =	14.35
Median =	5.11
Minimum =	0
Maximum =	133.1
Lower quartile =	1.738
Upper quartile =	21.45
Standard deviation =	26.28
Variance =	690.8

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APPENDIX A2

COMMUNITY	MEAN
ATALI	341.9
BAAGU	11.08
BACHALBADO	144.6
COSHAI	11.4
DIGBILA	367.5
GALUWIE	332
GBRIMANI	14.54
KPAKYIYILLI	49.17
KPALIGA	45.64
KPISINGA	263.8
KPLUNG	216.5
NADUNDU	15.28
NYONG	16.02
SAMBU-YESU	349.3
TAMALGU	11.17
YEPILGU	23.24
YING	182.4
ZIONG	33.37
ZOGGU	14.35
MAXIMUM	367.5
MINIMUM	11.08

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APPENDIX B1

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on: 1-Jul-2013 19:36:54

Identifier	Values	Missing	Levels
COMMUNITY	18	0	9

Identifier	Minimum	Mean	Maximum	Values	Missing
AFLATOXIN	4.300	19.93	179.8	18	0

Skew

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Summary statistics for AFLATOXIN: COMMUNITY DENYOKPALIGU

Number of observations = 2

Number of missing values = 0

Mean = 11.15

Median = 11.15

Minimum = 8.1

Maximum = 14.2

Lower quartile = 8.1

Upper quartile = 14.2

Standard deviation = 4.313

Variance = 18.60

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Summary statistics for AFLATOXIN: COMMUNITY KYIRIKIYILLI

Number of observations = 2

Number of missing values = 0

Mean = 9.63

Median = 9.63

Minimum = 8.96

Maximum = 10.3

Lower quartile = 8.96

Upper quartile = 10.3

Standard deviation = 0.948

Variance = 0.898

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Summary statistics for AFLATOXIN: COMMUNITY MOGLAA

Number of observations = 2

Number of missing values = 0

Mean = 13.06

Median = 13.06

Minimum = 7.49

Maximum = 18.63

Lower quartile = 7.49

Upper quartile = 18.63

Standard deviation = 7.877

Variance = 62.05

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Summary statistics for AFLATOXIN: COMMUNITY NANTON-KURUGU

Number of observations = 2

Number of missing values = 0

Mean = 4.9

Median = 4.9

Minimum = 4.8

Maximum = 5

Lower quartile = 4.8

Upper quartile = 5

Standard deviation = 0.141

Variance = 0.02

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Summary statistics for AFLATOXIN: COMMUNITY NYETUA

Number of observations = 2

Number of missing values = 0

Mean = 7.8

Median = 7.8

Minimum = 5.7

Maximum = 9.9

Lower quartile = 5.7

Upper quartile = 9.9

Standard deviation = 2.970

Variance = 8.82

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Summary statistics for AFLATOXIN: COMMUNITY SANDU

Number of observations = 2

Number of missing values = 0

Mean = 4.35

Median = 4.35

Minimum = 4.3

Maximum = 4.4

Lower quartile = 4.3

Upper quartile = 4.4

Standard deviation = 0.0707

Variance = 0.005

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Summary statistics for AFLATOXIN: COMMUNITY TARIKPAAR

Number of observations = 2

Number of missing values = 0

Mean = 110.0

Median = 110.0

Minimum = 40.24

Maximum = 179.8

Lower quartile = 40.24

Upper quartile = 179.8

Standard deviation = 98.68

Variance = 9738

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Summary statistics for AFLATOXIN: COMMUNITY YILKPANI

Number of observations = 2

Number of missing values = 0

Mean = 8.445

Median = 8.445

Minimum = 7.72

Maximum = 9.17

Lower quartile = 7.72

Upper quartile = 9.17

Standard deviation = 1.025

Variance = 1.051

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Summary statistics for AFLATOXIN: COMMUNITY YONG

Number of observations = 2

Number of missing values = 0

Mean = 10.06

Median = 10.06

Minimum = 8.4

Maximum = 11.72

Lower quartile = 8.4

Upper quartile = 11.72

Standard deviation = 2.348

Variance = 5.511

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APPENDIX B2

community	mean aflatoxin
Denyokpaligu	11.15
Kyirikiyilli	9.63
Moglaa	13.06
Nanton-kurugu	4.90
Nyetua	7.80
Sandu	4.35
Tarikpaar	110
Yilkpani	8.45
Yong	10.06

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APPENDIX C1

COMMUNITY	UPROOTING DAYS
DENYOKPALIGU	1.5
MOGLAA	1.0
NANTONKURUGU	1.5
NYIRIKIYILLI	1.5
NYETUA	3.0
SANDU	1.0
TARIKPAAR	4.0
YILKPANI	2.5
YONG	1.0

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APPENDIX C2

COMMUNITY	DAYS ON FIELD
DENYOKPALIGU	1.5
MOGLAA	1.0
NANTONKURUGU	3.5
NYIRIKIYILLI	1.0
NYETUA	4.0
SANDU	1.5
TARIKPAAR	4.5
YILKPANI	2.5
YONG	1.5

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APPENDIX D1

COMMUNITY	PLUCKING DAYS
DENYOKPALIGU	1.0
MOGLAA	1.0
NANTONKURUGU	1.0
NYIRIKIYILLI	1.0
NYETUA	1.0
SANDU	1.5
TARIKPAAR	1.0
YILKPANI	1.0
YONG	1.0

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APPENDIX D2

COMMUNITY	DRYINGDAYSTARP
DENYOKPALIGU	9.0
MOGLAA	8.0
NANTONKURUGU	7.0
NYIRIKIYILLI	7.0
NYETUA	7.0
SANDU	6.0
TARIKPAAR	11.0
YILKPANI	7.5
YONG	8.0

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APPENDIX E1

COMMUNITY	DRYING STAUS QUO
DENYOKPALIGU	8.5
MOGLAA	9.0
NANTONKURUG	6.5
NYIRIKIYILLI	7.5
NYETUA	4.5
SANDU	8.0
TARIKPAAR	12.5
YILKPANI	9.5
YONG	5.0

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APPENDIX E2

COMMUNITY	DRYING DAYS RACK
DENYOKPALIGU	15.5
MOGLAA	15.0
NANTONKURUGU	12.5
NYIRIKIYILLI	12.5
NYETUA	11.5
SANDU	10.0
TARIKPAAR	13.0
YILKPANI	12.5
YONG	13.5

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APPENDIX F1

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on: 3-May-2013 8:00:51

Identifier	Values	Missing	Levels
community	46	0	9

Identifier	Values	Missing	Levels
Treatment	46	0	3

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Summary statistics for aflatoxin_concentration: Treatment tarp+jute+pallet

Number of observations = 16

Number of missing values = 0

Mean = 37.89

Median = 6.805

Minimum = 3.52

Maximum = 447.0

Lower quartile = 4.145

Upper quartile = 17.93

Standard deviation = 109.4

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Summary statistics for aflatoxin_concentration: Treatment tarp+plastic+pallet

Number of observations = 15

Number of missing values = 0

Mean = 21.03

Median = 13.43

Minimum = 1.66

Maximum = 115.4

Lower quartile = 5.228

Upper quartile = 25.1

Standard deviation = 27.91

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Summary statistics for aflatoxin_concentration: Treatment tarp+status quo+pallet

Number of observations = 15

Number of missing values = 0

Mean = 26.31

Median = 14.45

Minimum = 1.37

Maximum = 137.8

Lower quartile = 8.435

Upper quartile = 23.99

Standard deviation = 36.78

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APPENDIX F2

Intervention	Means
tarp+jute+pallet	37.89
tarp+plastic+pallet	21.03
tarp+status quo+pallet	26.31

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APPENDIX G1

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Data imported from Clipboard

on: 3-May-2013 18:53:49

Identifier	Values	Missing	Levels
community	42	0	9

Identifier	Values	Missing	Levels
Treatment	42	0	3

Identifier	Minimum	Mean	Maximum	Values	Missing
Aflatoxin_conc	6.430	3980	16907	42	0

Skew

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Summary statistics for Aflatoxin_conc: Treatment rack+jute+pallet

Number of observations = 16

Number of missing values = 0

Mean = 4644

Median = 2319

Minimum = 6.62

Maximum = 13681

Lower quartile = 67.75

Upper quartile = 9407

Standard deviation = 5410

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Summary statistics for Aflatoxin_conc: Treatment rack+plastic+pallet

Number of observations = 14

Number of missing values = 0

Mean = 4645

Median = 3070

Minimum = 6.43

Maximum = 16907

Lower quartile = 774.4

Upper quartile = 6055

Standard deviation = 5387

Summary statistics for Aflatoxin_conc: Treatment rack+status quo

Number of observations = 12

Number of missing values = 0

Mean = 2318

Median = 886.2

Minimum = 7.9

Maximum = 12228

Lower quartile = 26.37

Upper quartile = 2799

Standard deviation = 3612

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APPENDIX G2

INTERVENTION	MEAN
RACK+JUTE+PALLET	4644
RACK+PLASTIC+PALLET	4645
RACK+STATUSQUO+PAL	2318

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APPENDIX H1

Data imported from Clipboard

on: 3-May-2013 19:28:44

Identifier	Values	Missing	Levels
community	48	0	9

Identifier	Values	Missing	Levels
Treatment	48	0	4

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Identifier	Minimum	Mean	Maximum	Values	Missing
Aflatoxin	1.220	116.6	1639	48	0

Skew

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Summary statistics for Aflatoxin: Treatment statua quo+jute+pallet

Number of observations = 1

Number of missing values = 0

Mean = 502.8

Median = 502.8

Minimum = 502.8

Maximum = 502.8

Lower quartile = 502.8

Upper quartile = 502.8

Standard deviation = 0

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Summary statistics for Aflatoxin: Treatment status quo+jute+pallet

Number of observations = 14

Number of missing values = 0

Mean = 128.9

Median = 11.94

Minimum = 1.62

Maximum = 1639

Lower quartile = 7.11

Upper quartile = 22.15

Standard deviation = 434.6

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Summary statistics for Aflatoxin: Treatment status quo+plastic+pallet

Number of observations = 17

Number of missing values = 0

Mean = 84.56

Median = 20.65

Minimum = 1.68

Maximum = 530.0

Lower quartile = 5.305

Upper quartile = 98.87

Standard deviation = 146.5

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Summary statistics for Aflatoxin: Treatment status quo+status quo

Number of observations = 16

Number of missing values = 0

Mean = 115.8

Median = 13.16

Minimum = 1.22

Maximum = 825.0

Lower quartile = 7.78

Upper quartile = 48.47

Standard deviation = 251.5

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APPENDIX H2

INTERVENTION	MEAN
STATUS QUO+JUTE+PALLET	128.90
STATUS QUO+PLASTIC+PALLET	84.56
STUSQUO+STATUSQUO+PALLET	115.80

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APPENDIX 2

MOISTURE ANALYSIS DATA OF TREATMENT

	TARPAULINE(g)			STATUS QUO (g)			RACK (g)		
Communities	Mass	Dry Mass	% M	Mass	Dry Mass	%M	Mass	Dry Mass	%M
Dinyokpaligu	5	4.7	6.4	5	4.7	6.4	5	4.7	6.4
Tarikpaar	5	4.6	8.7	5	4.6	8.7	5	4.7	6.4
Nyarikiyilli	5	4.7	6.4	5	4.7	6.4	5	4.7	6.4
Moglaa	5	4.7	6.4	5	4.8	4.2	5	4.8	4.2
Nyetua	5	4.7	6.4	5	4.7	6.4	5	4.9	2
Sandu	5	4.7	6.4	5	4.7	6.4	5	4.7	6.4
Yilkpani	5	4.8	4.2	5	4.8	4.2	5	4.8	4.2
Yong	5	4.6	8.7	5	4.7	6.4	5	4.7	6.4
Nanton-kurugu	5	4.7	6.4	5	4.6	8.7	5	4.7	6.4

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