UNIVERSITY FOR DEVELOPMENT STUDIES

EFFECT OF FERTILISER AND STORAGE ON AFLATOXIN CONCENTRATION AND NUTRIENT COMPOSITION OF GROUNDNUT (*Arachis hypogaea* L.)

EVANS ADINGBA ALENYOREGE

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EVANS ADINGBA ALENYOREGE (BSc. Agriculture Technology)

(UDS/MPHT/0011/13)

A THESIS SUBMITTED TO THE DEPARTMENT OF AGRICULTURAL MECHANISATION AND IRRIGATION TECHNOLOGY, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN POSTHARVEST TECHNOLOGY

2015

DECLARATION

DECLARATION BY CANDIDATE

I hereby declare that the work presented in this thesis is the result of my own original research carried out as a requirement for the award of the degree (MPhil) at the University for Development Studies. All sources have been accurately reported and acknowledged and that this research work has not been submitted previously in its entirety or in part to any other university or institution for a degree or diploma.

Evans Adingba Alenyorege ....

(UDS/MPHT/0011/13) Signature Date

DECLARATION BY SUPERVISORS

We hereby declare that the candidate under our supervision carried out the work reported in this thesis.

Ing. Albert Yawson ....

(Main Supervisor) Signature Date

Ing. Dr. Felix K. Abagale ....

(Co-Supervisor) Signature Date

Dr. Nelson Opoku ....

(Co-Supervisor) Signature Date

ABSTRACT

Plant nutrient deficiency is a major limitation to crop production efficiency and nutritional quality, and a predisposing factor for infections. The study was aimed at determining the proximate composition and aflatoxin concentration levels in groundnuts cultivated using Yaralegume and Humate Green OK ferti

lisers at four (4) communities in the Lambusie-Karni District of the Upper West Region of Ghana. The experiment was laid and replicated thrice in a randomized complete block design (RCBD). Fluorometric and Proximate analyses were employed respectively in the measurement of aflatoxin concentration and proximate composition. The results indicated that moisture content, crude protein, crude fat, crude fibre, ash and carbohydrate contents ranged between (3.15- 4.16 %), (21.54- 28.54 %), (39.98- 47.78 %), (2.37- 10.24 %), (1.29- 3.18 %) and (13.79- 28.61 %) for fresh groundnuts and (3.46- 4.14 %), (25.13- 30.38 %), (44.67- 55.33 %), (3.16- 11.08 %), (1.59- 2.59 %) and (3. 36- 18.43 %) for stored groundnuts respectively. After storage, 83.33 %, 58.33 % and 66.67 % of the samples recorded increase in moisture and crude protein, fibre and ash content, and crude fat correspondingly. However, 91.67 % of the samples had reduced levels of carbohydrate after storage. All groundnut samples tested contained detectable concentrations of total aflatoxins (B_1, B_2, G_1 and G_2). Fresh and stored groundnuts contained aflatoxins in the range of 3.59- 13.21 ppb and 10.43- 93.43 ppb respectively indicating high concentrations after storage. Aflatoxin concentration in fresh groundnuts where fertiliser was not applied increased from 34.19-62.05 % after storage whilst it reduced significantly from 24.41-19.23 % and 37.40-18.72 % in fields where Yaralegume fertiliser only and Yaralegume + Green OK fertilisers were used. Two stored groundnut samples however recorded aflatoxin levels of 93.43 ppb and 52.92 ppb which were noted to be above Ghana Standards Authority maximum allowable limit of 20 ppb. Insignificant ($p > 0.05$) positive and negative correlations existed between aflatoxin concentration and parameters of proximate composition for both categories of groundnuts. Fertiliser application reduced pre-harvest and post-harvest aflatoxin concentration with no change in proximate composition of the groundnuts. Storage, however increased aflatoxin concentration with no significant change in proximate composition. The potential of reducing the concentration of aflatoxins in fresh and stored groundnuts cultivated using the Yaralegume fertiliser and the combination of Yaralegume and Humate Green OK fertilisers was observed and can be considered by farmers for use in groundnut cultivation especially in the study areas.

DEDICATION

This piece of research is dedicated to my son Alenyorege Amaaliya Ethan and the entire Alenyorege family.

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ABBREVIATIONS

AF	Aflatoxin
AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFBO	AFB_1-8,9-epoxide
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFM_1	Aflatoxin M_1
AFM_2	Aflatoxin M_2
AOAC	Association of Official Analytical Chemists
DNA	Deoxy-ribose nucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
HBV	Hepatitis B viruses
HCV	Hepatitis C viruses
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
LSD	Least Significant Difference
ppb	Parts per billion
TLC	Thin Layer Chromatography
USAID	United States Agency for International Development
UV	Ultraviolet
jig/Kg	Microgram per kilogram

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CHAPTER ONE
INTRODUCTION

1.1 Background

Regardless of the rapid growth of Ghana's economy, virtually 2 million Ghanaians remain exposed to seriuos food insecurity (USAID, 2011). As in other developing countries, the most food insecure and malnourished are primarily rural people in deprived areas. Even as the whole country experienced rapid transition into middle-income status, the 2010 census found that almost half of all Ghanaian households were still engaged in agricultural activities and that close to 80 % of them were cultivating 1.2 hectares or less (USAID, 2011). Malnutrition and poverty remains particularly prevalent in the Northern part of the country, where limited and erratic rains, depleted soils and limited market access makes it difficult for households to either elevate agricultural productivity or find non-farm work (USAID, 2011). According to Dar (2002), the lack of adequate and quality food to the ever-increasing world population is putting more and more pressure on the agricultural industry to deliver higher crop yields with better quality. This is more significant in sub-Saharan Africa, which has a population growth rate of 2-3.5 % and is home to about 10 % of the world's population. With the disparity in population growth against food production, the region has emerged as a major location of hunger (Dar, 2002).

Although there has been some progress in agricultural productivity growth in sub-Saharan African (SSA) during the past several decades, current productivity growth lags far behind that in other regions of the world and is well below the growth required to meet food security and poverty reduction goals set forth in national and regional plans. Food security in Africa will

remain elusive until a decisive action is taken to assist small-scale farmers to grow more valuable crops. Poor soil fertility and nutrient depletion continue to represent major obstacles to securing needed harvests. In many tropical soils, the re-establishment of organic matter is a long-term process, and in lateritic soils such as those found throughout most parts of Africa, restoration might even be impossible. Without nutrient replacement most African farmers risk losing their soil reserve base permanently.

Improving access to fertilisers is a necessary counter-measure, particularly when farmers develop skills in selecting the fertilisers that are required and how to best derive benefits from their application. Better management of soil fertility is an imperative option for sub-Saharan Africa. Sanchez (1994) reinforces this view by identifying soil fertility depletion on smallholder farms as the fundamental biophysical root cause of declining per capita food production in Africa and advocated more integrated problem-solving approaches. African smallholder farmers historically managed the fertility of their farmlands mainly by allowing fields in fallow regularly and, in some parts of the continent, by applying animal manure. In most important agricultural areas, however, increasing rural population densities are shrinking the land available for crops and pasture production, posing serious problems for both approaches to soil fertility management and conservation. As such, considerably greater use of fertilisers is presently an important issue to ensure that farmers in Africa are able to farm profitably and to increase production to meet the food needs of the public.

The continuous production of crops without adequate supply of fertilisers coupled with other factors of production gave rise to soil nutrient depletion and consequently declining yields hence food shortages in parts of Africa including Ghana (Bagarama *et al.,* 2012). Although nutrient losses are high, there is very low use of organic or inorganic fertilisers on most crops

in this part of the world (Okello *et al.*, 2010). This was attributed to high fertiliser cost (Angadi *et al.* 1990) and limited financial resource base of many smallholder farmers in developing countries (Moyo *et al.*, 2007). It is often reported that many soils in SSA are known for their limited inherent supply of the major nutrients. Vlek *et al.* (1997) indicated loses of 22, 3, and 17 kg of Nitrogen (N), Phophorus (P) and Potassium (K) ha^{-1} yr^{-1} respectively in sub-Saharan Africa. Continuous nutrient mining was noted (Giller and Cadisch, 1995), with phosphorus identified as the most limiting element (Graham and Vance, 2000) in African soils. In view of these observed losses and the ever reported low levels of major nutrient elements in these soils, Graham and Vance (2000) concluded that soil fertility replenishment strategies are necessary to reduce the deficiencies of these nutrients.

Application of fertilisers could improve groundnut (*Arachis hypogaea* L.) output per unit area up from the current yield of 0.8 t ha^{-1} (Okello *et al.*, 2010) which is far below the potential yield level of 3 t ha^{-1}. Ghana's estimated groundnut yield level is 1.4 mt ha^{-1} and close to the world average, in between the African and Asian averages of about 1 and 2 mt ha^{-1} respectively. Like most groundnut production in parts of Africa, Ghana's crop is produced almost entirely without irrigation and with few soil amendments. Groundnuts (*Arachis hypogaea* L.) in terms of significance ranks sixth among oilseed crops and thirteenth among food crops cultivated in the world. In addition to providing high quality edible oil (48-50 %), easily digestible protein (26-28 %) and nearly half of the 13 indispensable vitamins as well as seven (7) of the essential minerals required for normal human growth and develoment, it produces good quality fodder for farm animals. It therefore plays a significant role in the livelihoods of farmers through economic and nutritional security.

In spite of its significance as food, the incidence of aflatoxin contamination has the potential to limit the importance of groundnut in the human diet since food safety is a fundamental measure for food security in sub-Saharan Africa where major food losses, health challenges and human fatalities have stemmed from contamination of major staples by fungal pathogens (Gong *et al*., 2002; Lewis *et al*., 2005). One of the major problems in groundnut production and supply globally is aflatoxin contamination, which is of great concern in groundnuts consumption as this toxin can cause carcinogenic and teratogenic effects in humans and animals (D' Mello, 2003). Infection of groundnut by *Aspergillus flavus* occurs not only under post-harvest but also during cultivation and harvest conditions (Waliyar *et al*., 2007).

The Food and Agriculture Organization (FAO, 2002) estimates that 25 % of world food crops are affected while the Center for Disease Control (CDC, 2004) estimates that more than 4.5 billion people in the developing world are exposed to aflatoxins. The incidence of these toxins in Ghanaian groundnuts and other crops has been well documented for many years. Awuah and Ellis (2001) found groundnut samples from 21 selected markets in 10 regions of Ghana with high levels of the toxin; infection was found in 31.7 % of the damaged kernels and 12.8 % of undamaged kernels.

Aflatoxins are a naturally-occurring mycotoxins produced by many species of *Aspergillus*, a fungus, the most prominent ones being *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin contamination most often occurs when crops suffer stress such as high rainfall, high temperatures, drought, and insect infestation which allows the fungi to grow on the outer surface of the groundnut pod and spreads inward reaching the kernel.

Several approaches to reducing or managing aflatoxin contamination have been proposed (Dorner *et aI*., 1998; Cardwell and Cotty, 2000; Bandyopadhyay *et al*., 2005; Abbas *et aI*.,

2006). They include; pre-harvest, at-harvest and post-harvest management practices; use of tolerant seed varieties and biocontrol measures. On-farm tests have been conducted in several countries in Asia and Africa to investigate not just technologies, such as the use of varieties that are tolerant or resistant to *Aspergillus flavus*, but also cultural practices, such as the use of soil amendments, and post-harvest handling on yield and aflatoxin contamination. A number of agronomic practices have been explored in reducing pre-harvest infection by *Aspergillus flavus* (Waliyar *et al*., 2007). Among them are the applications of lime and farmyard manure. Studies have shown that application of lime alone can reduce aflatoxin contamination by 72 %, while application of farmyard manure reduces aflatoxins by 42 % under field conditions. When combined, the two treatments can result in aflatoxin contamination reduction up to 84 %. Good human and animal health is dependent on healthy plants that are only available from fertile soils. The nutritional status of a plant decides its histological or morphological structure and properties, the function of tissues to speed up or slow penetration and disease development, and its nutritional value for feed or food (Datnoff *et al*., 2007). The dangers of most diseases can be significantly decreased or reduced by appropriate and timely nutrient management.

1.2 Problem Statement and Justification

The Food and Agriculture Organization in 2001 estimated that mycotoxins contaminate 25 % of agricultural crops worldwide and remain a threat to food safety. Food and feed contamination by aflatoxins is a significant food safety issue in the developing countries sometimes because of lack of detection, monitoring and regulating measures to safeguard the food supply. It is estimated that approximately 4.5 billion people living in developing

countries are chronically exposed to large uncontrolled amounts of aflatoxins that result in severe changes in immunity and nutrition (Williams *et al.*, 2004). In Ghana, agriculture is typified by low yields, a heavy reliance on rainfed cropping system and poor utilization of improved technologies. Household diets are often largely based on two to three major food crops (maize, rice and groundnuts), thus lacking the diversity that would improve food and nutrition security while simultaneously increasing the occurrence of and potentially aggravating the impact of aflatoxin outbreaks.

The realization of the Millennium Development Goal (MDG) of reducing by half the number of people suffering from hunger will require a significant increase in the amount of food grains, legumes and tubers produced in developing countries. However, food quality and safety issues resulting from aflatoxin contamination present a serious obstacle to improving nutrition, enhancing agricultural production and linking smallholder farmers to markets. Pre-harvest infection of groundnuts by *Aspergillus flavus* and subsequent aflatoxin contamination is one of the food safety factors that most often weaken groundnut productivity and human and animal health, especially in tropical regions of Africa. Evidence exists for the effectiveness of various interventions to reduce aflatoxin contamination of foods in developed countries, but it is unclear whether these are applicable in developing countries (Waliyar *et al.*, 2007).

An intervention to reduce exposure to aflatoxins can occur at various stages of food crop production and storage. Before crops are planted, efforts can be made to reduce the future burden of aflatoxins. Interventions can also occur before harvest, during harvest, and after harvest. The appropriate intervention or combination of interventions may differ depending on the crop and the country. Therefore further evaluation is needed with consideration towards the sustainability, cultural acceptability, economic feasibility, ethical implication, and overall

effectiveness of potential interventions. Although increased levels of aflatoxin contamination in post-harvest groundnut samples have been reported (Kladpan *et al.* 2004), the advent of readily available fertilisers has brought about the reduction or termination of many pathogenic diseases through improved plant resistance, disease escape, altered pathogenicity, or microbial interactions. Efficient fertility programs can enhance plant resistance to pathogens, reduce the impact of environmental stress, and increase the nutritional quality of the food and feed that are produced. Effective infection management improves crop quality and quantity to result in surplus food production, lower prices for consumers, and an abundance of quality food products. Ensuring nutrient sufficiency to maintain resistance to pathogens and abiotic stress is necessary to provide food safety, abundance and nutrient quality. An abundant supply of affordable, safe and nutritious food and feed is essential to meet society's needs. With the availability and utilization of some organic and inorganic fertilisers in groundnut production, an assessment of their effect on aflatoxin contamination of groundnuts and subsequent impact on nutritional quality was important.

1.3 Objectives of the Study

The main objective of the study was to establish the aflatoxin levels and nutritional quality of fresh and stored groundnuts cultivated with Yaralegume and Humate Green OK fertilisers. Specifically, the study sought to determine;

i. the effect of organic and inorganic fertilisers on aflatoxin concentration levels in groundnuts,
ii. the effect of storage duration on aflatoxin concentration levels in groundnuts,
iii. the effect of fertilisers on the proximate composition of groundnuts,

iv. the effect of storage duration on the proximate composition of groundnuts,

v. the manner relationship between aflatoxin concentration levels and proximate composition of groundnuts.

1.4 Hypotheses

The study sought to test the following hypotheses:

i. **H_0**: level of aflatoxin in groundnuts with fertiliser is equal to aflatoxin level without fertiliser

H_A: level of aflatoxin in groundnuts with fertiliser is different from aflatoxin level without fertiliser

ii. **H_0**: level of aflatoxin concentration in groundnuts before storage is equal to level of aflatoxin in stored groundnuts

H_A: level of aflatoxin concentration in groundnuts before storage is different from level of aflatoxin in stored groundnuts

iii. **H_0**: level of proximate composition in groundnuts with fertiliser is equal to proximate composition level without fertiliser

H_A: level of proximate composition in groundnuts with fertiliser is different from proximate composition level without fertiliser

iv. **H_0**: level of proximate composition of groundnuts before storage is equal to proximate composition level in stored groundnuts

H_A: level of proximate composition of groundnuts before storage is different from proximate composition level in stored groundnuts

v. **H_0**: level of aflatoxin concentration is not significantly correlated to proximate composition level of groundnuts (r = 0)

H_A: level of aflatoxin concentration is significantly correlated to proximate composition level of groundnuts (r 1 0)

CHAPTER TWO

LITERATURE REVIEW

2.1 Groundnut-Origin and Distribution

The cultivated groundnut (*Arachis hypogaea* L.) is an early crop of the New World, which originated in South America precisely southern Bolivia and North West Argentina where it was grown as early as 1000 B.C (Krapovickas, 1973). According to Gregory *et al.* (1980), Hammons, (1982) and Isleib *et al.* (1994) the distribution of the groundnut crop to Africa, Asia, Europe and the Pacific Islands occurred most probably between the sixteenth and seventeenth centuries with the finding prowess of the Spanish, Portuguese, British and Dutch. At present, it is grown in areas between 40 ^{o}C South and 40 ^{o}C North of the equator, where average rainfall is 500 to 1, 200 mm and mean daily temperatures are higher than 20 ^{o}C. The crop is cultivated in almost 110 countries on about 22.2 million hectares, of which 13.69 million ha are in Asia (India 8 million ha; China 3.84 million ha), 7.39 million ha in sub-Saharan Africa, and 0.7 million ha in Central and South America. Average pod yields on a global scale increased slightly from 1.08 mt ha^{-1} in the 1980's to 1.15 mt ha^{-1} in the 1990's, and the global production is nearly 30 million tonnes of pods. India, China, and the United States are the leading producers and produce in total about 70 % of the world's groundnuts (CGIAR, 2000).

2.2 Botany of Groundnuts

Groundnut as a plant belongs to the family *Leguminosae,* subfamily *Papilionoidae,* tribe *Aeschnomeneae,* sub-tribe *Stylosanthinae,* genus *Arachis* and species *hypogaea* (Isleib *et al.*,

1994). The genus name *Arachis* stems from a-rachis (Greek, meaning without spine) in reference to the lack of erect branches. The species name *hypogaea* stems from hupo-gè (Greek, meaning below ground) and relates to the gynophore that grows downward into the earth with the pod developing underground. Remarkably *A. hypogaea,* the only cultivated species, is not known in its wild state (Chapman, 1990). Sub-specific and varietal classifications are mostly based on location of flowers on the plant, patterns of reproductive nodes on branches, number of trichomes and pod morphology. There are two major sub-species of *A. hypogaea* that mainly differ in their branching pattern (Gibbons *et al.,* 1972): sub-species *hypogaea* with alternate branching and sub-species *fastigiata* with sequential branching (Table 2.1). Within the *hypogaea* sub-species are two botanical varieties; var. *hypogaea* (Virginia and runner types) and var. *hirsuta* (Peruvian humpback and Chinese dragon). Subspecies *fastigiata* is also divided into botanical varieties *fastigiata* (Valencia type) and *vulgaris* (Spanish type) (Chapman, 1990; Singh and Simpson, 1994).

Table 2.1 Varieties and Botanical Types of Groundnuts (*Arachis hypogaea* L.)

Subspecies	Variety	Botanical type	Branching pattern	Growth tendency	Maturation
hypogaea	*hypogaea*	Virginia	Alternate	Bunching	145-165 days
	hirsuta	Runner	Alternate	Spreading	145-165 days
fastigiata	*fastigiata*	Valencia	Sequential	Erect	90-120 days
	vulgaris	Spanish	Sequential	Erect	90-120 days

Source: Adapted and Modified from Shokes and Melouk (1995).

2.3 Groundnut Morphology and Growth

Groundnut seed consists of two cotyledons, stem axis and leaf primordia, hypocotyls and primary root. The hypocotyl functions to drive the seed to the soil surface during germination, and its length is determined by planting depth. The hypocotyl stops elongating as soon as light strikes the emerging cotyledon. Thus, groundnut emergence is intermediate between the epigeal (hypocotyl elongates and cotyledons emerge above ground) and hypogeal (cotyledons remain below ground) types. The taproot grows very fast, reaching a mean length of 100 – 120 mm within four to five days. Lateral roots appear about three days after germination (Gregory *et al.*, 1973). Initial plant growth is slow, with more rapid growth being observed between 40 and 100 days after emergence (Ramanatha-Rao, 1988). Groundnut is a self-pollinating, annual, herbaceous legume growing upright, and has an indeterminate growth habit. Natural cross pollination occurs at rates of less than 1 % to greater than 6 %. The plant is sparsely hairy and generally grows 12 to 65 cm high. Plants develop three major stems; the main stem develops from the terminal bud on the epicotyl while the two lateral stems equal in size to the central stem develop from the cotyledonary auxiliary buds. Groundnut produces a well developed taproot with many lateral roots. The taproot has four series of spirally arranged lateral roots with abundant branching and usually with a large number of nodules (Ramanatha-Rao, 1988). Groundnut plants start flowering about 30 to 40 days after planting and maximum flower production occurs 6 to 10 weeks after planting. The flowers are self-pollinated around sunrise, and wither within 5-6 hours. Within one week of fertilisation, the tip of the ovary bearing from 1–5 ovules grows out from between the floral bracts, bearing with it the dried petals, calyx lobes and hypanthia; creating a unique floral structure - the carpophore, commonly known as a peg (Ramanatha-Rao, 1988). The peg quickly elongates, and growth is positively geotropic

until it penetrates several centimeters (5-10 cm) into the soil when the tip becomes diageotropic, and the ovary starts developing into a pod (Ramanatha-Rao, 1988). Because flowering continues over a long period, and because of the relationship between the number of pods per plant and rainfall pattern, pods are in all stages of development at harvest. (Ramanatha-Rao, 1988). The pod is an elongated sphere with different reticulation on the surface and /or constriction between the seeds, and contains one to five seeds (Gregory *et al.*, 1973). Pods reach maximum size after 2 to 3 weeks in the soil, maximum oil content in 6 to 7 weeks, and maximum protein content after 5 to 8 weeks (Ramanatha-Rao and Murty, 1994). Considerable variability exists in groundnut morphological traits: seed size (0.15 to more than 1.3 g seed^{-1}), seed colour (white, light rose, rose, red, purple, white blotched with purple red), number of seeds pod^{-1} (1-5), pod length (11-83 mm) and pod breadth (9-27 mm) (Retamal *et al.*, 1990; Ramanatha-Rao and Murty, 1994).

2.4 Ecology and Soil Fertility Requirements

Groundnut requires abundant sunshine and warmth for normal development, but does not appear to be especially sensitive to day-length, though it generally produces more flowers under long day conditions (Retamal *et al.*, 1990). Temperature significantly influences the rate of development and growth of groundnut, the optimum range for vegetative and reproductive growth being between 25 and 30^{o}C (Cox, 1979; Leong and Ong, 1983). Groundnut grows in regions with an average annual rainfall of 500 – 1200 mm; thrives best when more than 500 mm of rain is evenly distributed during the growing season (Sellschop, 1967). Moisture stress during reproductive development causes embryo abortion, reduces seed development by

restricting calcium uptake by the pods, and increases aflatoxin contamination of the seeds (Cox, 1979).

Groundnut is grown mostly on light-textured soils ranging from coarse and fine sands to sandy clay loams with moderately low amounts of organic matter (1–2 %) and good drainage (Sellschop, 1967). The well-drained soils provide good aeration for the roots and nitrifying bacteria. Groundnut does not grow well in soils with a high water retention capacity (Cox, 1979), and grows best in slightly acidic soils with optimum pH ranging from 5.5 to 6.2 (Gibbons, 1980).

Groundnut requires considerable amounts of nutrients for high yields, however, responses to applied fertilisers have been observed to be very erratic, justifying the name "the unpredictable legume". It has often been accepted that groundnut has the ability to utilize soil nutrients that are relatively unavailable to other crops, and can therefore make good use of residual fertility (Sellschop, 1967; Reid and Cox, 1973).

Many changes have occurred in the recommended use of fertilisers on groundnuts over the years as a result of agricultural research. More reliance is being placed on the results from soil testing for fertiliser requirements for groundnuts. With improvements of recent years, larger quantities of more complete fertilisers are being found profitable, and broadcasting is the recommended method of applying lime and fertilisers (Sellschop, 1967). This avoids injury to the seed and plants. Much of the increase in yield has been due to better use of fertilisers, particularly to those crops immediately preceding groundnuts in rotation. Several months before planting and as a part of soil preparation, enough lime should be added to bring the pH between 6.0 and 6.4 (Perry, 1963). If the soil is low in phosphorus and potassium, 180 to 450 kg of 0-10-20 (nitrogen-phosphorus-potassium), fertiliser should be broadcasted per acre.

Certain micro-nutrients, particularly copper, boron, and sulphur, may be beneficial. Since groundnuts are leguminous, nitrogen fertiliser should not be required. Small amounts (4-12-12 or 5-10-15), however, are beneficial. Application of lime (0.5 t/ha), farm yard manure (10 t/ha) and cereal crop residue (5 t/ha) at the time of sowing helps reduce *Aspergillus flavus* seed infection and aflatoxin contamination by 50-90 %. Lime, a source of calcium, enhances groundnut wall thickness and pod filling and decreases fungal infection. Organic supplements, such as farmyard manure and crop residues, favour growth of native microbial antagonists and suppress soil and seed-borne infections. These three components also improve the water-holding capacity of the soil, minimising the effect of end-of-season moisture stress, and thereby limiting aflatoxin accumulation in groundnuts. Lime and farmyard manure are cheap and easily available. (Karthikeyan, 1996; Rosolem *et al.,* 1997). Davidson *et al.* (1983) reported that application of gypsum to groundnuts grown in Georgia increased germination and reduced aflatoxin content by 40 %.

2.5 Importance and Uses of Groundnuts

The uses of groundnut are diverse; all parts of the plant can be used. The seed (kernel) is a rich source of edible oil, containing 36 to 54 % oil and 25 to 32 % protein (Gibbons, 1980). About two thirds of world production is crushed for oil, which makes it an important oilseed crop (Woodroof, 1983). The oil is used primarily for cooking, manufacture of margarine, shortening and soaps. Seeds are consumed directly either raw or roasted, chopped in confectioneries, or ground into groundnut butter. Young pods may be consumed as a vegetable, while young leaves and tips are utilized as a cooked green vegetable (Duke and Ayensu, 1985).

Scorched seeds may serve as a coffee substitute (Duke, 1981). Non-food products such as soaps, medicines, cosmetics, pharmaceuticals, emulsions for insect control, lubricants and fuel for diesel engines can be made from groundnut. The oil cake, a high protein livestock feed, may be used for human consumption. The haulms are excellent high protein hay for horses and ruminant livestock. Groundnut shells may be used for fuel, as a soil conditioner, for sweeping compounds, as filler in cattle feed, as a raw source of organic chemicals, as an extender of resin, as a cork substitute, and in the building trade as blocks or hardboard (Gibbons, 1980). In folk medicine, groundnut is used for aphrodisiac purposes, inflammation, cholecystosis, nephritis and as a decoagulant. In China, the oil is taken with milk for gonorrhea, and used externally for the treatment of rheumatism, while in Zimbabwe the groundnut is used in folk remedies for plantar warts (Duke and Wain, 1981; Duke and Ayensu, 1985).

2.6 Nutritional Value of Groundnuts

Relative to other staple crops, groundnuts are a high-value, readily marketable and nutritious food, used as an ingredient in many traditional dishes and snacks as a major source of energy, protein, vitamins and minerals (Table 2.2). Groundnuts are a good source of total energy, fat (especially mono unsaturated fat) and vitamins and minerals including niacin, folate, copper and manganese (USDA, 2005). With additional vitamin and mineral fortificants, groundnut butter products have come to be utilized globally for the treatment and prevention of malnutrition in infants and young children, and groundnut-based snack foods can also be fortified to offer low cost products of very high nutritional value to reach the base of the economic pyramid.

Table 2.2: Nutritional Composition of Raw Groundnut

Nutrient	Unit	Amount per 100g
Energy	kcal	567
Protein	g	25.8
Fat	g	49.2
Fatty acids, total saturated	g	6.8
Fatty acids, total monounsaturated	g	24.4
Fatty acids, total polyunsaturated	g	15.5
Carbohydrate	g	16.1
Fiber	g	8.5
Calcium (Ca)	mg	92
Iron (Fe)	mg	4.6
Magnesium (Mg)	mg	168
Phosphorus (P)	mg	376
Potassium (K)	mg	705
Sodium (Na)	mg	18.0
Zinc (Zn)	mg	3.27
Thiamin	mg	0.64
Riboflavin	mg	0.13
Niacin	mg	12.1
Vitamin B-6	mg	0.35

Source: USDA (2005).

Examining food availability data from the UN Food and Agriculture Organization (FAO) database for 2009, groundnuts contributed similar levels of energy and fat compared to animal products as well as a small amount of protein. Groundnuts contributed to 3 % of total energy availability per capita and 16 % of total fat and 6.5 % of total protein availability per capita compared to 4.4 % total energy, 27 % total protein and 13 % total fat from animal products.

2.7 Proximate Composition of Groundnuts

Usefulness of agricultural crops as food for man and animals depends on their useful nutritional components. This may be obtained using food evaluation procedures like Proximate Analysis. Proximate analysis procedures involve a series of chemical determinations, which in turn reflect the food's feeding value. The procedure separates food materials into different fractions, which reflect the feeding value of the food as moisture, crude protein, crude fat, crude fibre, ash and carbohydrate (AOAC, 2007).

2.8 Mycotoxins

Mycotoxins are secondary metabolites produced naturally by fungi and can be defined based on three major determinants, fungal origin, chemical structure, and biological activity (Moss, 2002). They contaminate crops during production, harvest, storage and processing. Although thousands of mycotoxins exist, few pose significant risks with regards to food safety (Betina, 1989). In this view, three genera of mycotoxin-producing fungi are central and are the well studied with respect to their ability to cause risk- *Aspergillus*, *Fusarium* and *Penicillium* (Murphy *et al.*, 2006). According to Koster (2001) mycotoxins differ in their chemical structures, as well as biological activities such as oestrogenic, nephrotoxic, hepatotoxicity, neurotoxic, dermatoxic carcinogenicity, or immunosuppressive activities. Based on their bio-origin, Bhatnagar *et al.* (2002) in their research on toxins of filamentous fungi, classified mycotoxins into polyketides (aflatoxins, ochratoxins, zearalenone and fumonisins), trichothecenes (terpenes), tetramic acids (cyclopiazonic acid and tenuazonic acid) and nitrogen-containing metabolites (ergotamine). They have a diverse range of pharmacological effects in humans and other mammals (IARC, 1993). Incidence of mycotoxin contamination in

feeds and foods varies from region to region (Clark, 2003). This depends on the prevailing climatic conditions i.e. temperature and relative humidity (Valarezo *et al*., 1997), chemical composition (fat, moisture, protein) of the substrate, food habits as well as the local regulations of the food quality control strategies. Fumonisin B (FB) is common in maize, aflatoxin (AF) in groundnuts in the tropics, ochratoxin A (OTA) in barley in the Scandinavian countries, while deoxynivalenol (DON) is predominant in wheat in America, Canada and Europe (CAST, 2003). The most widely studied and dangerous mycotoxins are aflatoxins (Smith and Moss, 1985; Scott, 1991).

2.8.1 Toxigenic Aspergillus species

Several species of *Aspergillus* have been identified as possible toxin-producing secondary metabolites (Cole and Cox, 1981). The mycotoxins produced by various species of *Aspergillus* differ in number and toxicity. Flannigan and Pearce (1994) reported that, the most toxic of the *Aspergillus* mycotoxins are aflatoxins produced by *A. flavus*, *A. paraciticus* and *A. nomius*; ochratoxin A produced by *A. ochraceus*, *A. carbonarius*, and occasionally *A. niger*; steringmatocystin, produced primarily by *A. versicolor* and *Emericella* species; and cyclopiazonic acid produced by *A. flavus* and *A. tamarii*. In addition, citrinin, patulin, penicillic acid and the tremorgenic toxins are also produced by *Aspergillus* species and cause a wide range of effects (Flannigan and Pearce, 1994).

2.8.2 Aflatoxins

Notwithstanding their early discovery in 1960 by a set of British researchers investigating mycotoxicosis outbreaks in poultry (Wogan and Busby, 1980), studies on aflatoxins still dominate the research efforts on mycotoxins today, because they are the most dangerous of all

mycotoxins (Nektaria, 2007). They are produced largely by *Aspergillus flavus, A. parasiticus* (Pittet, 1998; Coulombe *et al.*, 2005) and some strains of *A. tamari* and *A. nomius* (Chu, 2002) with optimal production at temperatures between 25 and 30°C (Valarezo *et al.*, 1997) and kernel moisture content of about 18 % (Herrman, 2002). The water activity (aw) of the substrate and the relative humidity of the surroundings are the most important factors that contribute to increased aflatoxin production during storage (Wilson and Payne, 1994). Bhatnagar *et al.* (2002) indicated a water activity of 0.90-0.99 as optimum condition for aflatoxin production by *A. flavus* and *A. parasiticus*.

Aflatoxins can be found on a wide range of crop species including groundnuts, maize, sorghum, cassava, cottonseed, Brazil nuts, pistachios, spices, dried coconut and figs (Murphy *et al.*, 2006; Mkoka, 2007). Those common in cereals and legumes are produced by two species of *Aspergillus- A. flavus and A. parasiticus.* The native habitat of *Aspergillus* is the soil, decaying vegetation, hay and grains undergoing microbiological deterioration. The chemical structures of Aflatoxin are shown in Figure 2.1. Four chemical 'types' of aflatoxins are known- B_1, B_2, G_1 and G_2 named from the fluorescence produced when exposed to ultraviolet radiation (B for blue and G for green). Aflatoxin B_2 and G_2 are dihydroxylated derivatives of B_1 and G_1 while aflatoxins M_1 and M_2 are hydroxylated derivatives of B_1 and B_2 found in milk of cows that have been fed aflatoxin contaminated fodder (Lu, 2003). Some important physical and chemical properties of the aflatoxin are presented in Table 2.3.

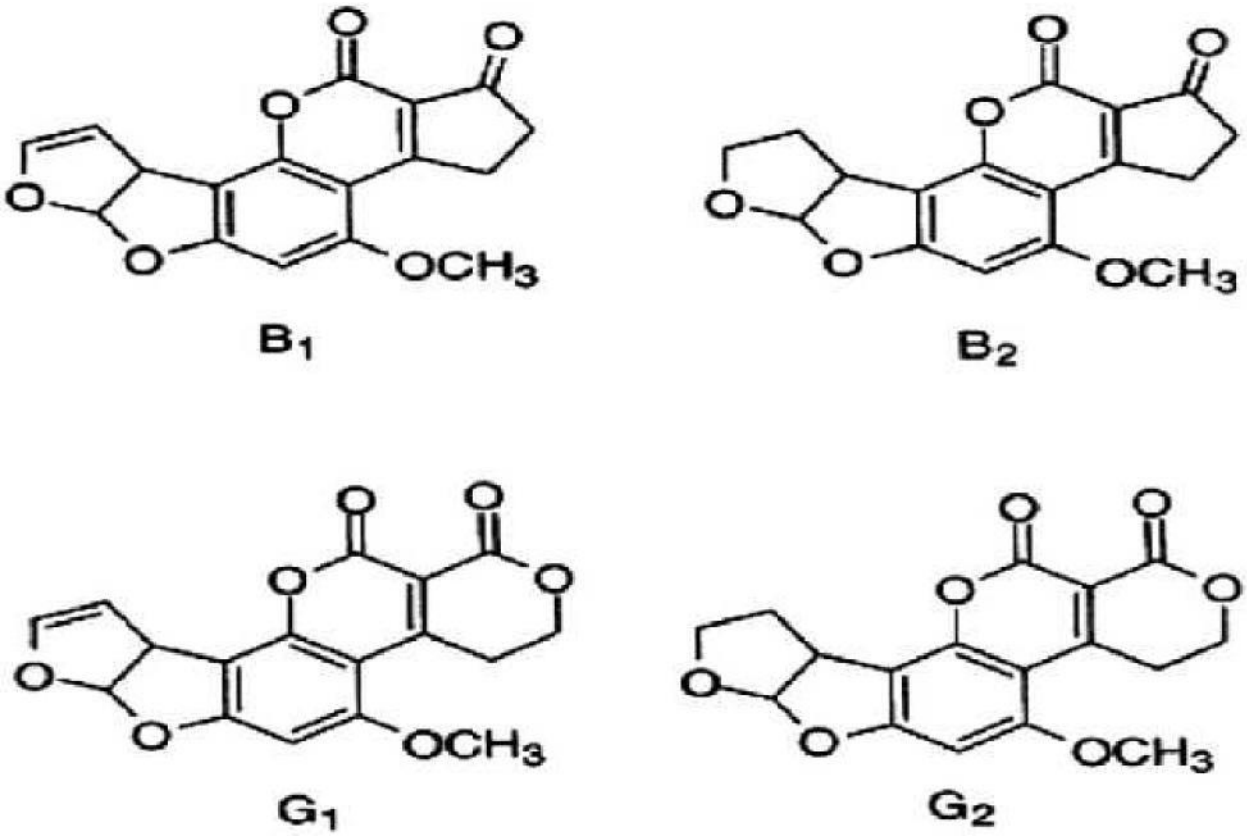


Figure 2.1: Chemical Structures of Aflatoxin B_1, B_2, G_1 and G_2. **Source:** Alexander (2011).

Table 2.3: Physico-chemical Properties of Aflatoxins

Aflatoxin	Molecular Formula	Molecular Weight (units)	Melting Point (°C)
B_1	$C_{17}H_{12}O_6$	312	268-269
B_2	$C_{17}H_{14}O_6$	314	286-289
G_1	$C_{17}H_{12}O_7$	328	244-246
G_2	$C_{17}H_{14}O_7$	330	237-240

Source: Adapted and Modified from Reddy and Waliyar (2000).

In pure form aflatoxins are heat stable and their levels or quantities are therefore not affected by normal food processing operations such as cooking or pasteurization (Reddy and Waliyar, 2000). Aflatoxins are soluble in methanol, chloroform, acetone and acetonitrile (Reddy and Waliyar, 2000). They are also stable for many years as chloroform or benzene solutions stored in the dark and cold, but are relatively unstable when exposed to light and air, especially when dissolved in highly polar solvents (Reddy and Waliyar, 2000). Aflatoxins are also intensely fluorescent when exposed to long wavelength UV light, which enables their detection at very low levels and also provides the practical basis for most methods used for their quantification (Reddy and Waliyar, 2000).

2.8.3 Aflatoxin Producing Types of *Aspergillus* Species

The ubiquity of the aflatoxigenic molds, *A. flavus* and *A. paraciticus*, is demonstrated by their presence on nuts, spices, corn, rice, and other grains during pre-harvest and post-harvest periods (Gams *et al.*, 1985). Cereals and spices are common substrates for *A. flavus*, and subsequent aflatoxin production in these foodstuffs is almost always due to poor drying, handling, or storage (Arim, 1995). Thus, aflatoxin levels are normally higher in tropical countries where crops, such as corn, groundnuts, and other nuts and oilseeds are often grown under marginal conditions where drying and storage facilities are limited (Lubulwa and Davis, 1994).

2.8.4 Pathogenicity and Prevalence of*Asspergillusflavus*

Aspergillus flavus is considered an opportunistic pathogen of plants. In the field, high *A. flavus* populations occur continuously but fungal populations increase during dry and hot weather conditions (McMillian, 1983). Infection propagules may be conidia, sclerotia or mycelia. Since

A. flavus is also a saprophyte, it can grow well on crop debris, dormant tissues, and/or damaged or weakened crops.

2.9 Evidence of Aflatoxins in Groundnuts

In order to estimate the relative exposure of Ghanaians to aflatoxins, Awuah and Ellis, in 2001 discovered the presence of aflatoxin in damaged and undamaged groundnuts sampled randomly from two (2) commercial markets each in all ten (10) regions of Ghana. High levels of infection were found in 31.7 % of the damaged kernels examined, and 12.8 % of the undamaged kernels.

In a 2010 IFPRI sponsored study, seventy (70) samples of various kinds of groundnut products were examined for aflatoxin contamination and the results were revealed as shown in Table 2.4 below.

Table 2.4 Aflatoxin Levels in Selected Groundnut Products

Type of Product	Average Total Aflatoxin Content (ppb)
Raw Groundnuts	
New Crop	1.7
In shell	7.6
Old crop	88.8
Rejects	288.8
Processed Products	
Roasted Groundnuts	1.0
Nkati (groundnut) cake	7.6
Dakwa	10.9
Pounded raw groundnuts	15.8
Groundnut Paste	52.6
Kulikuli	76.9
Manufactured Products	
Crispy Nut Cracker	1.1
Uni-mix (product #1)	1.9
Burger	5.0

Source: Florkowski and Kolavalli (2013).

In the West African country of Nigeria, where climatic conditions favour fungal growth and subsequent mycotoxin contamination, a number of studies have been conducted to examine the presence of aflatoxins in a broad range of foods and foodstuffs. Akano and Atanda (1990) reported the occurrence of aflatoxin in groundnut cake samples and found AFB_1 levels ranging from 375 to 455 jig/kg in 28 (87.5 %) of the 32 samples tested. Bankole *et al.* (2005) also

analysed dry-roasted groundnuts for aflatoxins by Thin Layer Chromatography (TLC) method and reported a mean presence of 25.5 jig/Kg of AFB_1 in 64.2 % of the samples. Barro *et al.* (2002) and Mphande *et al.* (2004) reported the presence of aflatoxigenic fungi and aflatoxins in groundnuts in Botswana with the former reporting some levels of aflatoxin occurrence ranging from 12 to 329 jig/ kg in raw groundnuts. Ghali *et al.* (2008) and Juan *et al.* (2008) in the North African countries of Tunisia and Morocco respectively found the existence of aflatoxins in foods including groundnuts with the later using liquid chromatography and fluorescence detection methods to expose the presence of 5 % of total aflatoxins and 5 % of AFB_1 in groundnuts. In Senegal, groundnut and its products are subjected to several reviews probably due to favourable conditions suitable for fungal growth and aflatoxin contamination (Diop *et al.,* 2000). Park and Njapau (1989) analysed over seventy (70) samples of groundnuts and it was found that all of the samples contained AFB_1 (20 to 200 jig/kg). It was revealed by Ndiaye *et al.* (1999) that groundnut oil samples tested for aflatoxins by HPLC method had 85 % of the samples containing an average of 40 jig/ kg AFB_1. According to several newspaper reports, groundnut butter used to feed school children in South Africa was contaminated with total aflatoxins and AFB_1 at concentrations of up to 27 jig/Kg,163 jig/Kg and 16 505 jig/Kg (Williams *et al.,* 2004).

2.10 Factors Affecting Aflatoxin Contamination of Groundnuts

2.10.1 Pre-harvest Contamination Factors

Pre-harvest contamination control is critical to success because once infection occurs; it is difficult to completely eliminate it. This stage of control focuses on controlling critical factors that predispose crops to mycotoxin contamination which although difficult in nature, have high

potential to mitigate the contamination and its effects. Use of poor quality seed increases pest and disease susceptibility facilitating infection by *Aspergillus* spp, as does poor plant nutrition. Pre-harvest contamination by aflatoxin is associated with drought stress (facilitates pod damage and exposure to mould), particularly at the end of the planting season and insect damage (providing entry point for fungus) in the field. Pre-harvest infection is difficult to control without irrigation and pesticide application (FAO, 2001; Craufurd *et al.*, 2006). Additional contamination may occur at harvest (Craufurd *et al.*, 2006). A pre-harvest treatment combination including fertiliser and disease management options resulted in permissible levels of aflatoxin contamination when compared with farmer practice in India (FAO, 2001). Aflatoxin contamination was found mainly in small and damaged pods while well-filled pods had no aflatoxin.

2.10.2 Post-harvest Contamination Factors

Pre-harvest contamination is very much related to post-harvest accumulation as higher aflatoxin loads at harvest provide inoculum sources for subsequent contamination during storage (Craufurd *et al.*, 2006). After harvest, *Aspergillus* infection and growth is likely if crops are not dried adequately within a short period of time (FAO, 2001). Post-harvest practices such as physical separation (sorting) of kernels are very effective in the reduction of mycotoxin levels, with reductions of up to 91 % reported (Lopez-Garcia and Park, 1998; Fandohan *et al.*, 2005). Studies conducted in Guinea where a post-harvest package was compared to usual post-harvest practices showed that aflatoxin concentration in blood samples in intervention villages was less than 50 % of that in control villages (Turner *et al.*, 2005). Winnowing, washing, crushing and dehulling of maize have also been reported to effectively reduce aflatoxin contamination (Fandohan *et al.*, 2005). ICRISAT has shown that drying

methods (avoiding high moisture, slow drying, and air circulation) are common practices that can help reduce or stop contamination (ICRISAT, 2006; Diaz Rios and Jaffeee, 2008).

2.11 Conditions Suitable for Occurrence of Aflatoxin

It was once thought that aflatoxin formation only occurred during post-harvest, i.e. during storage, but it is now well documented that aflatoxin production also occurs in the field prior to harvest. Aflatoxin contamination has been associated with prolonged high day and night temperatures during the growing season and severe drought conditions during grain fill. Risk factors for aflatoxin contamination include three or more weeks drought during pod formation (end of season drought), high moisture/ relative humidity (83±1 % or higher at 30 ^{o}C varying with substrate and length of incubation period) and high temperature with optimum temperatures between 25 ^{o}C and 35 ^{o}C (Schroeder, 1969; Hill *et al.*, 1983) or more, rainfall at the end of the growing season that postpones harvest and prevents dry-down. In terms of storage conditions, grains with moisture levels above 9 % and moderate temperatures (28 ^{o}C to 33 ^{o}C) increase the risk of aflatoxin contamination (Hill *et al.*, 1983). Grain damage by insects, rodents, birds, as well as drought stress, which predispose the crop to colonization by the fungus, can lead to aflatoxin occurrence in groundnuts and maize (Williams *et al.*, 2004; Desai *et al.*, 2008).

2.11.1 Moisture Content

The amount of moisture affects both the grade and storability of groundnuts and has a critical effect on mould growth and mycotoxin production (FAO, 1998). It is one of the most important considerations in determining whether aflatoxin will develop in groundnuts after harvest. *Aspergillusflavus* grows when the moisture content exceeds 9 %, at 80-85 % relative

humidity and above (Okello *et al.,* 2010). Soil moisture stress has also been reported to enhance pre-harvest aflatoxin contamination. Groundnuts exposed to drought stress in the field have been reported to have more *Aspergillus flavus* infected kernels than in irrigated plots. Excessive drought causes strains on groundnut pods thus providing entry points for infection by fungi (Okello *et al.,* 2010).

2.11.2 Temperature

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

2.11.3 Handling and Drying

Mechanical damage to kernels makes them much more vulnerable to invasion by storage moulds, including *Aspergillus flavus*. Under any given environmental conditions fungal growth is several times faster in damaged compared to intact kernels. Cracks and breaks in grains are caused mainly during harvesting and shelling, although insect and rodent feeding may also be responsible for breaks in the pericarp (Sauer and Tuite, 1987). Traditional

groundnut drying techniques in developing countries like Ghana involving field and bare ground drying are a major source of fungal contamination. Due to rains that normally persist at harvesting and drying times, it is difficult to achieve the recommended moisture level for safe storage. In addition, the crop is persistently exposed to soil contamination which is the source of fungi (Kaaya *et al.*, 2007; Okello *et al.*, 2010).

2.11.4 Storage Conditions

The fundamental reason why commodities are stored dry is to increase storability and in part, prevent growth of storage fungi. If commodities are incorrectly stored, that is, in an improperly dried state or under high humidity with inadequate protection, fungi will inevitably grow. Duration of storage is an important factor when considering mycotoxin formation. The longer the retention in storage the greater will be the possibility of building up environmental conditions conducive to groundnut fungi growth (Kaaya *et al.*, 2006). Storage structures commonly used by farmers in sub-Saharan Africa are traditional and may not maintain an even, cool and dry internal atmosphere. They do not provide adequate protection from insects and rodents, are not easy to clean, and above all they are not waterproof. All of these conditions invite mold growth and aflatoxin production (FAO, 1998).

2.11.5 Insect Infestation or Damage

Insect infestation during storage is one of the major problems that can contribute to fungal colonization in different ways. Fungal spores can be carried by insects. Also toxin-producing fungi can infect growing crops, due to insect damage, and can produce toxins pre- and post-harvest and during storage. During storage, insects, due to their metabolic heat and water, can

increase the water activity and temperature of groundnuts to levels suitable for fungal growth. Thus, it is important that insects are controlled both pre and post-harvest (Hell *et al.*, 2000).

2.12 Organic and Inorganic Fertilisers as Sources of Plant Nutrients

2.12.1 Organic Fertilisers

According to Lekasi (2003), organic fertilisers also referred to as organic sources are described as those organic materials that are used in agriculture as external or recycled inputs to produce crops either for subsistence or for commercial purposes. The ability of organic fertilisers to liberate nutrients is dependent on the presence of microbial decomposers, climate, and their quality such as C: N ratio, lignin and polyphenolic content (Murwira *et al.*, 2002). Organic fertilisers have been reported to improve the soil fertility, promote good soil aggregation, improve moisture infiltration and increase the water holding capacity of the soil, increase the soil organic carbon, soil available nutrients (N, P, and K), soil enzymes (dehydrogenase and alkaline phosphatase), and microbial biomass in the top 0-15 cm soil, improve fertiliser use efficiency to a great extent, and have the ability to prevent nutrient losses due to irregular and heavy rainfall (Bala *et al.*, 2011). Additionally, they have the ability to increase the P availability of the already present P by rendering it more accessible to crops through reducing the soil P absorption capacity, increasing the pH by decreasing the exchangeable acidity and aluminium in soil solution through chelation, and increasing the biological activity of the soil (Bationo *et al.*, 2007; Mukuralinda *et al.*, 2010).

2.12.1.1 Humate Green OK Fertiliser

Humic fertilisers contain a series of essential elements in the form of humates extracted from coal mass in complex NPK-type matrices which, after their incorporation in the soil, ensure

the assimilation of the nutrient ions contained and intensify the nutrition process (Lubal *et al.*, 2000). The organic matter and the humic substances are at the same time the energetic substrate of the microflora activity in the rhizosphere and represent an important reservoir of chelate-type compounds, which have a great capacity to bind various metal ions (B, Cu, Ca, Fe, Mg, Mo, Zn) and to form organo-metallic complexes with an important role in the formation of the soil characteristics and plant nutrition. A large number of nutritive ions (PO_4^{3-}; SO_4^{2-}; Zn^{2+}; Cu^{2+}; Fe^{2+}; Ca^{2+}) are retained by chelating processes, in the form of compounds of various solubility. For humic acids, the average chemical formula $C_{187}H_{186}O_{89}N_9S$ is accepted. Some studies have shown improved germination, seedling growth after germination, increased shoot and root growth, enhanced nutrient uptake, and increased microbial population and activity (Senesi *et al.*, 1991). From a quantitative standpoint water is the most important substance derived by plants from the soil. Humic substances help create a desirable soil structure that facilitates water infiltration and helps hold water within the root zone (Lubal *et al.*, 2000).

2.12.2 Inorganic Fertilisers

The use of inorganic or mineral fertilisers is indispensable in alleviating nutrient constraints and is central in Integrated Soil Fertility Management (ISFM) practices for improved crop production (Sanginga and Woomer, 2009). Inorganic fertilisers have a high concentration of nutrients that are rapidly available for plant uptake and they can be formulated to supply the appropriate ratio of nutrients to meet plant growth requirements. Today, a wide range of inorganic fertilisers are required to maintain soil fertility and sustainable agricultural systems. Generally, inorganic fertilisers containing N, P, K and S do not only increase crop yield but also improve nutritional quality of crop yields, such as protein, oil, starch, essential amino

acids and vitamins in pulses, oil seeds, tubers, and vegetables (Wang *et al.,* 2008). This is because nutrients in mineral fertilisers are immediately available for plant uptake (Nyamangara *et al.,* 2003). Empirical data has proved that sole application of inorganic fertilisers does not provide sustainable soil fertility management. However, they can serve as an entry point for a comprehensive sustainable soil fertility management (Sanginga and Woomer, 2009; Vanlauwe *et al.,* 2010).

2.12.2.1 Yaralegume Fertiliser

Groundnuts have important nutritional requirements, and maximum yields are possible only when producers meet them and other basic production factors. Using the best varieties and cultural practices will not allow groundnuts to reach full potential unless soil fertility is properly managed. Yara Ghana Limited therefore designed and tested with Savannah Agricultural Research Institute (SARI) in Tamale a dedicated balanced crop nutrition formula called Yaralegume fertiliser (0+18+13+31+4+2 as N, P_2O_5, K_2O, CaO, S and MgO). This fertiliser is suitable to leguminous crops requirements taking into account the symbiotic N_2 fixation by the nodules on the roots. Yaralegume fertiliser takes advantage of the soil acidity to dissolve and adjust accordingly.

2.12.3 Combined Effect of Organic and Inorganic Fertilisers

The combined use of mineral and organic sources of nutrients in soil fertility management is a new approach which evolved from long experiences in soil fertility management (Bationo and Waswa, 2011). Combining mineral fertilisers with organic inputs can significantly improve the agronomic efficiency of the nutrient use compared to the same amount of nutrients applied through either source alone (Sanginga and Woomer, 2009). According to Vanlauwe *et al.*

(2010), the ability of inorganic fertilisers to produce enough residues for sustainable soil fertility management on one hand, and the ability of organic fertilisers to rehabilitate less responsive soils and make them responsive to fertilisers have been proved by experimental results from Nigeria and Zimbabwe. Sanginga and Woomer (2009) also attributed the increment in fertiliser use efficiency to two main reasons: (1) common inorganic fertilisers lack the micro-nutrients essential for crop growth while organic fertilisers contain them. Organic fertilisers on the other hand are not able to meet crop major nutrients needs (N, P, and K) unless it is applied in huge quantities more than 10 tha^{-1} of dry matter. Therefore, combining both sources enable reasonable supply of all needed nutrients for a balanced growth and (2) a combination of both sources results in improvement in soil fertility status, increased soil organic matter content which improves nutrient retention, turnover and availability; enhanced P availability due to organic resources, alleviation of soil acidity and aluminium toxicity, improvement of the soil structure leading to reduced erosion, enhanced water infiltration and storage, and improved crop root development.

2.13 Impact of Fertilisation on Plant Infection, Control and Management

2.13.1 Interacting Factors of Fertilisation and Plant Disease

Infectious plant disease is the expression of the interaction of the plant, a pathogen, and the environment over time (Huber and Haneklaus, 2007). Disease control is most effectively achieved when the interacting factors of these three primary components are recognized and understood to make them less conducive for disease development. All interactions between the plant, pathogen and environment are affected by nutrition and all of the essential mineral elements are reported to influence disease incidence or severity (Huber and Graham, 1999; Datnoff *et al.*, 2007; Huber and Haneklaus, 2007). Although nutrients, as a component of the

environment, influence the plant's resistance, and a pathogen's virulence, each of the three primary components also influences the availability of nutrients. Nutrition of the plant can be drastically altered by many disease organisms through their effect on the uptake, translocation and distribution, or utilization of nutrients, and it is frequently difficult to clearly differentiate between the biotic and abiotic factors that interact to cause a plant nutrient deficiency or excess.

2.13.2 Managing Fertilisation to Control Plant Infections

Each of the 14 plant-essential mineral elements and several functional elements are known to influence infection severity (Huber, 1980). Infection suppression through manipulation of nutrient availability may be achieved by direct application of a nutrient to enhance resistance, by cultural practices which modify abiotic and biotic environments influencing nutrient availability, and by modifying the plant genotype relative to its nutrient uptake or interaction with the abiotic or biotic environment (Datnoff *et al.*, 2007). A well-balanced nutrition program, integrated with other crop production practices, permits a broad utilization of this cultural infection control, and generally provides the best opportunity for maximum infection suppression.

2.13.3 Mechanisms of Infection Control with Fertilisation

As indicated by Huber and Thompson (2007), resistance to an infection or disease is a property of the plant that describes the relative incompatibility of the plant-pathogen interaction, while tolerance describes the ability of the plant to produce even though infected, compatible plant-pathogen relationship. Virulence is a characteristic of the pathogen to cause infection, and infection escape refers to environmental conditions that are not conducive to

infection even though the pathogen and plant might be present (Huber and Haneklaus, 2007). Nutrients suppress infection by maximizing the inherent genetic resistance of plants, by facilitating infection escape and shortening the infection period, increasing tolerance through stimulating plant growth and yield in the presence of a pathogen, and by modifying the abiotic or biotic environment to reduce the survival or activity of pathogens (Marschner, 1995).

2.13.4 Fertiliser Effects on Infection Tolerance

Infection severity, and subsequent yield loss, may be limited by supplying sufficient nutrient quantity to offset the deleterious effects of a pathogen (Marschner, 1995). Phosphorus, N, and Zn stimulate root growth of plants to compensate for tissue lost through root rots. Increased availability of nutrients can compensate for reduced uptake efficiency caused by soil-borne pathogens. Although N rates required for nutrient sufficiency can increase powdery mildew (*Blumeria graminis*) in some plants by 10-20%, yield is increased 50 % to show that the vigorous, N-fertilized plants are able to tolerate the increased disease burden (Huber and Thompson, 2007). Phosphorus, N, and Zn stimulate root growth to promote more efficient nutrient uptake and translocation to promote infection resistance.

2.13.5 Fertiliser Effects on Infection Escape

A response to fertilisation by increased growth may constitute a form of infection escape, especially if a susceptible growth stage is shortened for some plant-pathogen interactions. Plants adequately fertilized with B and Zn have fewer root and leaf exudates to break spore dormancy (fungistasis) or stimulate fungal pathogens (Marschner, 1995).

2.13.6 Fertiliser Effects on Pathogen Survival and Virulence

Mineral nutrients may reduce the ability of a pathogen to cause infection by inhibiting germination, growth, virulence or survival directly or through plant exudates (Huber, 1980). The need for an external source of nutrients for saprophytic growth of fungi prior to infection is common. *Botrytis cinerea, Typhula* species, *Fusarium* species, *Sclerotinia*, and *Armillaria mellea* infect healthy plants slowly unless an external source of nutrients is available from soil or decaying organic matter. Exogenous C and N are required for germination of dormant *Fusarium* chlamydospores. Zinc is required for appressorium formation of *Puccinia coronata* on oat leaves and infection of broadbean by *Botrytis*. Leaf exudation of arginine from K and N sufficient plants inhibits germination of *Phytophthora infestans* sporangia, and the levels of arginine generally increase as the sufficiency for K increases. Calcium suppresses extra-cellular macerating enzymes of pathogens required for pathogenesis. Iron, Mg, Mn, and Zn also suppress macerating pathogen enzymes (Huber, 1980).

2.14 Effect of Fertiliser on Plant Nutritional Quality

Plant nutrient deficiency is a major limitation to crop production efficiency and nutritional quality, and a predisposing factor for infection (Graham *et al.,* 2007). Plant nutrient deficiencies can reduce both the quantity and quality of nutritive components of plants. Soil quality and soil fertility have a direct influence on the nutrient content of food crops and the nutrient output of farming systems (Bruulsema *et al.,* 2012). Soil fertility improvements can increase productivity and allow for greater diversity of crops without increasing the area cultivated. Thus, attention should be given to the role that soil fertility can play in increasing the nutrient output of cropping systems (Graham *et al.,* 2007). When plants become deficient

in a particular nutrient, other nutrients also may be affected so that the vitamins, protein, carbohydrate, fat and other essential nutritional components that plants are grown for will be affected. As primary food and feed sources, plants must provide nutrients in adequate quantity, safety and nutritional quality. Factors that result in a nutrient deficiency for plants also affect their nutrient value or nutrient availability for animals or man. Major causes of nutrient deficiency are an inadequate supply, lack of access to forms of nutrients available for absorption, or disease denial of nutrients necessary to maintain plant health and nutrient quality (Graham *et al.,* 2007). Nutrient deficiencies can be overcome by increased availability, more efficient plant uptake, increased physiological efficiency, and improved disease control. Benefits of nutrient sufficiency of the plant are achieved through increased production efficiency and greater productivity of more nutritious and safer food. A healthy plant will be more efficient and able to meet its nutrient needs more effectively from the generally limited resources available (Datnoff*et al.,* 2007).

2.15 Importance of Infection Control on Nutritional Quality and Food Safety

The application of fertiliser is nearly a universal practice in the production of commercial crops. The associations between levels and availability of macro- and microelements, changes in disease severity, plant vigour, and yield have long been recognized (Graham *et al.,* 2007). As well, the effects of mineral nutrition on pre-harvest toxin contamination in some host/pathogen interactions are well-documented (Wilson *et al.,* 1989). Soil macronutrient fertilisers (e.g., fertilisers that contain nitrogen, potassium, phosphorus, sulfur, calcium and magnesium), soil amendments, such as lime, gypsum and organic matter, can affect the available forms of micronutrients in soils and in the nutritional quality of the crops produced

on them (Datnoff *et al.,* 2007). Nutritional quality is noticeably reduced by disease infection, and sometimes before a yield reduction is observed. Greatest losses are sustained in protein, vitamin, and mineral composition, and least in carbohydrates. The need for increased processing required to compensate for losses or contamination may by itself reduce nutritional value. Mycotoxin production initiated during crop production can continue in storage to expose large segments of a population to highly toxic or carcinogenic compounds (Graham *et al.,* 2007).

2.16 Health and Nutritional Effects of Aflatoxins

Aflatoxins are potent carcinogens in animals and humans (Murphy *et al.,* 2006). There are a range of possible consequences of exposure to aflatoxins, largely determined by the dose and the duration of exposure. In all cases, the young are more susceptible than adults. Known for decades, aflatoxin contamination of groundnuts and maize has gained global significance due to the improved knowledge of the deleterious effects that contaminants have on human and animal well-being and the heavy reliance of smallholder populations on the two crops (CDC, 2004). Sufficient evidence that AFB_1 and mixtures of B_1, G_1 and M_1 are proven carcinogens has been provided by the International Agency for Research on Cancer who classifies them as Group 1 carcinogens (IARC, 1993) while M_1 and B_2 are designated to Group 2B. The deleterious pathway is as follows: AFB_1 is metabolized (by the liver) to AFB_1-8,9-epoxide (AFBO) or to less mutagenic forms which then can either result in cancer, toxicity or be excreted from the organism. The cancer is thus a result of formation of DNA-adducts by AFBO bonding with genetic material (Shimada and Guengerich, 1989; Crespi *et al.,* 1991; IARC, 1993). Prolonged exposure to doses of 50 micrograms aflatoxin B_1/kg/day has

clinically significant effects. No animal species has been found to be immune to the effects of aflatoxins (Murphy *et al.*, 2006).

The effects of aflatoxins on humans, as with animals, are dependent upon dosage and duration of exposure. Acute exposure can result in aflatoxicosis, which manifests as severe, acute hepatotoxicity with a case fatality rate of approximately 25 % (CDC, 2004). Early symptoms of hepatotoxicity from aflatoxicosis can manifest as anorexia, malaise, and low-grade fever. Acute high level exposure can progress to potentially lethal hepatitis with vomiting, abdominal pain, jaundice, fulminant hepatic failure, and death. Outbreaks of acute aflatoxicosis are a recurring public health problem throughout the world (CDC, 2004). In humans, aflatoxins induce a wide range of diseases. Several studies have linked chronic and acute exposure to dietary aflatoxins with primary liver cancer in humans in many countries worldwide including Uganda, Canada, Germany, Kenya, Mozambique and China (Casado *et al.*, 2001). Exposure to dietary aflatoxins is considered an important risk factor for the development of primary hepatocellular carcinoma in individuals already exposed to hepatitis B (Bennett and Klich, 2003). Synergistic interactions between aflatoxins and hepatitis B have actually been noted on the etiology of liver cancer (Groopman and Kensler, 1996; Montesano *et al.*, 1997).

Evidence has also been found associating aflatoxins with neoplasms in extrahepatic tissues, particularly the lungs (Bennett and Klich, 2003). An example being Hayes *et al.* (1984), who found a correlation between both respiratory and total cancer in an epidemiological study of Dutch groundnut workers exposed to dust contaminated with aflatoxin B_1. Aflatoxins are thought to be involved in Reye's syndrome, a disease characterized by encephalopathy and fatty degeneration in the viscera of children and adolescents (Hayes, 1980). It has been suggested that kwashiorkor, a severe malnutrition disease, may actually be a form of pediatric

aflatoxicoses (Hendrickse, 1997). Aflatoxin exposure in West Africa has also been correlated with stunted growth in children who were exposed right from the neonatal stages (Gong *et al.*, 2002). Maxwell *et al.* (1998) stated that due to the capacity of aflatoxins to cross the placental barrier, they may cause genetic defects during the foetal stage. Additional effects of chronic exposure have not been widely studied but are thought to include immunologic suppression, impaired growth, and nutritional interference. Outbreaks of acute aflatoxicosis from highly contaminated food have been documented in Kenya, India and Thailand (CAST, 2003). The immunosuppressive effects of aflatoxins have also been shown to be transferred across the placenta and affect the unborn foetus in porcines, suggesting that unborn babies could equally be affected. Consequently, poor nutrition usually attributed to food insecurity, is clearly exacerbated by exposure to aflatoxins, leading to increased disease prevalence and further reduction in the ability of individuals to cope with mycotoxin exposure. Important also, is the linkage between aflatoxins and hepatic cellular carcinoma (HCC), hepatitis B and hepatitis C viruses (HBV and HCV, respectively). Many studies, some as early as 1965, have shown linkage between aflatoxin and HCC (Svoboda *et al.*, 1966; Sun *et al.*, 1999) and later HBV and HCV were also identified as 'etiological risks' (IARC, 1993). Thus, in many regions of the world where there is high aflatoxin contamination, HBV and HCV infections are prevalent and a strong synergism has been reported (Lu, 2003; Qian *et al.*, 1994; CDC, 2004).

2.17 Economic Effects of Aflatoxins

Aflatoxins in groundnuts, as in all crops, can have a direct economic impact that results in the loss of an agricultural product or the loss of market value. Aflatoxin due to the invasion of *Aspergillus flavus* of the groundnut pod is a serious problem in the international groundnut market and has seriously hampered the export business of developing countries, including

Ghana (FAO, 2002). In order to restrict exposure to this substance, many countries and governmental agencies set safety regulations, limiting the average concentration of aflatoxin on groundnut and groundnut products. Aflatoxins also increase costs for veterinary and human health services, costs for food-borne disease surveillance, and food monitoring. The presence of high levels of aflatoxins in groundnuts can make them unacceptable for marketing, causing financial loss to the farmer and the food retailers. Depending on the size of the market, economic losses can reach 100 %, when the entire product is rejected by the market if aflatoxin levels are higher than acceptable standards. It is estimated that Africa loses over USD670 million annually due to requirements for EU aflatoxin standards (Otsuki *et al.,* 2001). Worldwide, billions of dollars are lost by farmers and traders due to aflatoxin contamination. It is therefore essential that contamination from mycotoxins in groundnuts be minimized as much as possible (Guo *et al.,* 2009).

2.18 Aflatoxin Detection, Measurement and Analysis

Numerous methods have been developed to meet analytical requirements from rapid tests for factories and grain silos to regulatory control in official laboratories. Common methods include thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) in combination with fluorescence detection with or without derivatisation, liquid chromatography tandem mass spectrometry (LC/MS) and immunochemicals methods, such as enzyme linked immunosorbent assay (ELISA), immunosensors, dipsticks and strip-test (Manetta, 2011).

2.18.1 Chromatographic Methods

Aflatoxins possess significant UV absorption and fluorescence properties, so techniques based on chromatographic methods with UV or fluorescence detection have always predominated.

Originally the chromatographic separation was performed by TLC: since when aflatoxins were first identified as chemical agents, it has been the most widely used separation technique in aflatoxin analysis in various matrices, like corn, raw groundnuts, cotton seed, eggs (Trucksess *et al.,* 1977), milk (Van Egmond *et al.,* 1978) and it has been considered the AOAC official method for a long period. This technique is simple and rapid and the identification of aflatoxins is based on the evaluation of fluorescence spots observed under a UV light. AFB_1 and AFB_2 show a blue fluorescence colour, while it is green for AFG_1 and AFG_2. TLC allows qualitative and semi-quantitative determinations by comparison of sample and standard analysed in the same conditions. Moreover, given the significant advantages of the low cost of operation, the potential to test many samples simultaneously and the advances in instrumentation that allow quantification by image analysis or densitometry, TLC can be used also in laboratories of developing countries in alternative to other chromatographic methods that are more expensive and require skilled and experienced staff to operate (Nawaz *et al.,* 1992).

Overpressured-layer chromatographic technique (OPLC), developed in the seventies, has been used for quantitative evaluation of aflatoxins in foods (Otta *et al.* 1998) and also in fish, corn, wheat samples that can occur in different feedstuffs (Otta *et al.,* 2000). Because of its higher separation power, higher sensitivity and accuracy, the possibility of automating the instrumental analysis, High performance liquid chromatography (HPLC) is now the most commonly used technique in analytical laboratories. HPLC using fluorescence detection has already become the most accepted chromatographic method for the determination of aflatoxins. Liquid chromatographic methods for aflatoxins determination include both normal and reverse-phase separations, although current methods for aflatoxin analysis typically rely

upon reverse-phase HPLC, with mixtures of methanol, water and acetonitrile for mobile phases (Manetta, 2011). Despite the enormous progress in analytical technologies, methods based on HPLC with fluorescence detection are the most used today for aflatoxins instrumental analysis, because of the large diffusion of this configuration in routine laboratories (Manetta, 2011). The recent availability of analytical columns with reduced size of the packing material has improved chromatographic performance. Today, numerous manufacturers commercialize columns packed with sub-2 μm particles to use devices that are able to handle pressure higher than 400 bar, such as Ultra-Performance Liquid Chromatography (UPLC). This strategy allows a significant decrease in analysis time: aflatoxins runs are completed in 3-4 min with a decrease of over 60 % compared to traditional HPLC. In addition, solvent usage has been reduced by 85 %, resulting in greater sample throughput and significant reduction of costs of analysis.

2.18.2 Immunological Methods

Manetta (2011) reported that high performance liquid chromatographic methods with fluorescent detection are mainly used in routine aflatoxins analysis. They are often arduous and time-consuming and require knowledge and experience of chromatographic techniques to solve separation and interference problems. The big demand in analytical chemistry to have sensitive, specific, but also simple and fast methods for an effective monitoring of aflatoxins in food and feed commodities, has produced analytical methods that combine simplicity with high detectability and analytical output. This can be realized by means of immunological methods in conjunction with a highly sensitive detection of the label.

Enzyme-linked immunosorbent assay is the best established and the most available immunoassay in aflatoxin rapid detection, using the 96 well plate microtiter format. Many commercial companies have developed and commercialized ELISAs which applicability, analytical range and validation criteria are well defined. Despite the increasing use of LC-MS techniques, antibody-based methods for aflatoxins analysis continue to be investigated. The development of these immunochemical methods and their evolution from single to multiple analyte screening, including topics on ELISA, immunosensors, fluorescence polarization and rapid visual tests (lateral-flow, flow-through and dipstick) have been developed. In the case of immunosensors for aflatoxins, antibodies are immobilized on the surface of a screen-printed electrode, magnetic beads held on the surface of a screen-printed electrode (Piermarini *et al.*, 2009), on piezoelectric quartz crystal immunosensor with gold nanoparticles (Jin *et al.*, 2009). A homogeneous assay for determining the aflatoxin content in agricultural products based on the technique of fluorescence polarization has been described (Nasir and Jolley, 2002). The disadvantage of this technique is that the aflatoxin contents are underestimated, probably because of the low cross-reactivity of the antibody with AFB_2, AFG_1 and AFG_2. The lateral flow device is one of the simplest and fastest immunoassay techniques have been developed. It is a screening test available in the format of strip or dipstick (Delmulle *et al.*, 2005). Immunodipstick or lateral flow immunoassay has recently gained increasing attention because it requires simple and minimal manipulations and little or no instrumentations. Colloidal gold conjugated anti-aflatoxin antibodies are immobilised at the base of the stick.

The recent development of biosensors has stimulated their application also to aflatoxin analysis: in literature many examples are reported, like DNA biosensor (Tombelli *et al.*, 2009), electrochemical immunosensor (Paniel *et al.*, 2010), electrochemical sensor (Siontorou *et al.*,

1998; Liu *et al.*, 2006), fluorometric biosensor (Carlson *et al.*, 2000). The advantages of biosensing techniques are: reduced extraction, clean-up analytical steps and global time of analysis (1 min or only few seconds); possibility of online automated analysis; low cost; skilled personnel not required. On the other side, sensitivity should be enhanced and their stability should be improved to allow long-term use. Because of the ease of use of these devices, many commercial systems continue to be developed not only for aflatoxins, but also for all mycotoxins.

2.19 Quality Standards for Aflatoxin

The Code of Practice for the prevention and reduction of aflatoxin contamination in groundnuts (GS 1003:2009) provides guidance in the production and handling of groundnuts for entry into international trade for human consumption (Table 2.5). All groundnuts should be prepared and handled in accordance with the recommended International Code of Practice-General Principles of Food hygiene, which is relevant for all foods being prepared for human consumption. The code has two main parts: (a) recommended Good Agricultural Practices (GAP) and, (b) Good Manufacturing Practices (GMP). The GAP recommends guidelines in post-harvest, harvest, transportation, segregation of aflatoxin contaminated lots and storage. The GMP provides guidelines for receiving and shelling, sorting, blanching and the packaging and storage of end products. The code recommends introduction in the future, a complementary management system that incorporates the Hazard Analysis Critical Control Point (HACCP) system in the effort to further reduce levels of Aflatoxin in groundnuts. Whilst the GS ISO 16050:2003 provides the methods to follow (High-Performance Liquid Chromatographic Method) in the determination of Aflatoxin B_1 and the total content of

Aflatoxins B_1, B_2, G_1 and G_2 in cereals, nuts and derived products, GS 313:2001 provides the quality requirements for groundnuts. The requirement for groundnut quality in Ghana is shown in Table 2.5.

Table 2.5 Ghana Standards Authority (GSA) Requirements for Groundnut Quality

Groundnuts		**Kernels**		
Maximum allowable limits	In shell	Grade 1	Grade 2	Grade 3
Extraneous matter content (%)	2	1	3	5
Damaged pods/kernels (%)	0.5	0.5	1.5	3
Shriveled kernels (%)	3	3	3	3
Skinned kernels (%)	-	0.5	0.5	0.5
Broken and split kernels (%)	-	8	10	12
Empty pods (%)	2	-	-	-
Admixtures of other varieties (%)	5	5	5	5
Aflatoxin content (µg /kg)	20	20	20	20

Source: Adapted from GSA (2013).

2.20 Management of Aflatoxin in Groundnuts

2.20.1 Pre-Harvest Management

A number of agronomic practices minimize pre-harvest infection by *A. flavus* (Table 2.6). Among them are the applications of lime (or any calcium source) and farmyard manure (FYM). Studies have shown that application of lime alone can reduce aflatoxin contamination

by 72 %, while application of FYM reduces aflatoxins by 42 % under field conditions. When combined, the two treatments result in aflatoxin contamination being reduced up to 84 %.

Table 2.6 Reduction in Aflatoxin Contamination by Single or Multiple Agronomic Practices

Agronomic Practice	Aflatoxin Reduction (%)
Cereal crop residues	28
Farmyard Manure (FYM)	42
Combination of FYM and residues	53
Lime application	72
Combination of lime and residues	82
Combination of FYM, lime, and residues	83
Combination FYM and lime	84

Source: Adapted from Waliyar *et al.* (2007).

Payne *et al.* (1986) demonstrated in an extensive four year study that the reduction of moisture stress was associated with lower levels of aflatoxin contamination. Despite the important link between moisture stress (drought) and higher mycotoxin levels and the fact that droughts occur commonly, breeding for drought resistance has received little or no interest (Moreno and Kang, 1999). Crop management practices such as weeding, reduces water usage and assists in reducing moisture stress and may therefore contribute to reduced mycotoxin contamination of grain (Moreno and Kang, 1999). According to Hell *et al.* (2003) other management practices such mixed cropping with vegetables have been found to reduce aflatoxin contamination of corn, whereas intercropping with cassava, groundnuts or cowpeas and ear damage on the field were found to increase aflatoxin contamination.

2.20.2 At-harvest and Post-harvest Management

Cultural practices, starting with harvesting the crop at the right maturity and wind drying, have been shown to be effective in reducing aflatoxin contamination in groundnuts. In addition, management practices—such as using appropriate drying techniques (including drying on raised surfaces or on mats), reducing kernel moisture content to 8 percent, proper threshing methods, and sorting the kernels before sale or consumption, significantly influence the level of aflatoxin contamination. Aflatoxin reduction under these practices can vary from 63 to 88 percent depending on location. Practices such as wetting groundnut shells to facilitate shelling increase the risk of aflatoxin contamination.

2.20.3 Host Plant Resistant Breeding

Rural farmers in developing countries are often resource poor and have a limited ability to implement integrated management approaches. Host plant resistance, when combined with pre- and post-harvest strategies, is thus often the most practical and effective approach (Bhatnagar, 2010). For the past decade, breeding groundnut varieties resistant to *A. flavus* infection has been a focus of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). A number of varieties with resistance to or tolerance of *A. flavus* infection and aflatoxin contamination have been released or are in various stages of testing (Bhatnagar, 2010). Study results indicate that, despite high variation in *A. flavus* infection and subsequent aflatoxin incidence, significant improvement in the level of varietal resistance (less than 20 ppb contamination) is possible. Breeding efforts have focused on reducing groundnut maturity periods to escape end-of-season drought, and the emphasis has been on the identification of short-duration farmer-preferred lines with resistance to or tolerance of *Aspergillus* species (Bhatnagar *et al.*, 2003).

2.20.4 Biocontrol Options for *Aspergillus* Species

Biocontrol of aflatoxin contamination or infection is becoming a promising technology. A biopesticide, consisting of a rhizosphere-competent non-aflatoxigenic strain of *Aspergillus* with competitive saprophytic capacity, may competitively prohibit contaminated strains from infecting the crop (Cole and Cotty, 1990). Fluorescent pseudomonads and several strains of *Trichoderma* species inhabit the rhizosphere of many crop plants and have been identified as potentially promising biocontrol agents against *A. flavus*. Since the beginning of the 21st century, a large number of *Trichoderma* and *Pseudomonas* isolates have been obtained from groundnuts rhizosphere and evaluated for their effect towards *A. flavus* and their ability to reduce pre-harvest kernel infection of groundnuts (Dorner *et al.*, 1998). Significant reduction of *A. flavus* populations and kernel infection occurred in both greenhouse and field experiments. Two *Trichoderma* isolates, Tv 47 and Tv 23, and two bacterial isolates *P. cepacia* (B 33) and *P. fluorescens* (Pf 2), were effective in reducing aflatoxin content in the kernels (Dorner *et al.*, 1998). The effectiveness of the biocontrol agents still needs to be established under African field conditions and simple, cheap and effective formulations developed for use in farmers' fields. Integration of these biocontrol agents with host plant resistance and agronomic management would provide an environmentally-friendly option for the management of aflatoxin contamination in groundnuts (Cole and Cotty, 1990).

2.20.5 Biotechnological approaches

Biotechnological approaches to increase host plant resistance through the use of anti-fungal and anti-mycotoxin genes also have begun. This approach received a major boost with the successful establishment of groundnut regeneration and transformation protocols, and led to the transformation of groundnut with a rice chitinase gene to help prevent invasion by fungal

pathogens (Abramson, 1998). These transgenic events are now in advanced generation, with some crops showing good resistance to *A. flavus* infection (less than 10 % infection) in *in vitro* seed inoculation tests. Such events can be used in conventional breeding to develop agronomically superior groundnut varieties that are highly resistant to aflatoxin contamination (Abramson, 1998).

2.20.6 Enterosorption and Chemoprotection

Researchers have developed mechanisms of detoxifying aflatoxins once consumed by animals. Chemoprotection against aflatoxins involves use of compounds that either increase the animal's detoxification process or prevent the production of compounds that cause damage to various parts of the body (Phillips *et al.*, 1995). The first progress was the discovery that certain zeolytic minerals could selectively adsorb aflatoxins tightly enough to prevent them from being absorbed through the intestine (Harvey *et al*., 1993). The utilisation of specially processed phyllosilicate/bentonite clays especially hydrated sodium calcium aluminosilicate (HSCA) that selectively bind and inactivate aflatoxins in the gastrointestinal tract of farm animals such as chickens, turkey poults, lambs, goats and pigs has been the most successful chemoprotection (Phillips *et al.,* 1995). These clays have been recommended to be incorporated in animal feeds as additives that provide protection from the toxins (Devegowda *et al.*, 1998).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The field experiments were conducted in four (4) communities; Samoa, Korro, Konguoli and Hiinneteng all in the Lambussie-Karni District of the Upper West Region of Ghana (Figure 3.1). The District is located in the North Western part of the region. It is the newest and smallest district in the Region and covers a total land area of 1,356.6 sq km. The Lambussie-Karni District falls in the Guinea Savanna climatic zone and experiences two major seasons with a single maxima **(**short rainy season and a long dry spell). The rainy season starts from June to October each year and gives way to the dry season from November to May. The rainfall distribution in the district varies from year to year sometimes with intermittent droughts and floods mostly peaking in August. Mostly, the rainfall ranges between 900 – 1,000 mm per annum. The occurrence of drought or floods affects crop growth thereby culminating in reduced crop yields each year, as additional nutrients intake by the crops is impaired. The on-farm fertilisation experiment was established in the wet season of 2014. Planting was done in July 2014 and harvested in October 2014.

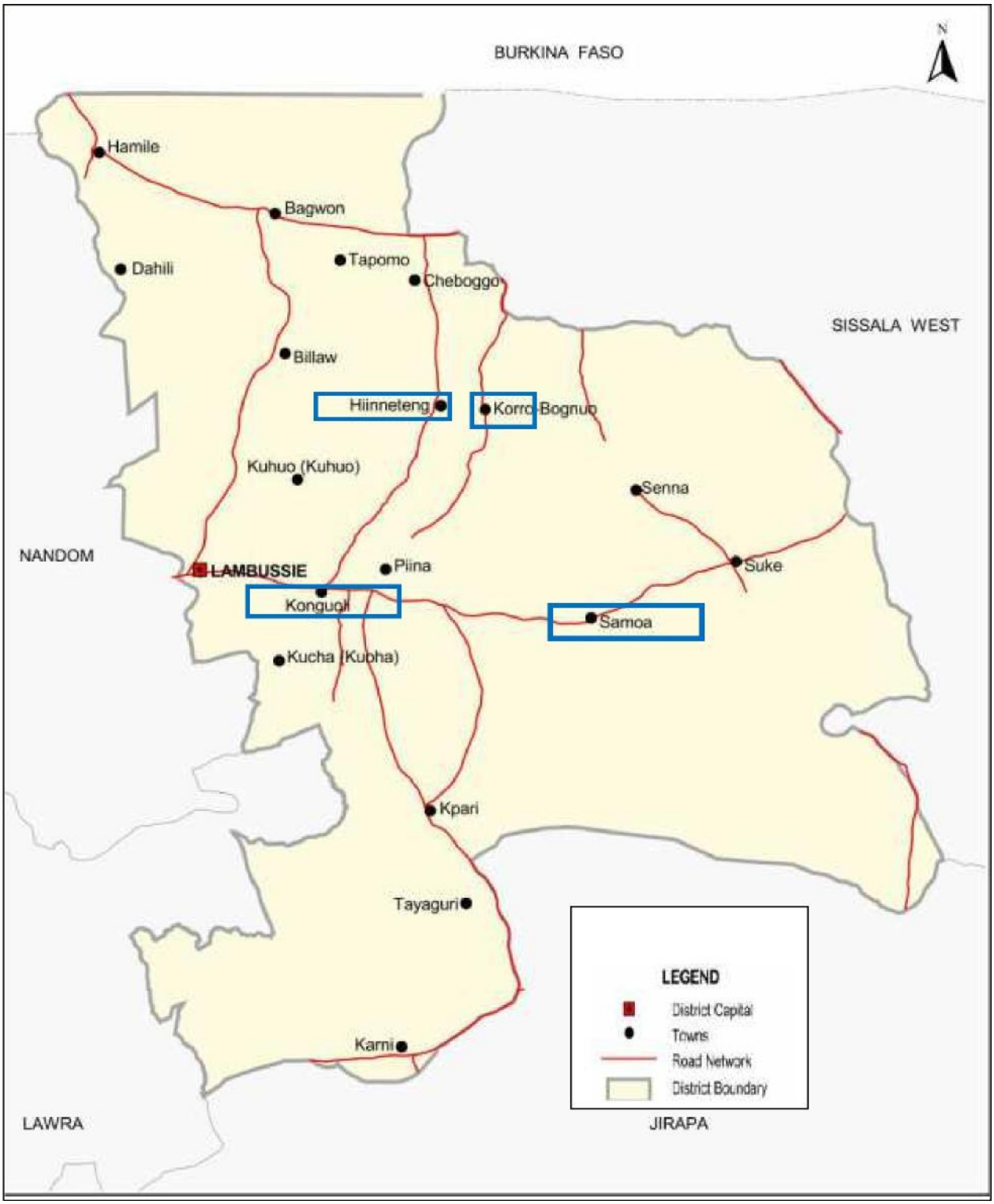


Figure 3.1: Map of Lambussie-Karni District indicating Sampling Communities**:** Samoa**,** Korro**,** Konguoli and Hiinneteng. **Source:** Ghana Statistical Service (2013).

3.2 Geology and Soils

There is an extensive Birimian soil formation in the District with a concentration of granite rocks around, Lambussie, Bawon and Billaw areas. The soils are mostly groundwater laterites and Sudan ochrosols. The soil of the District for the most part is sandy loam with underlying hard iron pans. The sandy loam is susceptible to severe sheet and gully erosion caused by surface run-off during the peak of the wet season. The wide spread erosion in effect adversely affects the fertility of the soil. According to the Ministry of Food and Agriculture (2001), the Soil Research Institute of the Centre for Scientific and Industrial Research in Kumasi carried out a research on the soil and reported the following; soil pH (6.0-6.8), organic matter (0.5-1.3 %), total nitrogen (0.01-0.07 %), available phosphorus (2.0-7.4 mg/kg soil) and available calcium (52-151.5 mg/kg soil).

3.3 Experimental Design

The experiment was laid and replicated thrice in a Randomized Complete Block Design (RCBD) with communities (Hiinneteng-HA; Konguoli-KG; Korro-KO; Samoa-SA) as blocks with three levels of fertilizers; 0, 3.75kg of Yaralegume (0.18.13+31CaO+4S+2Mg) and 1.88kg of Yaralegume + 3L of Humate Green OK (humic substances from Latvian peat). One groundnut variety (Chinese) obtained from the Savannah Agricultural Research Institute (SARI) was planted to all experimental plots. Plot size per treatment was 10 m x 10 m (100 m^2). Chinese is a*fastigiata* species and a valencia botanical type. Seeds were planted on the same day in all the plots after tillage. The plant spacing was 0.50 m inter-row and 0.10 m in-row. Yaralegume fertiliser was broadcasted uniformly after sowing to respective treatment

plots and Humate Green OK liquid fertiliser applied twice; at vegetative and flowering stages as foliar fertiliser using a knapsack sprayer.

3.4 Pre-germination of Groundnut Seeds

Groundnut seeds were soaked in ordinary water (1 L) for 12 hours before planting. This was done to speed up the germination process. Pre-germination treatment of groundnut seeds by soaking in water before planting resulted in a minimized lag period between sowing and seedling establishment (Polthanee, 1991). An early seed emergence leads to an early use of soil moisture, and crop maturity prior to experiencing water stress.

3.5 Preparation and Storage of Groundnuts

Groundnuts from each plot were harvested and dried. Mini (50 kg) polypropylene bags were used to store the unshelled groundnuts produced from the various plots in all four communities for five (5) months (November, 2014 to March, 2015).

3.6 Environmental Conditions of Communities

Data on temperature (maximum and minimum) for the entire storage period was obtained from the Lambussie – Karni District Meteorological Department and the relative humudity values estimated using online converters and psychrometric charts.

3.7 Sampling Structure

Podded groundnut samples (1.5 kg) were randomly gathered from the field and subsequently from storage, in order for the analysis to be representative of the whole lot. From respective lots, 1.0 kg of dried groundnuts was picked as fresh samples (without storage) and the

remainder stored. After storage, 1.0 kg of the groundnuts was picked again as stored samples from respective lots. Subsequently, each of the fresh and stored samples per experimental unit per community was hand-shelled separately; working samples (100 g and 60 g) were prepared accordingly and from which analytical samples (50 g and 30 g) were respectively taken for aflatoxin and proximate composition analysis.

Table 3.1: Fresh Groundnut Sampling Points in Communities

Community	Latitude (°)	Longitude (°)	Altitude (m)
Hiinneteng	N10.85800	W002.69651	312.72
Konguoli	N10.84229	W002.66427	291.69
Korro	N10.88371	W002.56889	287.43
Samoa	N10.83208	W002.56059	328.88

3.8 Fluorometric Quantification Analysis of Aflatoxins

The Romer FluoroQuant Afla Plus (COKFA3070) test kit used for quantification analysis of aflatoxin employed a solid-phase, single-step clean-up column followed by fluorometric analysis to determine aflatoxins in groundnuts (AOAC, 2007). Acetonitrile (ACN) with distilled or deionized water (86:14) was used for extraction. This quantitative method is rapid, accurate, and inexpensive and can be applied to individual samples.

A representative sample of the batch to be tested was taken. The sample was ground and a 50 g portion placed in a blender jar. The ground sample was blended with 100 mL of acetonitrile for 1 minute and filtered into a container. From this extract, 2000 µL of sample was placed on a SolSep 2001 clean-up column and extracted through the column. Following extraction, 1000

µL of sample and 1000 µL of diluent were added to the column and mixed with the pipette tips. The samples were then pushed through the column, and a 1000 µL portion of the purified extract placed into a clean scratch-free cuvette. A prepared developer (1000 µL) was added to the purified sample; the sample was then mixed using a vortex, and placed in a calibrated fluorometer. After a programmed 30 seconds delay, the fluorometer displayed the result in parts per billion (ppb).

3.9 Results Interpretation Criteria

Results of quantification analysis of aflatoxins represented the amount of total aflatoxin (B_1, B_2, G_1 and G_2) in parts per billion (ppb) present in the sample. Values less than the Limit of Quantification (LOQ) (2.4 ppb-5.0 ppb) are reported as <LOQ; values less than the Limit of Detection (LOD) (0.6 ppb-1.9 ppb) are reported as Non-Detect (ND).

3.10 Proximate Analysis of Groundnuts

3.10.1 Moisture Content Determination

The oven dry method was used to determine the percentage of water in a sample per the description of Kirk and Sawyer (1991) by drying the sample to a constant weight. The water content was expressed as the percentage by weight of the dried sample. Approximately 5–10 g of ground sample was weighed and placed in a drying oven at 105 °C for 12 hours. The sample was left to cool in a desiccator. The sample was weighed again with precaution against exposure to the atmosphere.

Calculations

$$\text{Moisture Content (\%)} = \mathbf{100}\left(\frac{\mathbf{B-A)-(C-A)}}{\mathbf{B-A)}}\right) \quad \textit{Equation (3.1)}$$

Where;

A–weight of clean dry can (g)

B–weight of can + weight of sample (g)

C–weight of can + dry sample (g)

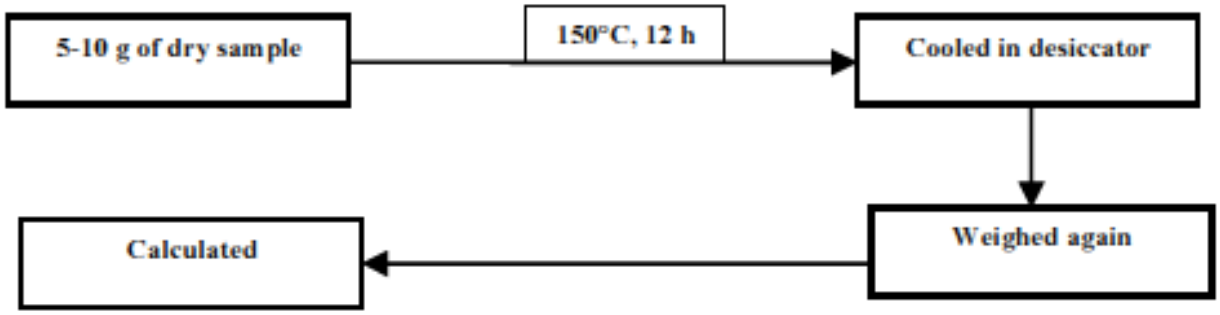


Figure 3.2: Determination of Moisture Content in Groundnut Samples. **Source:** Adapted from Kirk and Sawyer (1991).

3.10.2 Crude Protein Content Determination

Analysis was by Kjeldahl's method as described by Kirk and Sawyer (1991), which evaluates the total nitrogen content of the sample after it has been digested in sulphuric acid with mercury or selenium catalyst. Approximately 1 g of sample was weighed and placed in the Kjeldahl flask. Potassium sulphate (10 g), mercuric oxide (0.7 g) and 20 ml of concentrated sulphuric acid were added. The flask was placed tilted at an angle in the digester, brought to boiling point and retained until the solution was clear. It was heated for 30 minutes and antifoam added periodically when necessary. It was left to cool with approximately 90 ml of distilled, de-ionized water added gradually. 25 ml of sodium sulphate solution was added and stirred uniformly. Add one glass bead and Approximately 80 ml of 40 % sodium hydroxide

solution, keeping the flask tilted. The flasks was quickly connected to the distillation unit, heated and 50 ml of distillate containing ammonia in 50 ml of indicator solution collected.At the end of distillation, the receptor flask was removed and the end of the condenser rinsed. The solution was titrated with standard chlorhydric acid solution.

Calculations

Nitrogen in sample (%) = $\mathbf{100}\left[\frac{\mathbf{A \times B}}{\mathbf{C}}\ \mathbf{0.014}\right]$ .....*Equation (3.2a).*

Crude protein (%) = **Nitrogen in sample x 6.25** .....*Equation (3.2b).*

Where;

A−chlorhydric acid used in titration (ml)

B−normality of standard acid

C−weight of sample (g)

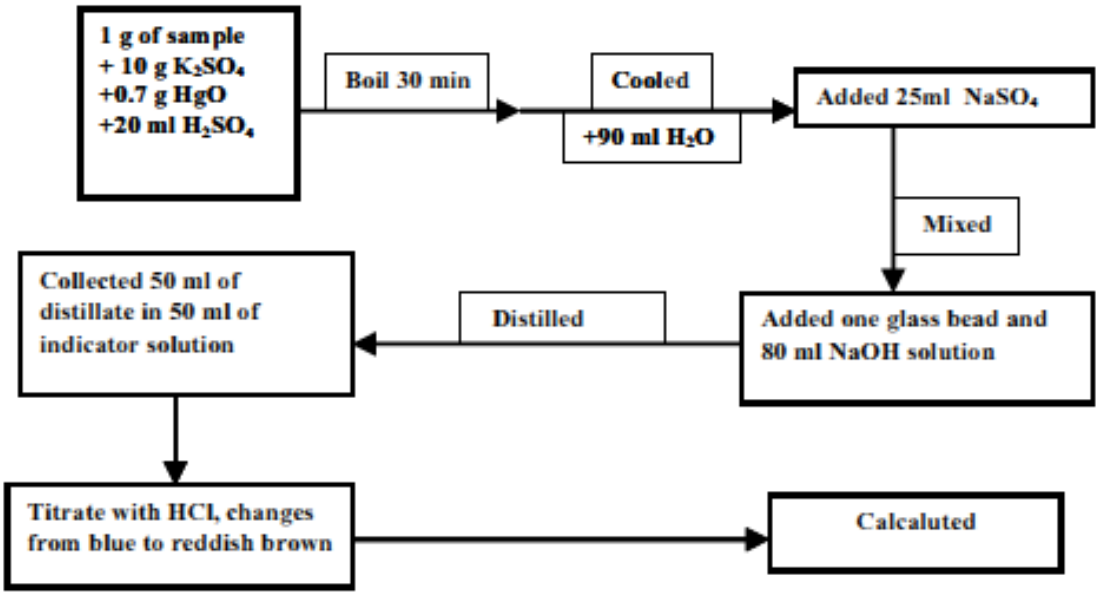


Figure 3.3: Determination of Crude Protein by Kjeldahl's Method. **Source:** Adapted and Modified from Kirk and Sawyer (1991).

3.10.3 Ash Content Determination

The dry ashing method as described by Kirk and Sawyer (1991) was used to determine ash content in all groundnut samples. Ash is considered as the total mineral content of the sample. A 2.5 to 5 g of dry sample was placed in a previously calcined crucible and brought to constant weight. The crucible was placed in a furnace and heated at 550 °C for 12 hours, left to cool and transferred to a desiccator. The crucible was carefully weighed again with the ash.

Calculations

Ash content (%) = **100** $\left(\frac{A-B}{C}\right)$ ..... *Equation (3.3).*

Where;

A–weight of crucible with sample (g)

B–weight of crucible with ash (g)

C–weight of sample (g)

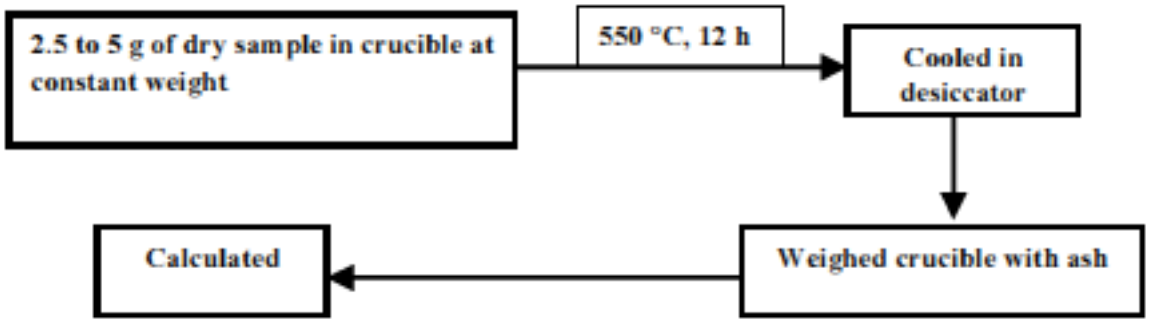


Figure 3.4: Determination of Ash Content in Groundnut Samples. **Source**: Adapted from Kirk and Sawyer (1991).

3.10.4 Crude Fat Determination

The Soxhlet's method of crude fat content determination was used as explained by Kirk and Sawyer (1991). In this method, the fats are extracted from the sample with petroleum ether and evaluated as a percentage of the weight before the solvent is evaporated.

Extraction flasks were removed from the kiln without touching them with the fingers, cooled in a desiccator and weighed. Approximately 3 to 5 g of dry sample was weighed into an extraction thimble. Handling with tongs, it was placed in the extraction unit. The flask containing petroleum ether at 2/3 of total volume was connected to the extractor. The setup was brought to boil and the heat adjusted to obtain about 10 refluxes per hour. The length of the extraction

depended on the quantity of fats in the sample. Fatty materials like groundnuts took 6 hours. The ether was evaporated in a rotoevaporator and flasks cooled in a desiccator and weighed. The defatted sample was used in determining crude fibre.

Calculations

Crude fat content (%) = **100** $\left(\frac{B-A}{C}\right)$ *Equation (3.4).*

Where;

A−weight of clean dry flask (g)

B−weight of flask with fat (g)

C−weight of sample (g)

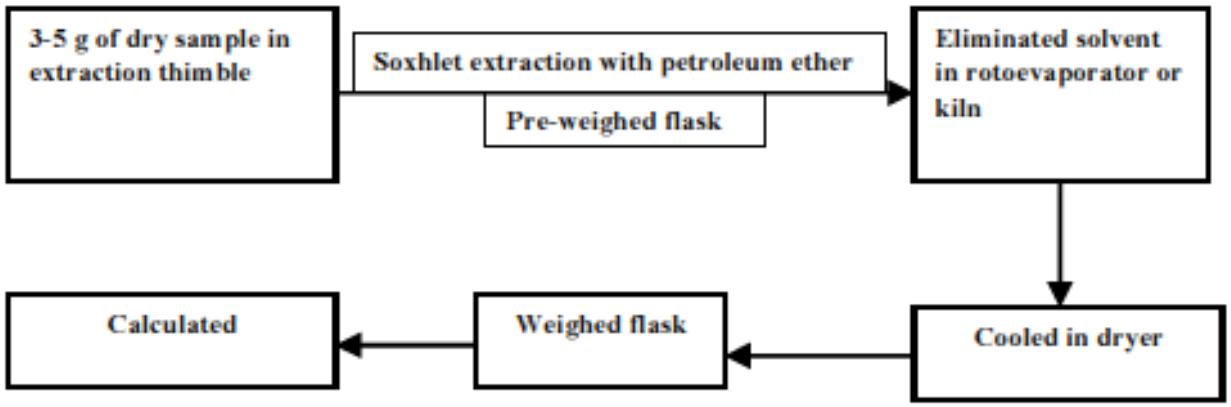


Figure 3.5: Determination of Fat Content of Groundnuts by Soxhlet's Method. **Source**: Adapted and Modified from Kirk and Sawyer (1991).

3.10.5 Crude Fibre Determination

The crude fibre content was determined by the Weende method (Sungsoo *et al.*, 1999). This method gives the crude fibre content of the sample after it has been digested in sulphuric acid and sodium hydroxide solutions and the residue calcined. The difference in weight after calcination indicates the quantity of fibre present. Approximately 2 to 3 g of defatted dry sample was weighed, placed in a flask and 200 ml of boiled sulphuric acid solution added. The flask containing the sample was attach to a condenser and brought to boiling point in a minute. Antifoam was added intermittently when necessary. The Boiling lasted for exactly 30 minutes and the volume of distilled water constantly maintained and periodic swirling of the flask done to remove particles adhering to the sides. The Buchner funnel was lined with filter paper and pre-heated with boiling water. At the same time, at the end of the boiling period, the flask was removed and delayed for a minute before the content was carefully filtered using suction. Filtration lasted for less than 10 minutes and the filter paper washed with boiling water. The residue was transferred into a flask using a retort containing 200 ml of boiling NaOH solution and boiled for 30 minutes and the volume of distilled water constantly maintained and periodic swirling of the flask done to remove particles adhering to the sides. The filtration crucible was preheated with boiling water and the hydrolyzed mixture carefully filtered after letting a rest of 1 minute. The residue was washed with boiling water, with the HCI solution and then again with boiling water, finishing with three washes with petroleum ether. The crucibles were placed in a kiln at 105°C for 12 hours and cooled in dryer or desiccator. The crucibles were quickly weighed with the residue inside and placed in the furnace at 550° C for 3 hours. It was left to cool in a desiccator and weighed afterwards.

Calculations

Crude fibre content (%) = $100\left(\frac{A-B}{C}\right)$ ..... *Equation (3.5).*

Where;

A–weight of crucible with dry residue (g)

B–weight of crucible with ash (g)

C–weight of sample (g)

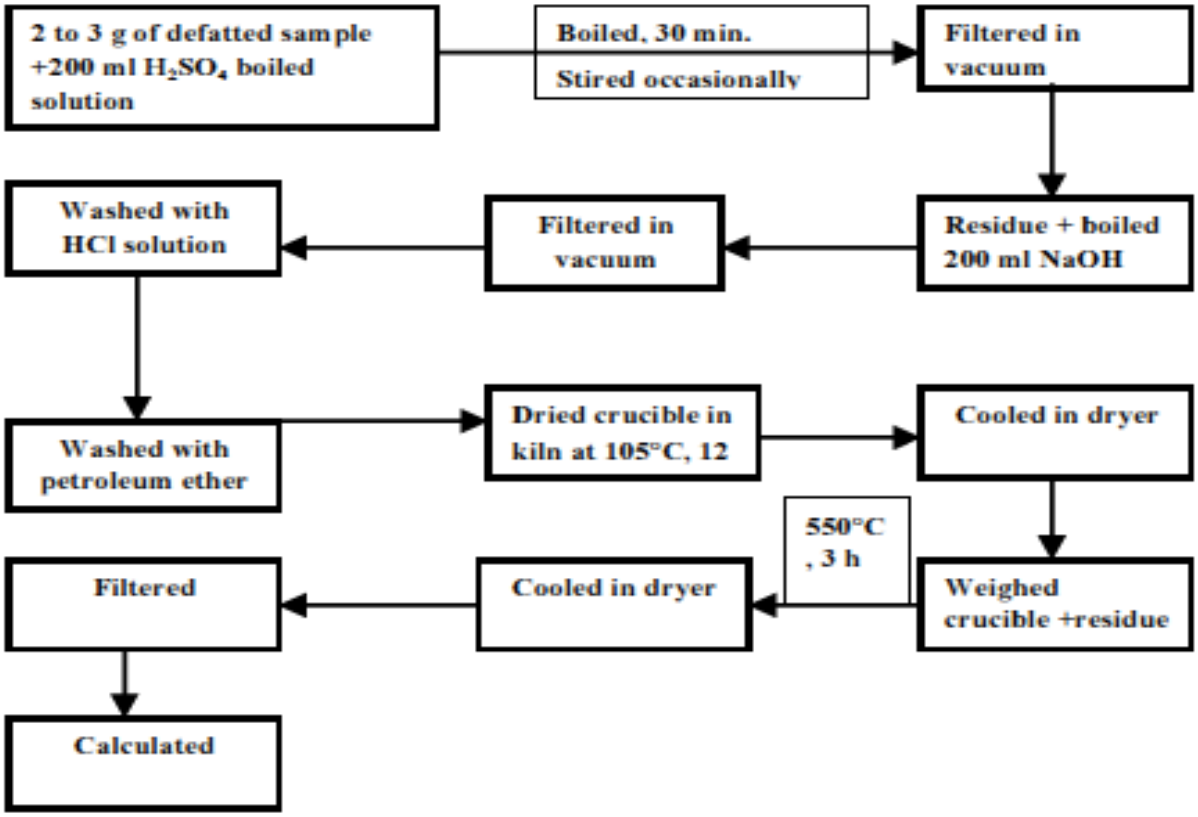


Figure 3.6: Determination of Crude Fiber Content. **Source:** Adapted and Modified from Sungsoo *et al.* (1999)

3.10.6 Carbohydrate Content Determination

This includes all the nutrients not assessed by the previous methods of proximate analysis. These are composed mainly of digestible carbohydrates, vitamins and other non-nitrogen soluble organic compounds. Since the result is obtained by subtracting the percentages calculated for each nutrient from 100, any error in evaluation will be reflected in the final calculation.

Calculations

Nitrogen-Free Extract (%) = **100** − [**A** + **B** + **C** + **D** + **E**] ..... *Equation (3.6).*

Where:

A−moisture content (%)

B−crude protein content (%)

C−crude fat content (%)

D−crude fibre content (%)

E−ash content (%)

3.11 Data Analysis and Results Presentation

Data collected was subjected to analysis of variance (ANOVA) using GenStat discovery edition 3 (VSN International Ltd). Statistically significant differences were reported at $p < 0.05$. If the overall F-test was significant ($p < 0.05$), then Fisher's Least Significant Difference

(LSD) test was used to compute the smallest significant difference between two means and alphabetical notations used as superscripts to mark the differences at significant levels. Pearson Product-Moment Correlation Analysis was also explored using SPSS version 16.0 (2007) to examine the relationship between aflatoxin contamination and proximate composition parameters at $p < 0.05$.

CHAPTER FOUR

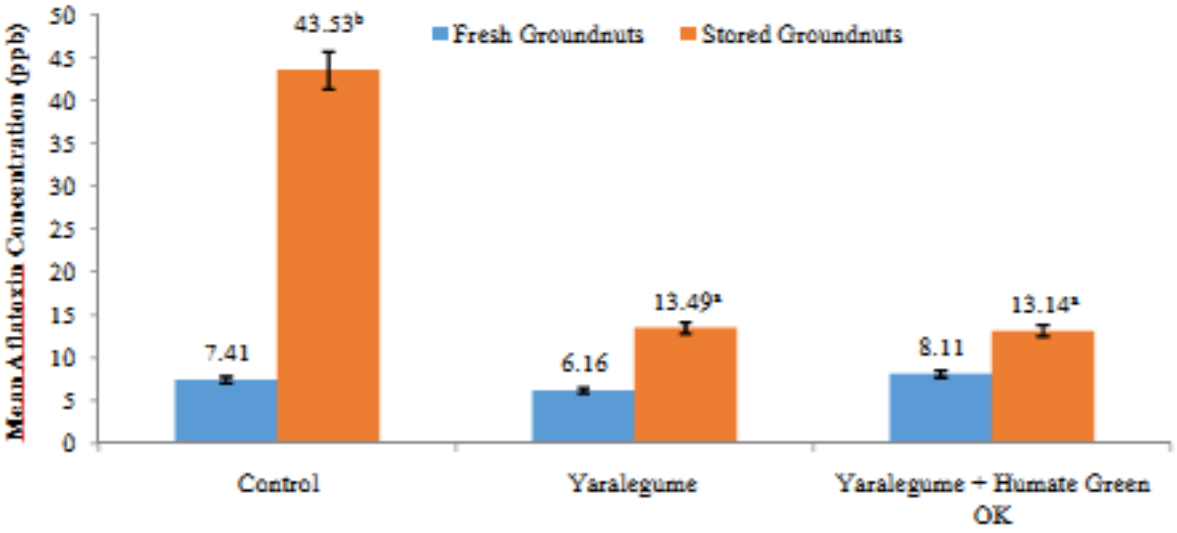
RESULTS AND DISCUSSION

4.1 Aflatoxin Concentration Levels in Fresh and Stored Groundnuts

Fertiliser treatments did not significantly (p = 0.354 and p = 0.233) affect Aflatoxin concentration levels in both fresh (3.59 -13.21 ppb) and stored (10.43 – 93.43 ppb) groundnuts. There however existed significant difference ($p < 0.05$) between no fertiliser treatment and fertiliser treatments in stored groundnuts (Figure 4.1). Aflatoxin concentration in all samples from all four (4) communities increased after storage. The highest (43.53 ppb) and lowest (6.16 ppb) mean aflatoxin concentration increase were observed under the control treatment (stored gorundnuts) and under Yaralegume treatment (fresh groundnuts) respectively. The increase was over 75 % pronounced in samples from the control plots with as high as 93.43 ppb and a low of 17.34 ppb depicting vulnerability to infection. This was also observed in the Yaralegume and Humate Green OK treated plots, making samples from Yaralegume treated plots reasonably safer and the Yaralegume fertiliser treatment more effective with storage. For fresh groundnuts, Yaralegume fertiliser treated plots produced samples that were less concentrated with aflatoxin in the range of 3.59 – 13.21 ppb. This treatment, in terms of protective effect is followed by the control treatment (5.44 – 7.84 ppb) with the combined effect of Yaralegume and Humate Green OK treated plots producing samples with concentration in the range of 5.46 to 13.21 ppb. Infection by *Aspergillus* species and subsequent aflatoxin contamination is influenced by many factors and can occur at any stage of groundnut production, from pre-harvest to storage (Campos *et al.*, 2008) and processing. According to Payne *et al.* (1986) seeds from plants receiving no nitrogen undergo nitrogen stress and produce more aflatoxin than those from plants receiving an optimum

nitrogen dose. The natural nitrogen-fixing capabilities of leguminous crops makes them less susceptible to aflatoxin infection. Jones, (1987) suggested that mineralization of nitrogen on highly organic soils, compared with sandy soils, tended to reduce aflatoxin contamination.

Ineffective management of post harvest factors such as storage moisture, temperature, aeration, insect activity and sanitation of storage facilities enhance mycotoxin problems in dried groundnuts (Campos *et al.,* 2008). Increased levels of aflatoxin contamination in postharvest groundnut samples have been reported (Kladpan et al., 2004; Kaaya et al., 2006). Storage time has also been discussed as a factor that would lead to increases in post-harvest aflatoxin contamination (Hell *et al.,* 2000). A previous study by Mutegi *et al.* (2009) also elucidated an increase in levels of aflatoxin over time in groundnuts after harvest. The longer the retention in storage the greater will be the possibility of building up environmental conditions conducive to groundnut fungi growth (Kaaya *et al.,* 2006). The length of time taken for aflatoxins to be detected in biologically significant quantities has been estimated to be about 5 months (Atehnkeng *et al.,* 2008). In their study, Atehnkeng *et al.* (2008) found that *Aspergillus* species was more frequent than other species of mycotoxin-producing fungi. This was due to the fact that *Aspergillus* species is more invasive than most other species and often dominant in groundnut seeds (Malaker *et al.,* 2008). *Aspergillus* species are able to grow and establish within a very short time on their substrate. In terms of competition, *Aspergillus* species is more competitive and its allelopathic relationship with other fungi is very strong. Fungal pathogens isolated from groundnuts have been reported to increase with increase in storage period (Bulaong and Dharmaputra, 2002). Saleemullah *et al.* (2006) reported faster growth of *Aspergillus* species with increase in humidity and prolonged storage of groundnuts for 12-18 months compared to short storage periods for 2-3 months.



Fresh Groundnuts (LSD = 2.798, P = 0.354) and Stored Groundnuts (LSD = 18.35, P = 0.233)

[a,b] Means that do not share a letter are significantly different at LSD ($p < 0.05$)

Figure 4.1: Effect of Fertiliser Treatments on Aflatoxin Concentration in Fresh and Stored Groundnuts

4.2 Effect of Fertiliser on Pre-harvest Aflatoxin Concentration in Groundnuts

Application of Yaralegume and Humate Green OK fertilisers had a reducing impact on the level of aflatoxin contamination as shown in Figure 4.1. The Yaralegume fertiliser treatment reduced aflatoxin concentration by 28.57 % in Hiinneteng community, 51.68 % in Konguoli community and 22.79 % in Korro community. On the other hand, the combined effect of Yaralegume and Humate Green OK could not reduce pre-harvest aflatoxin infection but rather increased it by 40.65 % in Hiinneteng community, 19.06 % in Konguoli community and 0.37 % in Korro community. Contrary to the above results, pre-harvest infection of groundnuts in the Samoa community reduced by 48.71 % in the Yaralegume and Humate Gren OK treated plot and increased by 20.55 % in the Yaralegume Only treated plot. In the absence of Humate Green OK, only Yaralegume fertiliser treatment, reduced pre-harvest infection in 3 out of the 4

communities in the range of 22.79 – 51.68 % whilst in combination (Yaralegume + Humate Green OK), prehavest infection increased in the range of 0.37 – 40.65 %. This could be attributed to the fact that when fertilisers (organic and inorganic) are combined and introduced to the soil, a biological process called mineralization takes place where organic substances are converted to plant available inorganic forms (Graham, 1983). This makes Yaralegume fertiliser effective compared to Yaralegume + Humate Green OK fertiliser. Mineral elements in mineral fertilisers like Yaralegume are directly involved in all mechanisms of a plant's defense to infection and disease as integral components of cells, substrates, enzymes, and electron carriers; or as activators, inhibitors, and regulators of metabolism. Resistance is generally a dynamic process involving the principles of metabolic regulation by substrate feedback, enzyme repression, and enzyme induction that are all controlled through mineral factors (Huber, 1980; Graham, 1983; Huber and Graham, 1999; Datnoff *et al.,* 2007). An adequate supply of nutrients is important in most of the defense mechanisms. Production of glycoproteins (lectins) associated with infection resistance also requires Mn (Graham, 1983). Calcium and Mg suppress tissue-macerating infections caused by bacteria and fungi by increasing the structural integrity of the middle lamella, cell wall components, and cell membranes to resist the extra-cellular enzymes produced by these pathogens. This result agrees partly with Florkowski and Kolavalli, (2013) who tested for aflatoxins in new and in-shell groundnuts in Ghana and found it in the range of 1.70 – 7.60 ppb. Pre-harvest infection is difficult to control without irrigation and pesticide application (Craufurd *et al*., 2006). The only form of input was the application of fertiliser. Stress due to drought during the ripening period of the seeds can lead to an aflatoxin infection (Augstburger *et al.,* 2002).The reductions in Hiinneteng and Konguoli communities were respectively greater than 28 % and 42 %

reduction achieved by Waliyar *et al.* (2007) when they used cereal crop residues and Farmyard Manure (FYM) respectively as treatments against pre-harvest aflatoxin infection.

4.3 Effect of Fertiliser and Storage on Post-harvest Aflatoxin Concentration in Groundnuts

Aflatoxin concentration increased significantly in all samples tested (Figure 4.1) despite the moisture content of all samples being less than 5 %. This was however more pronounced in the samples from the control plots (Figure 4.1). Post-harvest residual protective effect of fertilisers was observed in the samples from all the communities after analysis. In terms of overall total concentration of all communities, the combined treatment of Yaralegume and Humate Green OK was 2.65 % more protective of groundnuts in storage than the Yaralegume treatment. However, Yaralegume and Yaralegume + Humate Green OK fertilizer treatments were 69 % and 69.83 % more protective of groundnuts in storage against aflatoxin concentration increase than the control (No fertiliser) treatment respectively. Hence Yaralegume + Humate Green OK was 1 % less effective than Yaralegume in relation to the control (No fertiliser) effect. Hiinneteng community produced the most aflatoxin concentrated (93.33 ppb) sample and ranked first as the community with the highest overall infection (118.53 ppb). This could be attributed to water stress since the ground was fairly dry during harvesting. Korro and Samoa communities produced 10.43 ppb each as the least concentrated samples after storage. Korro however is the community with the overall least infected groundnuts after Samoa and Konguoli communities. Two groundnut samples from Hiinneteng and Konguoli communities had concentrations (93.43 and 52.92 ppb) above the maximum allowable limits of 20 and 50 ppb set by the Ghana Standards Authority as aflatoxin limits for consumption of groundnuts by humans and animals respectively.

The extent and severity of both invasion by *A. flavus* and the production of aflatoxin in the stored groundnuts could be influenced by several factors including moisture content and temperature of stored grain, condition of grain going into storage, storage bag type and length of storage. Moisture is the most important variable determining the rate of deterioration caused by fungi, with temperature being the second vital factor (Mutegi *et al.*, 2013). The impact of temperature is difficult to separate from the effect of moisture since under tropical conditions characteristic of Ghana, groundnuts and other products under storage are more vulnerable to infection by *Aspergillus* species than other mycotoxigenic fungal species (Pitt and Hocking, 1997). The most favourable temperature for aflatoxin production according to FAO (1998) is between 25ºC and 30 ºC, at 85 % relative humidity. Drying groundnuts to 9 % or less moisture content is an important strategy in reducing aflatoxin contamination during storage (Hill *et al.*, 1983). Malaker *et al.* (2008) found that the moisture content and black point incidence of seeds stored in different containers increased with the progress of storage and attributed the increase in moisture content and black point incidence of the stored seed to the activities of storage fungi. When groundnuts absorb moisture from the environment or when the environmental relative humidity exceeds the equilibrium relative humidity of the seeds, fungal growth occurs (Hayma, 2003). Increased levels of aflatoxin contamination in post-harvest groundnuts samples have been reported (Kladpan *et al.*, 2004; Kaaya *et al.*, 2006).

4.3.1 Effect of Storage Bags on Aflatoxin Level in Groundnut

The materials or bags used for storage plays the first role of protection against external environmental factors and insects in this part of the world under tropical conditions (Bulaong and Dharmaputra, 2002). The groundnut samples were stored in mini polypropylene bags (50 kg) for five months (November – March). The choice of bag was influenced by farmers practices and backed by previous works in other locations (Amoako-Atta *et al.*, 2011; Mutegi *et al.*, 2013). Bulaong and Dharmaputra (2002) reported that moisture content was significantly higher in groundnuts stored in jute than in polypropylene bags. The significantly higher ($p < 0.05$) moisture content of seeds stored in jute bags compared to polypropylene bags could be attributed to absorption of moisture from the environment. Groundnuts stored in polypropylene and polyethylene bags according to Mutegi *et al.* (2013) were 5.6 % and 13.4 % more contaminated with total aflatoxin than samples stored in jute bags, respectively. This could be attributed to retention of heat and moisture build up in the two bag types which promoted fungal growth and aflatoxin contamination compared to jute bags. The problem increases when groundnuts are stored in a facility where there is poor air circulation in the immediate environment (Mutegi *et al.*, 2013). On the other hand, high moisture content of seeds stored in polypropylene bags could result from lack of aeration within the bags. Amoako-Atta *et al.* (2011) observed that seeds stored in jute bags were predisposed to fungal activities as opposed to those stored in impermeable polypropylene bags.

4.3.2 Impact of Environmental Storage Temperature and Relative Humudity on Aflatoxin Levels in Groundnuts

Relative humidity ranged from a low of 42 % in the month of January to the highest of 56 % in November (Table 4.1). The highest average maximum temperature recorded for the district was 37 ^{o}C in February and March while the least minimum temperature was 20 ^{o}C in December and January. Mean maximum monthly temperature during storage hardly exceeded 37 ^{o}C with mean minimum temperature as low as 20 ^{o}C. Storage temperature was however higher (20- 37 ^{o}C) than the FAO stated favourable temperature range of 25 - 30 ^{o}C for aflatoxin production, hence making the groundnuts more vulnerable to *Aspergillus flavus* invasion and aflatoxin contamination. It can therefore be asserted that in-storage aflatoxin production in the seeds occured effectively in the last two (February and March) months of storage since contamination increases with increase in storage temperature as affected by the environmental temperature and humudity. According to Mutegi *et al.* (2013), storage of kernels at relatively low temperature and high relative humidity had the greatest effect on groundnut quality. According to Christensen *et al.* (1977), groundnuts stored at 19 °C and 64 % relative humudity retained the highest moisture content and had the greatest proportion of physical damage while samples stored at 24 °C and 56 % relative humidity had the lowest moisture content, physical damage and rancidity. Fungal growth in storage facilities was also favoured by high relative humidity of 83 – 85 % and above.

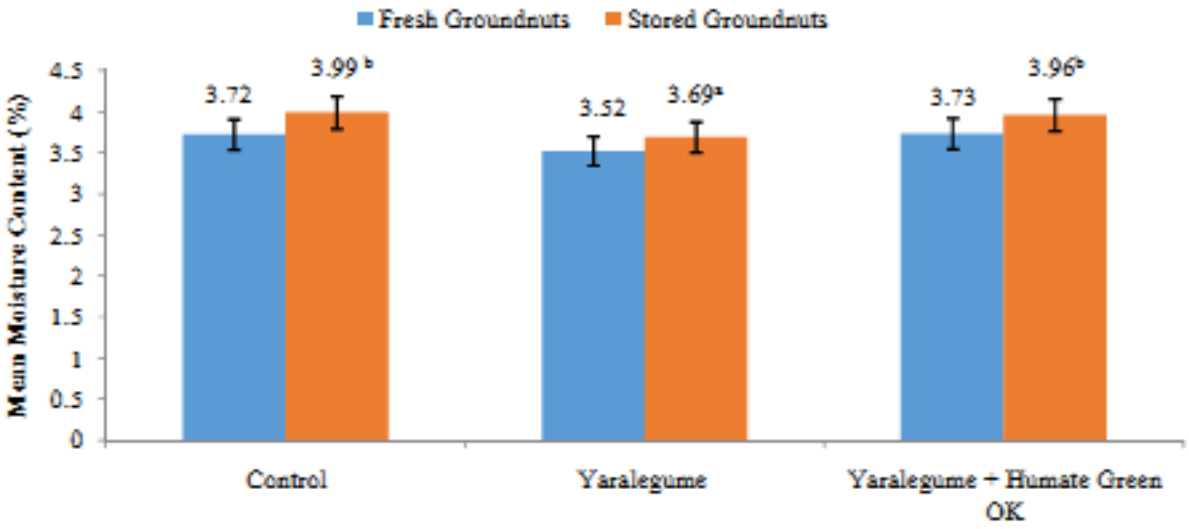
Table 4.1: Mean Monthly Temperature and Relative Humudity during Storage

Month/Year	Temperature ^{o}C		Relative Humudity
	Max	Min.	(%)
November, 2014	32.0	22.0	56.0
December, 2014	33.0	20.0	46.0
January, 2015	35.0	20.0	42.0
February, 2015	37.0	23.0	45.0
March, 2015	37.0	25.0	50.0

4.4 Effect of Fertiliser and Storage on Moisture Content in Groundnuts

Fertiliser application did not impact significantly ($p > 0.05$) on the moisture content of fresh groundnuts eventhough significant ($p < 0.05$) increase in moisture content of groundnuts was observed with storage (Figure 4.2). Significant difference ($p < 0.05$) existed between Yaralegume fertiliser treatment and the Yaralegume + Humate Green OK fertiliser treatment as well as the control in stored groundnuts. The control treatment produced groundnut with the highest mean moisture content (3.99 %) under storage and the lowest moisture content (3.52 %) observed in fresh groundnuts under Yaralegume treatment. Moisture content in fresh groundnuts increased from 3.72, 3.52 and 3.73 % to 3.99, 3.69 and 3.96 % after storage under control, Yaralegume and Yaralegume + Humate Green OK treatments. There was a general increase in moisture content after storage. Moisture content was in the range of 3.15 to 4.16 % in fresh groundnuts and 3.46 to 4.14 % in stored groundnuts. After storage, moisture content increased by 7.26 % , 4.83 % and 6.17 % under control, Yaralegume and Yaralrgume + Humate Green OK fertiliser treatments respectively. This makes samples from the control plots more susceptible to *Aspergillus* infection in storage and samples from Yaralegume

fertiliser treated plots less susceptible. As a determinant of quality and storability, moisture content plays a critical role in the evaluation of the legume as food (Kaaya *et al.*, 2006). El Tinay *et al.* (1989) stated that moisture content of groundnut seeds was not significantly affected by biological, inorganic or organic fertilisers but rather by relative humidity of the surrounding atmosphere at the time of harvest and during storage. With several days of sun drying, moisture content in both fresh and stored groundnuts was brought below 5 % hence lower than the 7 - 7.5 % indicated by Davidson *et al.* (1982); 6 - 8 % (Kaaya *et al.*, 2006) and 9 % (Hill *et al.*, 1983; MacRobert, 2009) as optimum levels for safe storage. However this moisture content agrees with 3.3 to 6.9 % by Mutegi *et al.* (2013), 3.40 % (Ayoola *et.al.*, 2012) and 4.11 % (Kumar *et al.*, 2013) for raw groundnut samples. At this moisture content (<5 %), both the fresh and stored groundnuts are below the minimum moisture content (7 %) for milling quality (McIntosh and Davidson, 1971). This could also be as a result of possible vigourous fungi activities (Malaker *et al.* 2008). The observed increase in moisture after storage could be due to the cause of possible fungi activities as explained by Ladele and Njoku (1984) as a likely result of metabolic or oxidation water and/or moisture absorbed from the environment during storage. Seed moisture content was strongly (r = 0.96) correlated with the relative humidity of the storage environment (Awuah and Ellis, 2001). Metabolism within the seeds is capable of producing about 110 g of water per 100 g of fat, 41.3 g of water per 100 g of protein and 55 g of water per 100 g of carbohydrates (Ladele and Njoku, 1984). All these energy-producing nutrients have been found in their right proportions, fat (40-55 %); protein (22-30 %) and carbohydrates (10-21 %) in the groundnu



Fresh Groundnuts (LSD = 0.479, p = 0.589) and Stored Groundnuts (LSD = 0.196, p = 0.007)

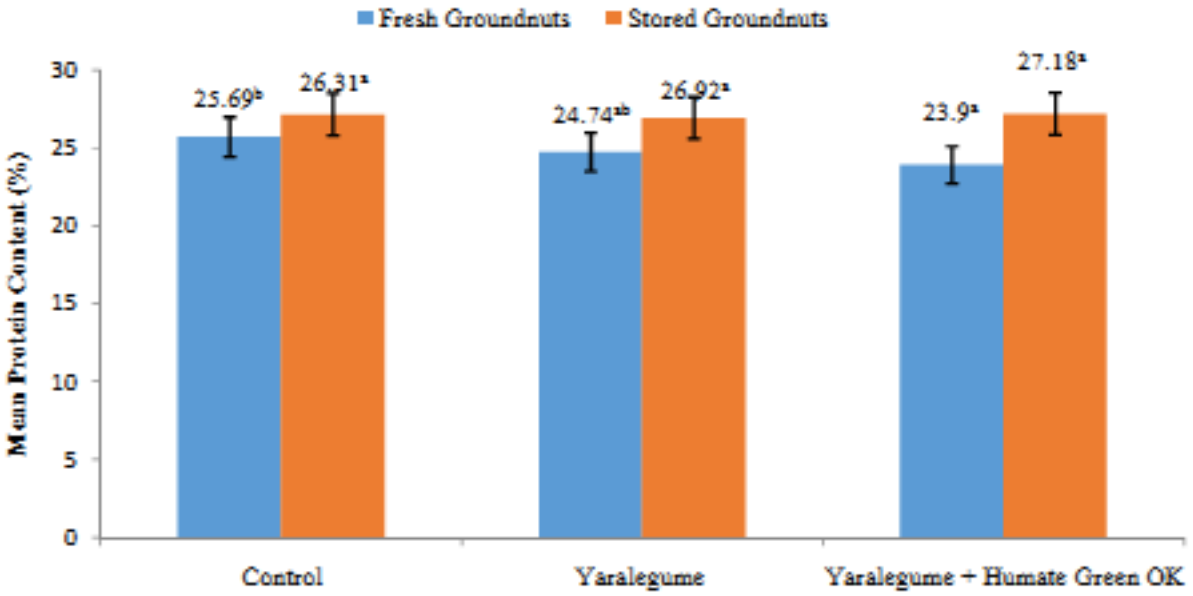
[a,b] Means that do not share a letter are significantly different at LSD ($p < 0.05$).

Figure 4.2: Effect of Fertiliser Treatments on Moisture content of Fresh and Stored Groundnuts

4.5 Effect of Fertiliser and Storage on Crude Protein Content in Groundnuts

The effect of fertiliser application was highly significant (p = 0.006) in fresh groundnuts and insignificant ($p > 0.05$) in stored groundnuts (p = 0.529) (Figure 4.3). The control treatment was significantly ($p < 0.05$) different from the fertiliser treatments in fresh groundnuts with all three treatments not significantly ($p > 0.05$) different in stored groundnuts. The lowest (23.90 %) and highest (27.18 %) mean protein contents were observed under Yaralegume + Humate Green OK treatment in fresh and stored groundnuts respectively. There was a general increase in mean protein content after storage. Proteins are easily absorbed by humans at the rate of 65– 80 % and appear to be the main 'building material' for cells of the human body (Badau *et al.*, 2013). Seeds of oil-bearing legumes are a good source of crude proteins and can accumulate 35–40 % of these compounds (Tarek *et al.*, 2001). This further explained the optimal protein

content of fresh groundnuts (22-30 %) which agreed perfectly with 22 to 30 % obtained by Savage and Keenan (1994); 29.12 % crude protein obtained by Kavitha and Parimalavalli (2014); 25.0 % (Badau *et al.*, 2013); 21.80 % (Ayoola and Adeyeye, 2010); and 24.40 % (Wakshama *et al.*, 2010). The presence of phosphate in Yaralegume fertiliser could have influenced the protein content since Deshmukh *et al.* (1993) found that the application of Phosphorus fertiliser to groundnut increased protein content. Gobarah *et al.* (2006) also reported that increasing rate of phosphorus from 30 to 60 kg P_2O_5/ha significantly increased vegetative growth, yield and its components as well as seed quality and protein content. Protein content increased by 2.41 % under control treatment, 8.81 % under Yaralegume treatment and 13.72 % under Yaralegume + Humate Green OK treatment after storage. This depicts the effectiveness of the fertilisers used. According to Rahman (2006) there is an increasing trend in qualitative characteristic like protein content of groundnut with the increase in the level of calcium from 0-100 kg/ha. Asibuo *et al.* (2008) observed in their studies on nutritional quality of groundnuts from Ghana that the mean protein content of subspecies *fastigiata* was higher (25.69 %) than sub species *hypogaea* (22.78 %). This confirms and denotes improvements in protein content as a result of the treatments and storage aside the variety.



Fresh Groundnuts (LSD = 1.042, p = 0.006) and Stored Groundnuts (LSD = 1.608, p = 0.529)

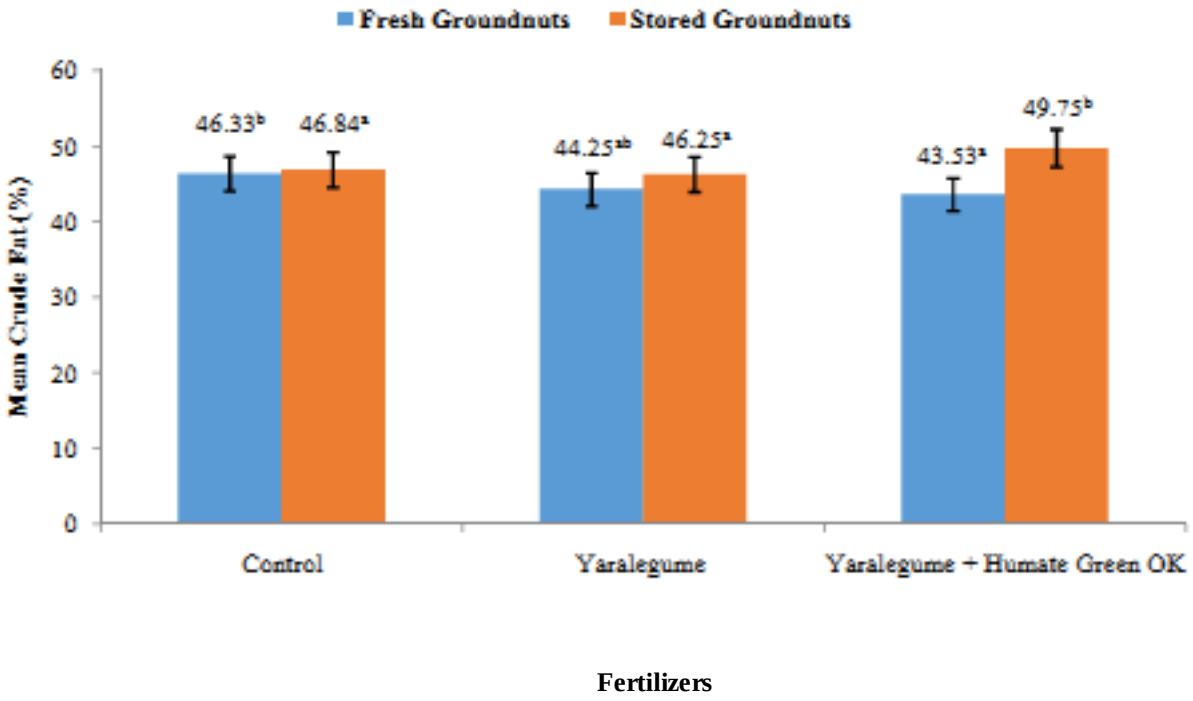
a,b Means that do not share a letter are significantly different at LSD ($p < 0.05$).

Figure 4.3: Effect of Fertiliser Treatments on Crude Protein Content of Fresh and Stored Groundnuts

4.6 Effect of Fertiliser and Storage on Crude Fat Content in Groundnuts

Fertiliser application significantly ($p < 0.05$) affected crude fat content in both fresh (p = 0.034) and stored (p = 0.001) groundnuts (Figure 4.4). Yaralegume + Humate Green OK fertiliser was significantly ($p < 0.05$) different from Yaralegume fertiliser and control treatments in stored groundnuts. The highest mean fat content (49.75 %) was observed in stored groundnuts and lowest fat content (43.53 %) observed in fresh groundnuts both under Yaralegume +Humate Green OK treatment. There was a marginal increase in fat content after storage under all treatments. There was a 1.1, 4.52 and 14.29 % increase in fat content under control (no fertiliser), Yaralegume and Yaralegume + Humate Green OK treatments respectively after storage. Over 65 % of the samples increased in fat content after storage,

indicating the possible residual effect of the fertilisers eventhough some samples from the control plots also appreciated in fat content. This could possibly be attributed to previous fertility status of the plots. Individual fat content for both fresh and stored samples ranged from 39.98- 47.78 % and 44.67- 55.33 % respectively. Plots without fertiliser treatments produced fresh groundnuts with higher fat content in 3 out of the 4 communities (75 %) and 2 out of the 4 communities (50 %) after storage indicating minimal impact of fertilisers if groundnuts are purposely for fat or oil production. It was evident that the fertilisers used had a 25 % and 50 % reducing effect on the fat content of fresh and stored groundnuts respectively. The fat content of samples agree with reports by Asibuo *et al.* (2008) and Savage and Keenan (1994) that fat content of groundnut ranged from 33.60 – 54.95 % and 42 to 52 % respectively. Bhatol *et al.* (1994) found that nitrogen fertilisers decreased the crude fat of groundnuts while phosphorus fertilisers increased it. Ranjit *et al*. (2007) reported that the oil content of groundnut differed significantly with the application of different levels of lime. Elshiekh and Mohamedzein (1998) showed that mycorrhizal inoculation and/or superphosphate significantly increased both oil and protein content of groundnut seeds.



Fresh Groundnuts (LSD = 2.163, p = 0.034) and Stored Groundnuts (LSD = 1.781, p = 0.001)

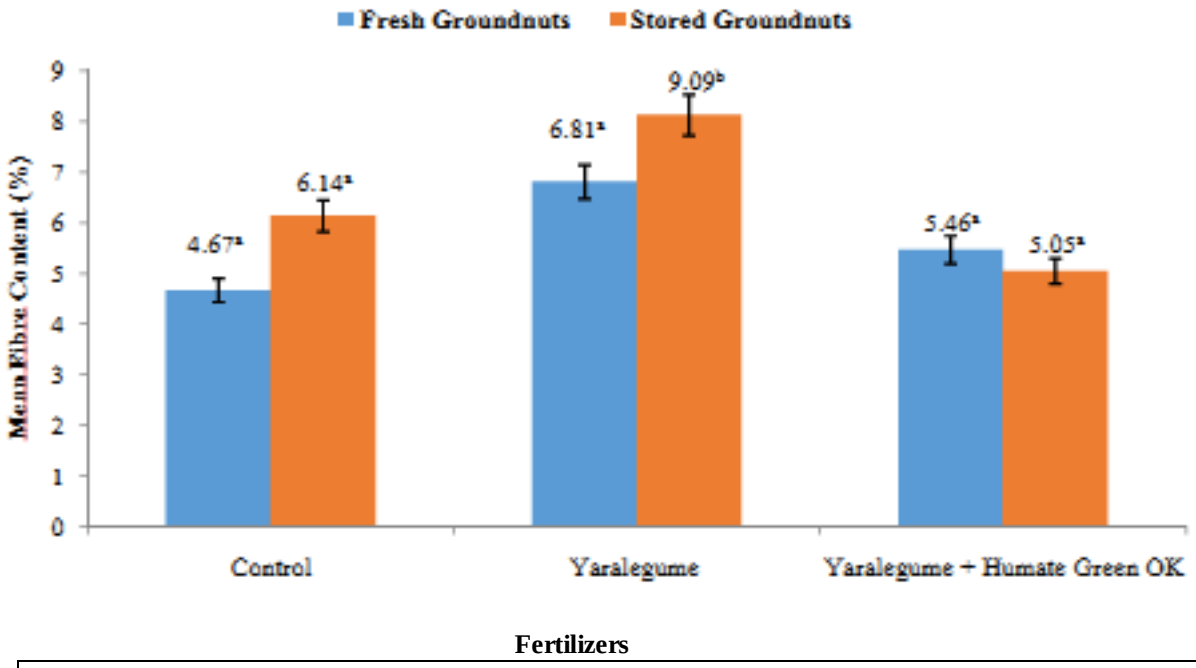
[a,b] Means that do not share a letter are significantly different at LSD ($p < 0.05$)

Figure 4.4: Effect of Fertiliser Treatments on Crude Fat Content of Fresh and Stored Groundnuts

4.7 Effect of Fertiliser and Storage on Crude Fibre Content in Groundnuts

In groundnut samples, the amount of crude fibre achieved varied from 3 to 11 %. There existed a near perfect run of significance ($p < 0.05$) for fresh ($p = 0.056$) and stored ($p = 0.001$) samples (Figure 4.5). There existed no significant difference among treatments in fresh groundnuts. Yaralegume fertiliser treatment (9.09 %) was however different significantly ($p < 0.05$) from the Control (6.14 %) and Yaralegume + Humate Green OK (5.05 %) treatments. Fibre content increased by 31.48 % under control treatment, 33.48 % under Yaralegume treatment and decreased by 8.12 % under Yaralegume + Humate Green OK treatment after storage. This might be attributed to uncontrolled release of nutrients in the soil through mineralisation of Humate Green OK fertiliser which might have facilitated poor crop growth.

The plots treated with only Yaralegume fertiliser, produced groundnuts with the highest fibre content both after harvest (10.24 %) and after storage (11.08 %) indicating effective release of nutrients during crop growth. The control plots produced groundnuts with fibre in the range of 3.01-7.00 % and 5.04- 9.12 % for fresh and stored samples respectively. This result is an improvement to that of 2.70 % (Kavitha and Parimalavalli, 2014); 3.17 % (Abdualrahman, 2013); 2.60 % (Badau *et al.,* 2013); and 2.43 % (Ayoola and Adeyeye, 2010). The high content of fibre mentioned above for both categories of samples is an indication of the importance of the groundnuts as food, since fibre-rich foods can be eaten directly or as supplements in processed foods. Not yet formally proposed as an essential macro-nutrient, dietary fibre is nevertheless regarded as important for the human body, with regulatory authorities in many developed countries recommending increases in fibre intake (Eastwood and Kritchevsky, 2005). According to Tarek *et al.* (2001), fibre, the indigestible component of carbohydrates stimulates the activity of bowels, combines heavy metals, cholesterol and bilious acids. It removes and cleanses the human body of these and other harmful materials, reducing the risk of arteriosclerosis, obesity and cancer. Tarek *et al.* (2001) indicated that, about 13–14 % of crude fibre is found in oil seeds.



Fresh Groundnuts (LSD = 1.751, p = 0.056) and Stored Groundnuts (LSD = 1.384, p = 0.001)

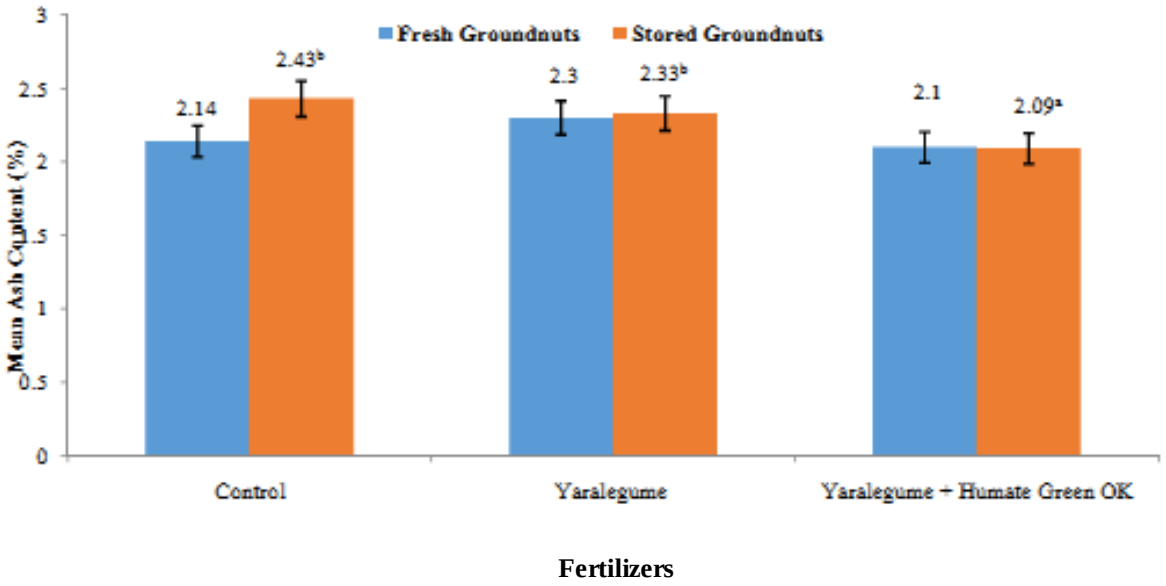
[a,b] Means that do not share a letter are significantly different at LSD ($p < 0.05$)

Figure 4.5: Effect of Fertiliser Treatments on Crude Fibre Content of Fresh and Stored Groundnuts

4.8 Effect of Fertiliser and Storage on Ash Content in Groundnuts

There was a significant (p = 0.013) and an insignificant (p = 0.493) response to fertiliser treatments in stored and fresh groundnuts respectively with regards to ash content. Yaralegume + Humate Green OK fertiliser treatment was significantly different from the Control and Yaralegume fertiliser treatment in stored groundnuts. The lowest mean ash content (2.10 %) was recorded in fresh groundnuts and the highest (2.43 %) recorded under stored groundnuts. This points to the fact that ash content increases with time as can be observed under all treatments. It was observed that fertiliser treatments restricted ash content increase in stored groundnuts as same could not be attributed to the control which resulted in ash content increase from 2.14 % to 2.43 % after storage. The study revealed that 58.33 % of the samples

increased in ash content in the range of 2.08- 2.59 % after 150 days of storage. Before storage, all samples which had ash content below 2 % ended up with ash content above 2 % but less than 3 %. This agrees with an ash content of 2.45- 2.78 % reported by Kavitha and Parimalavalli (2014); Badau *et al.* (2013) disagrees with 3.17 % and 3.50 % reported by Abdualrahman (2013) and Wakshama *et al.* (2010) respectively. Mineral constituents of groundnut ash include potassium, calcium, sodium and magnesium which are in large amount and alluminiun, iron, copper and manganese. Zinc, arsenic, iodine, fluorine and other elements are present in traces. Ash represent the total mineral content in foods. Although minerals represent less than 5 % of total composition, they play a physio-chemical and nutritional role in foods. Foods with low ash content are useful in controlling urinary track infections. On the other hand, food with high ash content causes the formation of crystals in the urinary track including the kidneys and bladder especially in already infected folks and animals.



Fresh Groundnuts (LSD = 0.352, p = 0.493) and Stored Groundnuts (LSD = 0.223, p = 0.013)

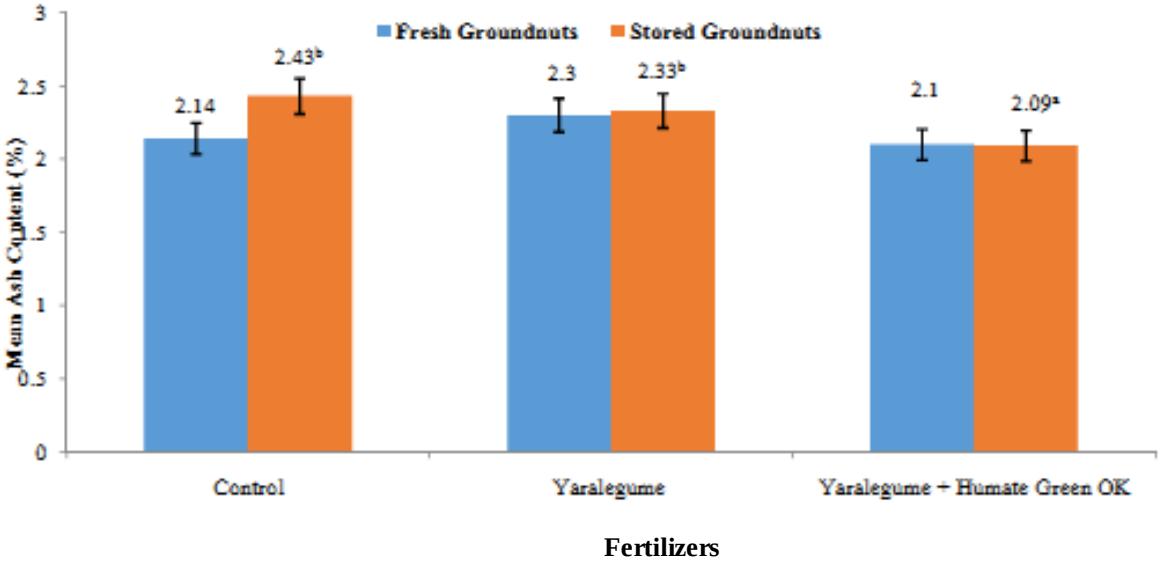
a,b Means that do not share a letter are significantly different at LSD ($p < 0.05$)

Figure 4.6: Effect of Fertiliser Treatments on Ash Content of Fresh and Stored Groundnuts

4.9 Effect of Fertiliser and Storage on Carbohydrate Content in Groundnuts

Treatment effect was significant (p = 0.029) in fresh groundnuts and not significant (p = 0.428) in stored groundnuts with respect to carbohydrate content. There was no significant ($p > 0.05$) treatment difference among treatments in stored groundnuts whilst there existed significant ($p < 0.05$) difference between Yaralegume + Humate Green Ok fertiliser treatment and the control treatment in fresh groundnuts. The control treatment produced fresh groundnuts with the lowest mean carbohydrate content of 17.50 % with 20.81 % as the highest from Yaralegume + Humate Green OK treated plots. After storage, the highest reductions in mean carbohydrate content were observed in samples from fertiliser treated plots. Carbohydrate content reduced by 31.58 % under control treatment, 61.19 % under Yaralegume treatment and 74.58 % under Yaralegume + Humate Green OK treatment after storage. The highest (28.61 %) carbohydrate content of fresh groundnuts harvested from Yaralegume +

Humate Green OK fertiliser treated plot after storage resulted in the lowest (3.36 %) carbohydrate containing sample after storage. This could be attributed to the high crude protein (27.93 %) and high crude fat (55.33 %) levels of the sample. Carbohydrate content was agreeably high in the range of 13.79 to 28.61 % in fresh samples and adversely low in the range of 3.36 to 18.43 % in stored samples. It was evident that Yaralegume + Humate Green OK produced fresh groundnuts with significantly high ($p < 0.05$) carbohydrate content whilst fresh groundnuts from control plots produced comparably low levels of carbohydrate. The reduction in carbohydrate content in over 90 % of the samples after storage could be attributed to the increased activities of *Aspergillus* sp. It is expected that the fungi present will use the carbohydrates as a source of energy for survival, establishment and multiplication (Tanuja *et al.*, 2012). During fungal infestation the carbohydrate content of groundnuts decreased due to the utilization of carbohydrates by the fungi through amylolytic activities (Somani and Pandrangi, 1992).



Fresh Groundnuts (LSD=2.419, p = 0.029) and Stored Groundnuts (LSD=2.771, p = 0.428)

[a,b] Means that do not share a letter are significantly different at LSD ($p < 0.05$)

Figure 4.7: Effect of Fertiliser Treatments on Carbohydrate Content of Fresh and Stored Groundnuts

4.10 Relationship between Aflatoxin Concentration and Moisture Content in Groundnuts

The strength of association between aflatoxin concentration and moisture content were established in the fresh and stored groundnuts. There was no significant ($p > 0.05$) correlation between aflatoxin concentration and moisture content for both fresh and stored groundnuts. The correlation coefficient between aflatoxin concentration and moisture content in fresh groundnuts was negative (r = -0.318) with a coefficient of determination, r^2 = 0.101 whilst that for stored groundnuts was positive (r = 0.113) with a coefficient of determinantion, r^2 = 0.013. The two parameters moderately correlated negatively and slightly correlated positively for fresh and stored groundnuts respectively. There was an inverse relationship between aflatoxin concentration and moisture content for fresh groundnuts whilst the relationship

between these two (2) variables was direct for stored groundnuts. Hence for fresh groundnuts, aflatoxin concentration increased with a decrease in moisture content whilst for stored groundnuts, aflatoxin concentration increased with an increase in moisture content. The relationship between aflatoxin concentration and moisture content for both fresh and stored groundnuts was not statistically significant at 95 % confidence level with levels of significance as 0.130 and 0.599 respectively. It can therefore be noted that 10.1 % variance was shared between aflatoxin concentration and moisture content in fresh groundnuts whilst 1.3 % variance was shared between aflatoxin concentration and moisture content in stored groundnuts. This means that, only 10.1 % and 1.3 % of the total variation or difference in moisture content can be accounted for by variation in aflatoxin concentration levels respectively for fresh and stored groundnuts. Equally, 89.9 % and 98.7 % variance was not shared between aflatoxin concentration and moisture content of both fresh and stored groundnuts respectively. These results agreed with Urvashi *et al.* (2013) who had a positive correlation between moisture and aflatoxin levels in groundnuts. Wagacha *et al.* (2013), also found a moderately positive correlation between moisture content and total aflatoxin in stored groundnuts.

4.11 Relationship between Aflatoxin Concentration and Crude Protein Content in Groundnuts

Both parameters (Aflatoxin Concentration and Protein Content) correlated negatively and were not statistically significant ($p > 0.05$). A moderately weak correlation strength existed for both fresh and stored groundnuts. The relationship between aflatoxin concentration and protein content for fresh groundnuts resulted in a correlation coefficient (r) of -0.352 ($r^2 = 0.124$) and a variance percentage of 12.4 %. As observed in the stored groundnuts, there existed a weak

negative correlation with a coefficient (r) of -0.217 (r^2 = 0.047) and a variance percentage of 4.7 %. Eventhough both groundnut groups had the two measured parameters correlating negatively and weakly, 12.4 % and 4.7 % of variance was shared between these variables or parameters for fresh and stored groundnuts respectively. This explains that, only 12.4 % and 4.7 % of the total variation in protein content of the groundnuts, can be attributed to variation in aflatoxin concentration levels in the fresh and stored groundnuts respectively. The levels of significance between the two parameters for both fresh and stored groundnuts are 0.092 and 0.308 respectively. This means that the relationship between aflatoxin concentration and protein content for both fresh and stored groundnuts was not significant at 95 % confidence level. Conversely, 87.6 % and 95.3 % variance was not shared between aflatoxin concentration and protein content in fresh and stored groundnuts respectively. The two variables are inversely related, hence with increasing aflatoxin concentration levels, protein content decreased. This study reported similar results as Urvashi *et al.* (2013), who found a negative correlation between aflatoxin concentration and protein content of groundnuts marketed locally. Contrary to the results of this study, some works showed an increase in the protein content of groundnuts during infestation by *Aspergillus parasiticus* (Mehan *et al.*, 1991) which could be attributed to the production of non-protein nitrogen by hydrolytic enzymes. Tanuja *et al.* (2012) found an increase in crude protein content with fungal infection.

4.12 Relationship between Aflatoxin Concentration and Crude Fat Content in Groundnuts

The correlation between aflatoxin concentration and fat content of fresh groundnuts was not statistically significant ($p > 0.05$). It slightly correlated positively with correlation coefficient

(r), coefficient of determination (r^2) and variance of 0.029, 0.001 and 0.1 % respectively. This could be described as a state of no correlation or relationship. There was a direct relationship between aflatoxin concentration and fat content of the fresh groundnuts, hence aflatoxin concentration increased with increase in fat content. However, the strength of association between these two parameters depicted the fact that, 0.1 % variance was shared between them. This explained that, only 0.1 % of the total variation in fat content can be accounted for by variation in aflatoxin concentration level. The fungi probably depended on the fat component of the groundnuts as a source of energy for multiplication and growth. This further explained the fact that as more fat is made available, the fungi population increased. It was however established that 99.9 % variance is not shared between these two parameters or variables. As established, the level of significance (0.894) between aflatoxin concentration and fat content of the fresh groundnuts was not statistically significant at 95 % confidence level.

For groundnuts that were stored before analysis, correlation between aflatoxin concentration and fat content was positive and negligible in the range of 0.01 to 0.19. The correlation coefficient (r), coefficient of determination (r^2) and variance were 0.106, 0.011 and 1.1 % respectively. There was a direct relationship between aflatoxin concentration and fat content of the stored groundnuts, hence aflatoxin contamination increased with increase in fat content. Just as for the fresh samples, fat provided a source of energy for the survival and multiplication of *Aspergillus* sp. For stored groundnuts, a 1.1 % variance was shared between aflatoxin concentration and fat content. This only explains the fact that, not more than 1.1 % of the total variation in fat content can be accounted for by variation in aflatoxin concentration level in the stored groundnuts. On the other hand, it can be observed that 98.9 % variance was not shared between aflatoxin concentration and fat content. The level of significance

(0.622) between aflatoxin concentration and fat content for stored groundnuts was not significant at 95 % confidence level. The results disagreed with Tanuja *et al.* (2012) who indicated a significantly negative correlation (r = -0.940) between aflatoxin concentration and fat content of groundnuts in India. Earlier studies on alterations in the fat content in stored groundnut seeds infected with *Aspergillus* species have also shown similar changes (Vaidya and Dharam, 1989).

4.13 Relationship between Aflatoxin Concentration and Crude Fibre Content in Groundnuts

The correlation between aflatoxin concentration and fibre content for fresh and stored groundnuts was moderate, statistically insignificant ($p > 0.05$) and negative in the range of - 0.30 to -0.39. Correlation coefficient (r), coefficient of determination (r^2) and variance were - 0.360, 0.130 and 13.0 % for fresh groundnuts and -0.306, 0.094 and 9.4 % for stored groundnuts respectively. These results were indicative of the fact that aflatoxin concentration and crude fibre content are inversely related for both categories of groundnuts. Increase in aflatoxin concentration resulted in a decrease in fibre content, hence 13.0 % and 9.4 % variance was shared between these variables. It can therefore be concluded that, 13.0 % and 9.4 % of total variation in crude fibre content can be attributed to variation in aflatoxin concentration levels correspondingly in fresh and stored groundnuts respectively. The fungi probably depended on the fibre as food for growth and multiplication. However, 87.0 % and 90.6 % variance was not shared between aflatoxin concentration and fibre content in fresh and stored groundnuts respectively. The levels of significance between the two parameters for both fresh and stored groundnuts are 0.084 and 0.145 respectively. This means that the relationship between aflatoxin concentration and fibre content for both fresh and stored groundnuts was not

significant at 95 % confidence level. Zubair *et al.* (2011) in their study on walnuts found a positive correlation between total aflatoxins and crude fibre content.

4.14 Relationship between Aflatoxin Concentration and Ash Content in Groundnuts

The two parameters measured were negative and significantly not correlated ($p > 0.05$). The correlation strengths between the parameters for both fresh and stored groundnuts were negligible or near zero (-0.01 to -0.19). The findings revealed a correlation coefficient (r) of - 0.187 and -0.049, a coefficient of determination (r^2) of 0.035 and 0.002 and a variance percentage of 3.5 % and 0.2 % respectively for fresh and stored groundnuts respectively. There existed an inverse relationship between aflatoxin concentration and ash content for both fresh and stored groundnuts. Increase in aflatoxin concentration resulted in a decrease in ash content. This indicated that, 3.5 % and 0.2 % variance was shared between aflatoxin concentration and ash content respectively for fresh and stored groundnuts. In addition, only 3.6 % and 0.3 % of total variation in ash content can be explained or accounted for by variation in aflatoxin concentration levels. Equally, 96.5 % and 99.8 % variance was not shared between aflatoxin concentration and ash content respectively for fresh and stored groundnuts. The level of significance between the two variables for both fresh and stored groundnuts are 0.383 and 0.819 respectively. This showed that the relationship between aflatoxin concentration and fibre content for both fresh and stored groundnuts was not significant at 95 % confidence level. Ash content was found to be positively correlated to aflatoxin in a study on walnuts (Zubair *et al*., 2011).

4.15 Relationship between Aflatoxin Concentration and Carbohydrate Content in Groundnuts

The Correlation coefficient for fresh groundnuts (r = 0.40), was twice higher than the positively correlated coefficient (r = 0.198) for stored groundnuts. The coefficient of determination, r^2 = 0.160 and r^2 = 0.039 resulted in a variance of 16 % and 3.9 % respectively for fresh and stored groundnuts. A direct relationship existed between aflatoxin concentration and carbohydrate content, with aflatoxin concentration increase resulting in an increase in carbohydrate content in both fresh and stored groundnuts. This meant that, 16 % variance was shared between aflatoxin concentration and carbohydrate content of fresh groundnuts whilst 3.9 % variance or difference was shared between aflatoxin concentration and carbohydrate content of stored groundnuts. This shows that, only 16 % and 3.9 % of total variation in carbohydrate content respectively for fresh and stored groundnuts, can be accounted for by variation in aflatoxin concentration levels. It can therefore be concluded that, 84 % and 96.1 % variance was not shared between aflatoxin concentration and carbohydrate content in fresh and stored groundnuts respectively. Energy containing nutrients like crude protein and fat may be partly responsible. There was no statistically significant correlation (p > 0.05) between aflatoxin concentration and carbohydrate content of the groundnuts. The levels of significance between the two (2) variables for both fresh and stored groundnuts were 0.055 and 0.353 respectively. This confirmed that the relationship between aflatoxin concentration and carbohydrate content for both fresh and stored groundnuts was not significant at 95 % confidence level.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The impact of organic and inorganic fertilisers on the proximate composition and aflatoxin contamination levels of fresh and stored groundnuts was investigated in four (4) communities. Both fresh and stored groundnuts yielded in optimal levels, moisture, crude protein, crude fat, crude fibre, ash content and carbohydrate. Detectable levels of aflatoxin contamination were revealed in both fresh and stored samples in all communities. Fertiliser treatments did significantly affect proximate composition parameters but minimal increased levels were observed in proximate composition parameters except carbohydrate content that decreased after storage. There existed significant difference in pre-harvest and post-harvest aflatoxin concentration levels as affected by fertiliser treatments. There was a significant increase in concentration with storage. The Yaralegume Only treatment as a soil nutrient proved efficient in reducing pre-harvest aflatoxin contamination and providing effective residual protection to groundnuts in storage. The combined effect of Yaralegume and Humate Green OK performed poorly compared to the control of no fertiliser application in reducing pre-harvest aflatoxin concentrations with the former producing groundnut samples with higher total aflatoxin concentration. However, Yaralegume + Humate Green OK treatment had a slightly effective residual post-harvest capacity in suppressing aflatoxin increase in groundnuts than the Yaralegume only treatment with the control (no fertiliser) treatment being the least protective.

From the results of the study, application of any of these two fertilisers might not be necessary if it is mainly applied for the purpose of advancing the proximate composition aspect of

nutritional quality but rather storing groundnuts for at least five (5) months could serve this purpose. On the other hand, for the purpose of reducing pre-harvest and post-harvest aflatoxin contamination or concentration levels, applying only Yaralegume fertiliser at full rate may achieve this purpose without affecting proximate composition significantly. Eventhough there might be natural increase in aflatoxin concentration and proximate composition parameters of stored groundnuts over time, the two fertilisers used proved generally to be effective in reducing pre-harvest and post-harvest aflatoxin concentration levels and maintaining optimal levels of proximate composition parameters. The relationship between aflatoxin concentration and parameters of proximate composition were found not to be significantly correlated in both fresh and stored groundnuts. However, there existed weak positve and negative correlations between aflatoxin concentration and parameters of proximate composition.

5.2 Recommendations

5.2.1 Recommendations for Farmers

1. Farmers from the Lambussie-Karni District especially farmers in Samoa, Korro, Konguoli and Hiinneteng may apply full rate (3.75 kg/100 m^2) of Yaralegume fertiliser to Chinese variety of groundnuts.
2. The proximate composition of the groundnuts did not change with fertiliser application, hence application of Yaralegume or Humate Green OK fertilisers may not be recommendable in this respect.
3. Raw groundnuts meant for consumption should not be stored for over five (5) months since aflatoxin concentration builds up in storage with time.

5.2.2 Recommendations for Future Research

1. The research should be repeated and groundnuts analysed for proximate composition and aflatoxin concentration at different periods of storage (eg. 2, 4, 6 and 8 months) to reveal the effect of long storage and trend of build up on aflatoxin levels and proximate composition.
2. A similar experiment should be conducted and the groundnuts stored in different storage bags to assess the additional impact of the storage bag on the concentration levels of aflatoxin and nutritional quality of the groundnuts.
3. A similar research should be carried out on other varieties of groundnuts.

REFERENCES

1. **Abbas, H.K., Zablotowicz, R.M., Bruns, H.A. and Abel, C.A. (2006).** Biocontrol of aflatoxin in com by inoculation with non-aflatoxigenic *Aspergillus flavus* isolates. *Biocontrol Science and Technology,* 16:437-449.
2. **Abdualrahman, M.A.Y. (2013)**. Chemical, *in-vitro* Protein Digestibility, Minerals and Amino Acids Composition of Edible Groundnut Seeds (*Arachis hypogaea* L.). *Science International*, 1: 199-202.
3. **Abramson, D. (1998).** Mycotoxin formation and environmental factors. In: Sinha, K.K., and Bhatnagar D., Eds., Mycotoxins in Agriculture and Food Safety. Marcel Dekker, Inc, New York, 255-277.
4. **Akano, D.A. and Atanda, O. (1990)**. The present level of aflatoxin in Nigerian groundnut cake ('kulikuli'). *Letters in Applied Microbiology,* 10(4): 187-189.
5. **Alexander, N. (2011)**. 'Aflatoxins', University of Leeds, Microbiology Teaching Support, FacultyofBiologicalSciences,http://www.bmb.leeds.ac.uk/mbiology/mycotoxins/aflatoxinshtml, viewed 11 September.
6. **Amoako-Attah, I., Awuah, R. T. and Jolly, V. M. (2011).** Efficacy of clove (*Syzygium aromaticum* (L) Merr and Perry) powder as a protectant of groundnut kernels in storage. *African Journal of Food, Agriculture, Nutrition and Development,* 11(6):5414-5430.
7. **Angadi, V. V., Patil, S. V., Shilvantar, M. N. and Chittapur, B. M. (1990).** Effect of NPK levels and split application of N on growth and yield of bunch groundnut in vertisol under irrigation system. *Karnataka Journal ofAgricultural Science,* 3: 9-14.
8. **AOAC (Association of Official Analytical Chemists). (2007)**. Official Methods of Analysis. 19th Edition, Association of Official Analytical Chemists, Arlington.

9. **Arim, R.H. (1995).** Present status of the aflatoxin situation in the Philippines. *Food Additives and Contaminants*, 12: 291-296.

10. **Asibuo, J. Y., Akromah, R, Adu-Dapaah, H. and O. Safo-Katanka (2008)**. Evaluation of nutritional quality of groundnut (*Arachis hypogaea* L.) from Ghana. *African Journal of Food, Agriculture, Nutrition and Development*, 8(2): 133-150.

11. **Atehnkeng, J., Ojiambo, P. S., Dorner, M., Ikotun, T., Sikora, R. A., Cotty, P. J. and Bandyopadhyay, R. (2008)**. Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. *International Journal of Food Microbiology*, 122:74-84.

12. **Augstburger, F., Berger, J., Censkowsky, U., Heid, P., Milz, J. and Streit, C. (2002)** *Organic Farming in the Tropics and Subtropics: Groundnuts*, Gräfelfing, Germany.

13. **Awuah, R. T. and Ellis, W. O. (2001)**. Effects of some packaging methods and protection with *Ocimum* and *Syzygium* powders on kernel infection by fungi. *Mycopathologia*, 154:29-36.

14. **Ayoola, P.B. and Adeyeye, A. (2010)**. Effect of heating on the Chemical Composition and Physico – Chemical Properties of *Arachis hypogaea* (Groundnut) Seed Flour and Oil. *Pakistan Journal of Nutrition*, 9(8): 751-754.

15. **Ayoola, P.B., Adeyeye, A. and Onawumi, O.O. (2012).** Chemical Evaluation of Food Value of Groundnuts (*Arachis hypogaea* .L) Seeds. *American Journal of Food Nutrition*, 2(3): 55-57.

16. **Badau, M.H., Hauwa Z., Agbara, G.I and Abdullahi, A.Y. (2013)**. Proximate composition, mineral content and acceptability of granulated maize dumpling (Dambu

Masara) with varying proportions of ingredients. *Global Advanced Research Journal of Agricultural Scence,*. 2(1): 7-16

17. **Bagarama, F. M., Shenkalwa, E. and Matata, Z. P. (2012)**. The effect of gypsum and NPK fertiliser on groundnut performance in Western Tanzania. Third RUFORUM Biennial Meeting, 24 - 28 September, 2012.Entebbe, Uganda, pp 389-394.
18. **Bala, A., Osunde, A. O. and Odofin, A. J. . (2011)**. Organic matter utilization and the determinants of organic manure use by farmers in the Guinea savanna zone of Nigeria. In A. W. Bationo, *Innovation as key to the Green Revolution in Africa. Exploring the Scientific Facts,* 2: 965 - 974.
19. **Bandyopadhyay, R., Kiewnick, S., Atehnkeng, J., Donner, M., Cotty, P.L. and Hell, K. (2005)**. Biological control of aflatoxin contamination in maize in Africa. *Proceedings of the Tropentag Conference on International Research for Development*, (11-13 *October2005,Hohenheim,Germany).*http://www.tropentag.de/2005/abstracts/full/398.pdf.
20. **Bankole, S.A., Ogunsanwo, B.M. and Eseigbe, D.A. (2005)**. Aflatoxins in Nigerian dry-roasted groundnuts. *Food Chemistry,* 89: 503-506.
21. **Barro, N., Quattara, C.A., Nikiema, P.A., Quattara, A.S. and Traore, A.S. (2002)**. Microbial quality assessment of some street food widely consumed in Ouagadougou, Burkina Faso. *Sante,* 12: 369-374.
22. **Bationo, A. and Waswa, B. S. (2011)**. New challenges and opportunities for integrated soil fertility management in Africa. In A. W. Bationo, *Innovation as key to the Green Revolution in Africa. Exploring the Scientific Facts*, 1:3 - 18.

23. **Bationo, A., Kihara, J., Vanlauwe, B., Waswa, B. S. and Kimetu, J. (2007).** Soil organic Carbon dynamics, functions and management in West African agro-ecosystems. *Agricultural Systems*, 94:13 - 25.

24. **Bennet, J. W. and Klich, M. (2003)**. Mycotoxins. *Clinical Microbiology Reviews*, 16: 497- 516.

25. **Betina, V. (1989).** Taxonomy of mycotoxin-producing fungi. In: Mycotoxin chemical, biological and environmental aspects. Elsevier. Pp 19-41.

26. **Bhatnagar, D. (2010)**. Elimination of Post-harvest and Pre-harvest Aflatoxins Contamination; 10th International Working Conference on Stored Product Protection, Section: Microbiology, mycotoxins and food safety: 425.

27. **Bhatnagar, D., Yu, J. and Ehrlich, K. C. (2002)**. Toxins of filamentous fungi. *Chemical Immunology*, 81: 167-206.

28. **Bhatnagar, D., Ehrlich, K.C., Yu, J. and Clevel, T.E. (2003)**. Molecular genetic analysis and regulation of aflatoxin biosynthesis. *Applied Microbiology and Biotechnology*, 61:83-93.

29. **Bhatol, D. P, Patel, N. A. and Pavaya, R.P. (1994).** Effect of nitrogen, phosphorus and zinc application on yield and uptake of nutrients by groundnut. *Indian Journal of Agricultural Research*, 28: 209- 213.

30. **Bruulsema,T.W., Heffer, M.Welch, H.R., I.Cakmak, and Moran, K. (2012).** Fertilizing crops to imporve human health: a scientific review. Vol. 1. Food and nutrition security. International Plant Nutrition Institute and International Fertiliser Industry Association, Norcross, GA and Paris, France.

31. **Bulaong, S.S.P. and Dharmaputra, O.S. (2002).** Fungal population, aflatoxin and free fatty acid contents of groundnuts packed in different bag types. *Biotropia*, 19:1-25

32. **Campos, S. G., Cavaglieri, L. R., Fernández, M. G., Dalcero, A. M., Krüger C., Keller, L. A., Magnoli, C.E. and Rosa C. A. (2008).** Mycobiota and aflatoxins in raw materials and pet food in Brazil. *Journal of Animal Physiology and Animal Nutrition*, 92 (3): 377 - 383.

33. **Cardwell, K.F., and Cotty, P.J. (2000)**. Interactions among U.S. and African *Aspergillus* spp. strains: Influence on aflatoxin production. *Phytopathology*, 90: 11.

34. **Carlson M. A., Bargeron, C. B., Benson, R. C., Fraser, A. B., Phillips, T. E., Velky, J. T., Groopman, J. D., Strickland, P. T. and Ko H. W. (2000)**. An automated, handheld biosensor for aflatoxin. *Biosensor andBioelectronics*, 14: 841-848.

35. **Casado, J. M., Theumer, M., Masih, D. T., Chulze, S., Rubenstein, H. R. (2001)**. Experimental subchronic mycotoxicoses in mice: individual and combined effects of dietary exposure to fumonisins and aflatoxin B_1. *Food and Chemical Toxicology*, 39: 579-586.

36. **CAST (Council for Agriculture Science and Technology). (2003)**. Mycotixins: Risks in plant, animal and human systems. Taskforce Report No. 139. Ames, IA: Council for Agriculture, Science and Technology.

37. **CDC (Centre for Disease Control). (2004)**. Outbreak of aflatoxin poisoning-eastern and central provinces, Kenya, January-July. MMWR Morb Mortal Weekly Reporter 53:790-793. Available at www.cdc.gov/nceh/hsb/chemicals/pdfs/mmwr5334p790.pdf. Accessed May 15, 2014.

38. **CGIAR (Consultative Group on International Agricultural Research). (2000)**. Groundnut (*Arachis hypogaea Linnaeus*). Report on CGIAR Priorities and Strategies for Resource Allocation during 1998-2000.

39. **Chapman, S.C. (1990).** The effect of drought during reproductive development on yield of cultivars of groundnut (Arachis hypogaea, L). PhD Thesis, Univ. Queensland, Brisbane.

40. **Christensen, C. M., Mirocha, C. J. and Meronuck, R. A. (1977)**. Molds, mycotoxins and mycotoxicoces. St. Paul, MN: American Association of Cereal Chemists.

41. **Chu, F. S. (2002)**. Mycotoxins. In: Foodborne Diseases. D. O. Cliver (Ed.) 2nd ed. Academic Press, New York. Pp 271-303.

42. **Clark, T. (2003)** Mycotoxins: International overview. *AFMA Matrix*, 12: 3-9.

43. **Cole, R.J and Cox, R.H. (1981)**. Handbook of toxic fungal metabolites. Academic Press, Publ., New York, N.Y.

44. **Cole, R.J. and Cotty, P.J. (1990)**. Biocontrol of aflatoxin production by using biocompetitive agents. In Robens, J., Huff, W. and Richard, J. (eds.) *A Perspective on Aflatoxin in Field Crops and Animal Food Products in the United States: A Symposium; ARS-83*. U.S. Department of Agriculture, Agricultural Research Service, Washington, D.C., pp. 62-66.

45. **Coulombe, R. A., Guarisco, J. A., Klein, P. J. and Hall, J. O. (2005)**. Chemoprevention of aflatoxicosis in poultry by dietary butylated hydroxytoluene. *Animal Feed Science and Technology*, 121:217-225.

46. **Cox, F.R. (1979)**. Effect of temperature treatments on groundnut vegetative and fruit growth. *Groundnut Science*, 6: 140-147.

47. **Craufurd, P.Q., Prasad, P.V.V., Waliyar, F. and Taheri, A. (2006)**. Drought, pod yield, pre-harvest Aspergillus contamination on groundnut in Niger. *Field Crops Research*, 98: 20-29.

48. **Crespi, C.L., Penman, B.W., Steimel, D.T., Gelboin, H.V., and Gonzales, F.J. (1991)**. The development of a human cell line stably expressing human CYP3A3: role in the metabolic activiation of aflatoxin B_1 and comparision to CYP1A2 and CYP2A3. *Carcinogenesis*, 12: 255-259.

49. **D'mello, J.P.F. (2003).** Mycotoxins in cereal grains, nuts and other plant products. In: D'Mello J.P.F. (ed.) *Foods safety: Contaminants and toxins*. Wallingford: Cab International Publishing. pp. 65-88.

50. **Dar, W. D. (2002)**. ICRISAT. Towards a Grey to Green Revolution. Turning Adversity into Opportunity. A compendium of speeches and presentations. Groundnut in the New *Millennium*, 18: 1-5.

51. **Datnoff, L.E., W.H. Elmer, and D.M. Huber. (2007)**. Mineral Nutrition and Plant Disease. APS Press, St. Paul, MN, 278 p.

52. **Davidson, J., Cole, R. and Sanders, T. (1983)**. Effect of gypsum and calcium carbonate on plants. http.//www.fao.org/doCrept032e05.htm. Accessed 13 April 2013.

53. **Davidson, J.I., Whitaker, T.B. and Dickens, J.W. (1982)**. Grading, cleaning, storage, shelling and marketing of groundnuts in the United States. *In* H.E. Pattee and C.T. Young, eds., Groundnut Science and Technology, pp. 571-623. American Groundnut Research and Education Society, Yoakum, TX.

54. **Delmulle B. S., De Saeger S. M. D. G., Sibanda L., Barna-Vetro I. and Van Peteghem C. H. (2005).** Development of an immunoassay-based lateral flow dipstick for

the rapid detection of aflatoxin B_1 in pig feed. *Journal of Agricultural and Food Chemistry*, 53(9): 3364-3368

55. **Desai, S., Thakur, R.P., Bandyopadhyay, A. and Nigam, S.N. (2008)**. Mycotoxins in groundnut, National Research Centre for Groundnut and International Crops Research Institute for the Semi Arid Tropics
56. **Deshmukh, V. N., Atre, A. H., Rangacharya, R. P. and Rewakar, S.S. (1993)**. Effect of phosphorus and Potassium application on growth, yield, quality and nutrient uptake by groundnut. *Journal of Potassium Research*, 9: 72-75.
57. **Devegowda, G., Raju, M.V.L.N and Swamy, H.V.L.N. (1998)**. Mycotoxins: novel solutions for their counteraction. *Feedstuffs*, 70:12-15.
58. **Diaz Rios, L. and Jaffee, S. (2008)**. Standards, Competitiveness, and Africa's GroundnutExports to Europe: Barrier, Catalyst, or Distraction? Agriculture and Rural Development Department. Discussion Paper 39 The International Bank for Reconstruction and Development/ The World Bank.
59. **Diop, Y.M., Ndiaye, B., Fall, M., Diouf, A., Sall, A., Ciss, M. and Ba, D. (2000)**. Aflatoxin contamination level in artisanal and industrial groundnut butter food in Dakar (Senegal). *Dakar Medical*, 45(2): 134-137.
60. **Dorner, J.W., Cole, R.J. and Blankenship, P.D. (1998)**. Effect of inoculum rate of biological control agents on pre-harvest aflatoxin contamination of groundnuts. *Biological Control*, 12: 171-176.
61. **Duke, J.A. (1981)**. Handbook of legumes of world economic importance. Plenum Press. NewYork

62. **Duke, J.A. and Ayensu, E.S. (1985)**. Medicinal plants of China. Reference Publications, Inc. Algonac, MI.

63. **Duke, J.A. and Wain, K.K**. **(1981).** Medicinal plants of the world. Computer index. Source: Complete list of references for Duke, Handbook of Energy Crops - James A. Duke, Plenum Press, NewYork.

64. **Eastwood, M. and Kritchevsky, D. (2005)**. Dietary fibre: how did we get to where we are? *Annuual Review of Nutrition*, 25: 1-8.

65. **El Tinay, A. H., Mahgoub, S. O., Mohamed, B. E. and Hamad, M.A. (1989)**. Proximate composition and mineral and phytate contents of legumes grown in Sudan. *Journal of Food Composition and Analysis*, 2: 69-78.

66. **Elsheikh, E. A. E and E. M. M. Mohamedzein (1998)**. Effect of Bradyrhizobium, VA mycorrhiza and fertilisers on seed composition of groundnut. *Annual Applied Biology*, 132: 325-330.

67. **Fandohan P., Zoumenou, D., Hounhouigan, D.J., Marasas, W.F.O., Wingfield, M.J. and Hell, K. (2005)**. Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. *International Journal of Food Microbiology*, 98: 249-259.

68. **FAO (Food and Agriculture Organization). (1998)**. Mycotoxin Prevention and Control in Food Grain. Food and Agricultural Organization of the United Nations. Rome.

69. **FAO (Food and Agriculture Organization). (2001)**. Manual on the application of HACCP system in mycotoxins prevention and control. FAO Food and Nutrition Paper No. 73. FAO, Rome, Italy. ISSN 0254-4725.

70. **FAO (Food and Agriculture Organization). (2002)**. Food and Agricultural Statistics. Rome, Italy. Pons W. A., Lee L. S. and Stoloff L. (1980). Revised method for aflatoxin

in cottonseed products and comparison of thin layer chromatography and high pressure liquid chromatography determinative steps: collaborative study. *Journal of the Association of Official Analytical Chemists*, 63: 899-906

71. **Flannigan, B. and Pearce, A.R. (1994).** Aspergillus spoilage: Spoilage of cereals and cereal products by the hazardous species *A. clavatus*. *In*: The Genus Aspergillus: From Taxonomy and Genetics to Industrial Application, eds. K.A. Powell, A. Renwick, and J.F. Peberdy, 115-127. Plenum Press, Publ., New York, NY.
72. **Florkowski, W.J. and Kolavalli, S. (2013)**. Aflatoxin control Strategies in the Groundnut Value Chain in Ghana. Working paper 33. Ghana Strategy Support Program.
73. **Gams, W., Christensen, M., Onions, A.H.S., Pitt, J.I. and Samson, R.A. (1985).** *Intrageneric taxa of Aspergillus*. *In*: Advances in *Penicillium* and *Aspergillus* Systematics, eds, R.A. Samson, and J.I. Pitt, 55-62. Plenum Press, Publ., New York, NY.
74. **Ghali, R., Hmaissia-khlifa, K., Ghorbel, H., Maaroufi, K. and Hedili, A. (2008)**. Incidence of aflatoxins, ochratoxin A and zearalenone in Tunisian foods. *Food Control*, 19: 921-924.
75. **Ghana Statistical Service (2013)**. 2010 Population and Housing Census (*Regional analytical Report*, Upper West Region). GSS, Accra.
76. **Gibbons, R. W., Bunting. A. H. and Smartt, J. (1972)**. The classification of varieties of groundnut (*Arachis hypogaea* L.). *Euphytica*, 21: 78-90.
77. **Gibbons, R.W. (1980)**. Adaptation and utilization of groundnuts in different environments and farming systems. *In* Summerfield, R.J. and Bunting, A.H. (eds). Advances in legume Science, Royal Botanic Gardens, Kew.

78. **Giller, K. E. and Cadisch, G. (1995).** Future benefits from biological nitrogen fixation: an ecological approach to agriculture. *Plant Soil,* 174: 255-277.

79. **Gobarah, M. E., Mohamed, M. H. and Tawfik, M.M. (2006)**. Effect of phosphorus fertiliser and foliar spraying with zinc on growth, yield and quality of groundnut under reclaimed sandy soils. *Journal of Applied Science Research*, 2(80): 491-496.

80. **Gong, Y.Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P.C., Hall, A.J., Wild, C.P. (2002)**. Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *British Medical Journal,* 325: 20-21.

81. **Graham, P. H. and Vance, C. P. (2000)**. Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Research,* 65: 93-106.

82. **Graham, R.D. (1983)**. Effect of nutrient stress on susceptibility of plants to disease with particular reference to the trace elements. *Advanced Botany Research*, 10: 221-276.

83. **Graham, R.D., Welch, R.M., Saunders, D.A., Bouis, H.E., Bonierbale, M., Haan, S., Burgos, G., Thiele, G., Liria, R., Meisner, C.A., Beebe, S.E., Potts, M.J., Kadian, M., Hobbs, P.R., Gupta, R.K. and Twomlow, S. (2007)**. Nutritious Subsistence Food Systems. p. 1-74. *In* L.S.Donald (ed.) *Advances in Agronomy*. Academic Press.

84. **Gregory, W.C., Gregory, M.P. and Krapovickas, A. (1973)**. Structure and genetic resources of groundnuts. p. 47-133. *In* Groundnuts – Culture and Uses, American Peanut Research and Education Association, Stillwater, Oklahoma.

85. **Gregory, W.C., Krapovickas, A. and Gregory, M.P. (1980)**. Structure, variation, evolution and classification in Arachis.p. 469-481. *In* Summerfield R.J. and Bunting, A.H. (eds). *Advances in Legume Science*. International Legume Conference, Royal Botanic Gardens, Kew

86. **Groopman, J. D. and Kensler, T. W. (1996).** Temporal patterns of aflatoxin-albumin adducts in hepatitis B surface antigen-positive and antigen-negative residents of Daxin, Qidong County, People's Republic of China. Cancer Epidemiology, *Biomarkers and Prevention*, 5: 253-261.

87. **GSA (Ghana Standards Authority). (2013).** Nuts - Specification for Groundnut. GSB Ref. No. GS 313:2001, Accra.

88. **Guo, B., Yu, J., Holbrook, C., Cleveland, T., Nierman, W., and Sully, B. (2009)**. 'Strategies in Prevention of Pre-harvest Aflatoxin Contamination in Groundnuts: Aflatoxin Biosynthesis, Genetics and Genomics' in *Groundnut Science*, vol. 36.

89. **Hammons, R.O. (1982)**. Origin and early history of the groundnut. p. 1-20. *In* Patee, H.E. and Young, C.T. (eds). Groundnut science and technology. American Groundnut Research and Education Society, Yoakum, Texas.

90. **Harvey, R.B., Kubena, L.F., Elissalde, M.H., and Phillips, T.D. (1993)**. Efficacy of Zeolitic Ore Compounds on the Toxicity of Aflatoxins to Growing Broiler Chickens. *Avian Diseases*, 37: 67-73.

91. **Hayes, A. W. (1980)**. Mycotoxins: a review of biological effects and their role in human diseases. *Clinical Toxicology*, 17: 45-83.

92. **Hayes, R. B., van Nienwenhuise, J. P., Raatgever, J. W., Ten and Kate, F. J. W. (1984)**. Aflatoxin Exposure in the Industrial Setting: An Epidemiological Study of Mortality. *Food and Chemical Toxicology*, 22: 39-43.

93. **Hayma, J. (2003)**. The storage of tropical agricultural products. Wageningen, the Netherlands: STOAS Digigrafi.

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

95. **Hell, K., Cardwell, K., Sétamou, M., and Peohling, H. (2000)**. 'The influence of storage practices on aflatoxin contamination in maize for four agroecological zones of Benin, West Africa' in *Journal of Stored Product Research*, vol. 36.

96. **Hendrickse, R. G. (1997).** Sick turkeys, kwashiorkor, malaria, perinatal mortality, heroin addicts and food poisoning: research on the influence of aflatoxins on child health in the tropics. *Annals of Tropical Medicine and Parasitology,* 91: 787-793.

97. **Herrman, T. (2002)**. Mycotoxins in feed grains and ingredients. MF-2061. Feed Manufacturing. Dept of Grain Science and Industry, Kansas State University.

98. **Hill, R. A., Blankenship, P. D., Cole, R. J. and Sanders, T.H. (1983)**. Effects of soil moisture and temperature on pre-harvest invasion of groundnuts by the *Aspergillus flavus* group and subsequent aflatoxin development. *Applied and Environmental Microbiology,* 45: 628–633.

99. **Huber, D.M. (1980)**. The role of mineral nutrition in defense. p. 381-406. *In* J.G. Horsfall and E.B. Cowling (eds.). Plant Disease, An Advanced Treatise Vol. V. How Plants Defend Themselves. Academic Press, New York. 534 p.

100. **Huber, D.M. and Graham, R.D. (1999).** The role of nutrition in crop resistance and tolerance to diseases. p. 169-204. *In* Z. Rengel (eds.). Mineral Nutrition of Crops, Fundamental Mechanisms and Implications. The Haworth Press, Inc., New York.

101. **Huber, D.M. and Haneklaus, S. (2007)**. Managing nutrition to control plant disease. *Landbauforschung Volkenrode,* 57: 313-322.

102. Huber, D.M. and Thompson, I.A. (2007). Nitrogen and plant disease. p. 31-44. *In* L.E. Datnoff, W.H. Elmer, and D.M. Huber (eds.). *Mineral Nutrition and Plant Disease*.APS Press, St. Paul, MN.

103. IARC (International Agency for Research on Cancer). (1993). Aflatoxins: naturally occurring aflatoxins (Group 1), aflatoxins M1 (Group 2B). *International Agency for Cancer Research* 56: 245.

104. ICRISAT (International Crops Research Institute Semi-Arid Tropics). (2006). Nurturing the seeds of success in the semi-arid tropics: ICRISTAT Annual Report.

105. Isleib, T.G., Wynne, J.C. and Nigam, S.N. (1994). Groundnut breeding. p. 552-620. *In* Smartt, J. (ed). The groundnut crop: A Scientific Basis for Improvement. Chapman and Hall, London.

106. Jin X., Chen L., Jiang J., Shen, G and Yu, R. (2009). Determination of aflatoxins in highpigment content samples by matrix solid-phase dispersion and high-performance liquid chromatography. *Biosensors and Bioelectronics*, 24(8): 2580-2585.

107. Jones, R. K. (1987). The influence of cultural practices on minimizing the development of aflatoxin in field maize. *In* "Aflatoxin in Maize" eds. M. S. Zuber, E. B. Lillehoj and B. L. *Renfro*, pp 136 - 144.

108. Juan, C., Zinedine, A., Moltó, J.C., Idrissi, L. and Maňes, J. (2008). Aflatoxins levels in dried fruits and nuts from Rabat-Salé area, Morocco. *Food Control,* 19: 849-853.

109. Kaaya, A. N., Kyamuhangire, W., and Kyamanywa, S. (2007). Factors Affecting Aflatoxin Contamination of Harvested Maize in the Three Agroecological Zones of Uganda. *Journal of Applied Sciences,* 6: 2401-2407.

110. **Kaaya, A.N., Eigel, W and Harris, C. (2006)** 'Groundnut Aflatoxin Levels on Farms and in Markets of Uganda in *Groundnut Science*, vol. 33.

111. **Karthikeyan, A. (1996).** Effect of organic amendments, antagonist Trichoderma viride and fungicides on seed and collar rot of groundnut: In *Plant Disease Research*, vol. 11.

112. **Kavitha, S. and Parimalavalli, R. (2014)**. Effect of Processing Methods on Proximate Composition of Cereal and Legume flours. *Journal of Human Nutrition and Food Science*, 2(4): 1051.

113. **Kirk, R.S and Sawyer, R. (1991)**. Pearson's Composition and Chemical Analysis of Foods, 9th Edition. Longman Scientific and Technical, Essex, England.

114. **Kladpan, S., Mahakachanakul, W., Yongmanitchai, V., Boonyaratanakornkit, M., and Chinbuti, A. (2004)**. Situation of aflatoxin contamination in groundnut products in Thailand in 2004. In: Proceedings of the 43rd Kasetsart University Annual Conference, Thailand, 1e4 February, 2005, pp. 557-564.

115. **Koster, H. (2001).** Mycotoxins. *AFMA Matrix*. Pp 24-25.

116. **Krapovickas, A. (1973)**. Evolution of the genus *Arachis*. p. 135-151. *In* Moav, R. (ed). Agricultural Genetics – Selected Topics. National Council for Research and Development, Jerusalem.

117. **Kumar, B.S., Sadagopan, R.S., Vasanthi, R.P., Kalapati, M.V. and Mabu, P. (2013).** Comparative Physico-chemical, Proximate and Mineral Analysis on Raw and Roasted seeds of Groundnuts. *Com Plant Science*. 3(3-4): 25-29.

118. **Ladele, E.A and Njoku, PC. (1984)**. Moisture distribution in plantain in relation with maturation. *Journal ofFood Biotechnology*, 412: 78-79.

119. Lekasi, J. K. (2003). Organic resource management in smallhold agriculture. In C. E. Savala, *Organic resource management in Kenya: Perspectives and guidelines* (pp. 6 - 12). Nairobi, Kenya: Forum for Organic Resource Management and Agricultural Technologies, FORMAT.

120. Leong, S.K. and Ong, C.K. (1983). The influence of temperature and soil water deficit on the development and morphology of groundnuts (*Arachis hypogaea* L.). *Journal ofExperimental Botany,* 34: 1551-1561.

121. Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., Ambrose Misore, A., DeCock, K. and Rubin, C. (2005). Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya. *Journal of Environmental Health Perspect*, 113(12): 1763–1768.

122. Liu Y., Qin Z. H., Wu X. F. and Hong J. (2006). Immune-biosensor for aflatoxin B_1 based bioelectrocatalytic reaction on micro-comb electrode. *Biochemical Engineering Journal,* 32: 211-217.

123. Lopez-Garcia, R. and Park, D.L (1998). Effectiveness of post-harvest procedures in management of mycotoxin hazards. In D. Bhatnagar and S. Sinha, eds. Mycotoxins in agriculture and food safety, p. 407-433. New York, Marcel Dekker.

124. Lu, F.C. (2003). Assessment of safety/risk vs. public health concerns: aflatoxins and hepatocarcinoma. *Environmental Health and Preventive Medicine,* 7: 235-238.

125. Lubal P., Fetsch, D and Iroký, D. (2000). Potentiometric and spectroscopic study of uranyl complexation with humic acids. *Talanta,* 51: 977-991.

126. **Lubulwa, A.S.G and Davis, J.S. (1994)**. Estimating the social costs of the impacts of fungi and aflatoxins. *In:* Stored Product Protection. Proceedings of the 6th International Working Conference on Stored-Product Protection, eds. E. Highley, E. J. Wright, H. J. Banks and B.R. Champ, 1017-1042. CAB International, Wallingford, Publ., Oxford, United Kingdom.

127. **MacRobert, J. F. (2009)**. Seed business management in Africa. Harare, Zimbabwe, CIMMYT.

128. **Malaker, P. K., Mian, I. H., Bhuiyan, K. A., Akanda, A. M. and Reza, M. M.A (2008)**. Effect of storage containers and time on seed quality of wheat. *Bangladesh Journal of Agricultural Research,* 33(3): 469-477.

129. **Manetta, A.C. (2011)**. Aflatoxins: Their Measure and Analysis, Aflatoxins - Detection, Measurement and Control, Dr Irineo Torres-Pacheco (Ed.), ISBN: 978-953-307-711-6, *InTech*:http://www.intechopen.com/books/aflatoxins-detection-measurement-and-control/aflatoxins-their-measureand-analysis

130. **Marschner, H. (1995)**. Mineral Nutrition of Higher Plants, Second Ed. Academic Press, London. 889 p.

131. **Maxwell, S. M., Apeagyei, F., de Vries, H. R., Mwanmut, D. D. and Hendrickse, R. G., (1998)**. Aflatoxins in breast milk, neonatal cord, blood and sera of pregnant women. *Journal of Toxicology,* 8: 19-29.

132. **McIntosh, F.P., and Davidson, J.I. (1971)**. Effect of temperature on shelling runner- and spanish-type groundnuts. Rep. 52-65, U.S. Department of Agriculture, Agricultural Research Service, Washington, DC.

133. **McMillian, W.W. (1983)**. Role of anthropods in field contamination. *In* Aflatoxin and *Aspergillus flavus* in corn, eds. U. L. Diener, R.L. Asquith and J.W. Dickens, 20-22. Alabama Agr. Exp. Sta., Auburn U., Auburn.

134. **Mehan V K, McDonald D, Haravu L G and Jayanthi, S. (1991)**. The Groundnut Aflatoxin Problem: Review and Literature Database, International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, A.P., India. P p. 387.

135. **Mkoka, C. (2007).** Purging Malawi's groundnuts of deadly aflatoxin. SciDevNet. http://www.scidev.net/en/features/purging-malawis-groundnuts-of-deadly-aflatoxin.html. Last accessed on 19 May, 2013

136. **Montesano, R., Hainaut, P. and Wild, C. P., (1997)**. Hepatocellular carcinoma: from gene to public health. *Journal of National Cancer Institute,* 89: 1844-1851.

137. **Moreno, O. J. and Kang, M. S. (1999)**. Aflatoxins in maize: The problem and genetic solutions. *Plant Breeding,* 118: 1-16.

138. **Moss, M (2002)**. Risk assessment for aflatoxins in foodstuffs. *International Biodeterioration and Biodegradation,* 50: 137-142.

139. **Moyo, S., Norton, G. W., Alwang, J., Rhinehart, I. and Deom, M. C. (2007)**. Peanut research and poverty reduction: Impacts of variety improvement to control peanut viruses in Uganda. *American Journal Agricultural Economics,* 89**:** 448-460.

140. **Mphande, F.A., Siame, B.A. and Taylor, J.E. (2004)**. Fungi, aflatoxins, and cyclopiazonic acid associated with groundnut retailing in Botswana. *Journal of Food Protection,* 67(1): 96-102.

141. **Mukuralinda, A., Tenywa., J. S, Verchot., L, Obua, J., Nabahungu, N. L., and Chianu, J. N. (2010)**. Phosphorus uptake and maize response to organic and inorganic fertiliser inputs in Rubona, Southern Province of Rwanda. *Agroforest Syst 80* , 211 - 221.

142. **Murphy, P.A., Hendrich, S., Landgren, C. and C.M. Bryant, (2006)**. Food mycotoxins: An update. *Journal of Food Science*, 71: 51-65.

143. **Murwira, H. K., Mutuo, P., Nhamo, N., Marandu, A. E., Rabeson, R., Mwale, M., and Palm, C. A. (2002)**. Fertiliser equivalency value of organic materials of different quality. In B. D. Vanlauwe, *Integrated plant nutrient management in Sub-Saharan africa: From concept to practice* (pp. 113 - 122). Trowbridge, UK: CAB International.

144. **Mutegi, C.K., Wagacha, J.M., Christie, M.E., Kimani, J. and Karaja, L. (2013)**. Effect of Storage Conditions on Quality and Aflatoxin Contamination of Peanuts (*Arachis hypogaea*. L). *International Journal of AgriScience*, 3(10): 746-758.

145. **Mutegi, C.K., Wagacha, J.M., Kimani, J., Otieno G., Wanyama, R., Hell, K and Christie, M.E. (2013)**. Incidence of aflatoxin in groundnuts (*Arachis hypogaea* Linnaeus) from markets in Western, Nyanza and Nairobi Provinces of Kenya and related market traits. *Journal of Stored Product Research*, 52:118-127.

146. **Mutegi, C.K., Ngugi, H.K., Hendriks, S.L. and Jones, R.B. (2009)**. Prevalence and factors associated with aflatoxin contamination of peanuts from western Kenya. *International Journal of Food Microbiology*, 130: 27-34.

147. **Nasir, M. S. and Jolley, M. E. (2002)**. Development of a fluorescence polarization assay for the determination of aflatoxins in grains. *Journal of Agricultural and Food Chemistry*, 50, pp. 3116-3121.

148. **Nawaz S., Coker, R.D. and Haswell, S. J. (1992)**. Development and evaluation of analytical methodology for the determination of aflatoxins in palm kernels. *Analyst*, 117: 67-74.

149. **Ndiaye, B., Diop, Y.M., Diouf, A., Fall, M., Thiaw, C., Thiam, A., Barry OCiss, M. and Ba, D. (1999)**. Measurement and levels of aflatoxins in small-scale pressed groundnut oil prepared in the Diourbel and Kaolack regions of Senegal. *Dakar Medical,* 44 (2): 202–205.

150. **Nektaria, P. (2007)**. Biomarkers of aflatoxin exposure and dietary intervention. Doctoral dissertation presented to the Faculty of Medicine, University of Kuopio.

151. **Nyamangara, J., Piha, M. I. and Giller, K. E. (2003)**. Effect of combined cattle manure and mineral nitrogen on maize N uptake and grain yield. *African Crop Science Journal,* 11(4): 289 - 300.

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

153. **Otsuki, T., Willson, J. and Sewadeh, M. (2001**). 'What Price Precaution? European Harmonisation of Alfatoxin Regulations and African Groundnut Exports' in *European review of Agricultural Economics*, vol. 28.

154. **Otta K. H., Papp E. and Bagócsi B. (2000)**. Determination of aflatoxins in food by overpressured-layer chromatography. *Journal of Chromatography A*, 882: 11-16.

155. **Otta K. H., Papp E., Mincsovics E. and Záray, G. (1998)**. Determination of aflatoxins in corn by use of the personal OPLC basic system. *Journal of Planar Chromatography*, 11, pp. 370-373

156. **Paniel, N., Radoi, A. and Marty, J. L. (2010)**. Development of an electrochemical biosensor for the detection of aflatoxin M1 in milk. *Sensors*, 10: 9439-9448.

157. **Park, D.L. and Njapau, H. (1989)**. Contamination issues and padding. *Journal of the American Oil Chemists' Society,* 66: 1402–1405.

158. **Payne, G. A., Cassel, D. A. and Adkins, C. R. (1986)**. Reduction of aflatoxin contamination in corn by irrigation and tillage. *Phytopathology*, 76: 679-684.

159. **Perry, A. (1963)**. *Groundnut production guide*, Circular 257, North Carolina: North Carolina Agricultural Extension Service.

160. **Phillips, T.D., Sarr, A.B., and Grant, P.G. (1995)**. Selective Chemisorption and Detoxification of Aflatoxins by Phyllosilicate Clay. *Natural Toxins*, 3: 204-213.

161. **Piermarini S., Volpe G., Micheli L., Moscone, D. and Palleschi, G. (2009)**. An ELIME-array for detection of aflatoxin B_1 in corn samples. *Food Control*, 20: 371-375.

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

163. **Pittet, A. (1998)**. Natural occurrence of mycotoxins in foods and feeds: An updated review. *Review Medicene Veterinaire*, 6: 479-492.

164. **Polthanee, A. (1991)**. Cultivation of groundnut after rice inrainfed areas of Northeast Thailand: Farmers' approach. *Journal of Agriculture.* 7(1): 70-76.

165. **Qian, G. S., Ross, R. K., Yu, M. C., Yuan, J. M., Gao, Y. T., Henderson, B. E., Wogan, G. N. and Groopman, J. D. (1994)**. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shang-hai, People's Republic of China. *Cancer Epidemiology and Biomarkers Prevention*, 3: 3-10.

166. **Rahman, M.A. (2006)**. Effect of Calcium and Bradyrhizobium inoculation of the Growth, Yield and quality of groundnut (*A. hypogaea* L.*). Bangladesh Journal of Science and Industrial Research*. 41(3-4): 181-188.

167. **Ramanatha-Rao, V. (1988).** Botany. In: *Groundnut* (Reddy, P. S., ed.) *Indian council of agricultural research*, New Delhi, pp. 24-64.

168. **Ramanatha-Rao, V. and Murty, U. R. (1994)**. Botany, Morphology and Anatomy. pp 43-89. *In* Smartt, J. (ed). The groundnut crop: A Scientific Basis for Improvement. Chapman and Hall, London.

169. **Ranjit, R., Dasog, G. S. and P. L. Patil .(2007)**. Effect of lime and phosphorus levels on the pod , Haulm and oil yield of two groundnut genotypes in acid soils of coastal AgroEcosystem of Karnataka. *Karnataka Journal of Agricultural Sciences*, 20(3): 627-630.

170. **Reddy, S. V. and Waliyar, F. (2000)**. Properties of aflatoxin and it producing fungi. URL: http://www.aflatoxin.info/aflatoxin.asp Environmental Health Criteria (EHC), 1979. Environmental Health Criteria 11. Mycotoxins. Geneva.

171. **Reid, P.H. and Cox, F.R. (1973)**. Soil properties, mineral nutrition and fertilisation practices. p. 271-297. *In* Groundnuts – Culture and Uses. *Am. Groundnut Res. Ed. Ass. Inc*., Stillwater, USA.

172. **Retamal, N., Lopez-Vences and Duran, J.M. (1990)**. Seed morphology of 75 genotypes of groundnut *Arachis hypogaea* L. grown in Spain. *Plant Genetic Resources Newsletter*, 80: 1-4.

173. Rosolem, C., Fernandez, E., Maringoni, A. and Oliveira, D. (1997). 'Fungus incidence on groundnut grains as affected by drying method and Ca nutrition' in *Field Crops Research*, vol. 52.

174. Saleemullah, I. A., Khalil, I. A. and Shah, H. (2006). Aflatoxin contents of stored and artificially inoculated cereals and nuts. *Food Chemistry,* 98: 699-703.

175. Sanchez, P.A. (1994). Tropical soil fertility research, towards the second paradigm, p. 65-88. In Trans. 15th World Congress of Soil Science, Acapulco, Mexico. 10-16 July 1994. ISSS, Wageningen, the Netherlands.

176. Sanginga, N and Woomer, P. L. (2009). *Integrated soil fertility management in Africa: Principles, practices, and developmental process.* Nairobi, Kenya: TSBF-CIAT.

177. Sauer, D.B. and Tuite, J. (1987). Conditions that affect the growth of *Aspergillus flavus* and production of aflatoxin in stored maize. In Zuber, M.S., Lillehoj, E.B., Renfro, B.L. (eds.), Aflatoxins in Maize: Proceedings of a Workshop. CIMMYT. 41-50.

178. Savage, G. P. and Keenan, J.I. (1994). The composition and nutritive value of groundnut kernels. In: Smart J(ed.). *The groundnut Crop: A scientific basis for improvement*: Chapman and Hall. London, pp 173-213.

179. Schroeder, H.W. (1969). Factors influencing the development of aflatoxins in some field crops. *Journal of Stored Products Research*, 5: 187-190.

180. Scott, P. (1991). 'Methods of Analysis for Mycotoxins: An Overview' in Rossell J. and Pritchard, J. (eds.) *Analysis of Oilseeds, Fat and Fatty Foods*, London, UK: Elsevier Applied Science.

181. Sellschop, J.P.F. (1967). Groundnuts-all aspects of cultivation. *Farming in South Africa*, pp 3-19.

182. **Senesi N., T.M. Miano, M.R. Provenzano and Brunetti, D. (1991)**. Characterization, differentiation, and classification of humic substances by fluorescence spectroscopy. *Soil Science*, 152: 259-271

183. **Shimada, T. and Guengerich, F.P. (1989)**. Evidence for cytchrome P-450NE, the nefedipine oxidase, being the principal enzyme involved in the bioactivation of aflatoxins in human liver. *Proceedings of the USA National Academy of Science*, 86: 462-465.

184. **Shokes, F.M. and Melouk, H.A. (1995)**. Plant health management in groundnut production. p. 1-6 *In* Melouk, H.A. and Shokes, F.M.(eds). Groundnut health management. APS Press, St Paul, Minnesota.

185. **Singh, A.K. and Simpson, C.E. (1994)**. Biosystematic and genetic resources. p. 96 - 137. *In* Smartt, J. (ed). The groundnut crop: A Scientific Basis for Improvement. Chapman and Hall, London.

186. **Siontorou, C. G., Nikolelis D. P., Miernik, A. and Krull, U. J. (1998)**. Rapid methods for detection of Aflatoxin M1 based on electrochemical transduction by self-assembled metalsupported bilayer lipid membranes (s-BLMs) and on interferences with transduction of DNA hybridization. *Electrochimica Acta*, 43: 3611-3617.

187. **Smith, J. and Moss, M. (1985)** *Mycotoxins: Formation, Analysis and Significance*, Chichester, West Sussex, UK: John Wiley and Sons

188. **Somani, R.B and Pandrangi, R.B. (1992)**. Biochemical status of healthy and mouldy grain of sorghum, SPH 388, *In: Proceedings of the XXII Annual Sorghum Workshop*, held on April 2-4, 1992, Surat, Gujarat, India, p. 27.

189. Sun, Z., Lu, P., Gail, M.H., Pee, D., Zhang, Q., Ming, L., Wang, J., Wu, Y., Liu, G., Wu, Y. and Zhu, Y. (1999). Increased Risk of Hepatocellular Carcinoma in Male Hepatitis B. Surface Antigen Carriers With Chronic Hepatitis Who Have Detectable Urinary Aflatoxin Metabolite M1

190. Sungsoo, C., Jonathan, W.V and Leon, P. (1999). Dietary Fibre Analysis and Application. AOAC International Gaithersburg, Maryland, USA.

191. Svoboda, D., Grady, H. and Higginson, H. (1966). Aflatoxin B_1 injury in rat and monkey livers. *American Journal ofPhytopathology*, 49: 1023-1051.

192. Tanuja, K., Kavitha, G., Karuna, R., and Sashidhar, R. B. (2012). Substrate suitability of neem seed kernel for the growth and elaboration of aflatoxins by *Aspergillus parasiticus* (NRRL 2999). *Indian Journal of Natural Products and Resources*, 3(3): 395-406

193. Tarek, A., El-Adawy, K. and Taha,M. (2001). Characteristics and composition of different seeds oils and flours. *Food Chemistry*, 74: 47–54.

194. Tombelli S., Mascini M., Scherm B., Battacone G. and Migheli Q. (2009). DNA biosensors for the detection of aflatoxin producing *Aspergillus flavus* and *A. parasiticus*. *Monatshefte für Chemie / Chemical Monthly*, 140: 901-907.

195. Trucksess M. W., Stoloff L., Pons W. A., Cucullu A. F., Lee L. S. and Franz, A. O. (1977). Thin layer chromatographic determination of aflatoxin B_1 in eggs. *Journal of the Association of Official Analytical Chemists*, 60(4): 795-798.

196. Turner, P., Sylla, A., Gong, Y., Diallo, M., Sutcliffe, A., Hall, A. and Wild, C. (2005). Reduction in exposure to carcinogenic aflatoxins by post-harvest intervention

measures in west Africa: a community-based intervention study. *The Lancet*, 365(9475): 1950 – 1956.

197. **Urvashi, P., Divya, V., Pushpa, S. and Kavita, B. (2013)**. Effect of aflatoxin contamination on protein content of locally marketed food grains. *Jornal ofIndian Food*, 4(5): 235-246

198. **USAID (United States Agency for International Development). (2011)**. Feed the Future Ghana: FY2011-2015 Multiyear Strategy. Washington, DC.

199. **USDA (United States Department of Agriculture). (2005)**. National Nutrient Database for Standard Reference, Release 18. Washington, DC: Nutrient Data Laboratory (http://ndb.nal.usda.gov).

200. **Vaidya, A and Dharam, V. (1989)**. Changes in the oil in stored groundnut due to *Aspergillus niger* and *Aspergillus flavus*. *Indian Phytopathology*, 42: 525.

201. **Valarezo, S., Jacque, K. A., Weir, J. and Obregon, H. (1997)**. Comparative effects of antibiotic, mannanoligosaccharide and mycotoxin absorbent on performance of commercial broilers fed pelleted diets. In: *Mycosorb technical Dossier*. *Alltechnology*, Pp 2.

202. **Van Egmond, H. P., Paulsch, W. E. and Schuller, P. L. (1978)**. Confirmatory test for aflatoxin M1 on thin layer plate. *Journal of the Association of Official Analytical Chemists*, 61: 809-812

203. **Vanlauwe, B., Bationo, A., Chianu, J., Giller, K. E., Merckx, R., Mokwunye, U., Ohiokpehai, O., Pypers, P., Tabo, R., Shepherd, K. D., Smaling, E. M. A., Woomer, P. L. and Sanginga, N. (2010)**. Integrated soil fertility management: Operational

definition and consequences for implementation and dissemination. *Outlook on Agriculture*, 39(1): 17 - 24.

204. Vlek, P. L. G., Kuhne, R. F. and Denich, M. (1997). Nutrient resources for crop production in the tropics. *Philosophical Transaction*, 352: 975-984.

205. Wagacha, J.M., Mutegi , C.K., Maria E. C., Karanja, L.W and Kimani, J. (2013). Changes in Fungal Population and Aflatoxin Levels and Assessment of Major Aflatoxin Types in Stored Groundnuts (*Arachis hypogaea* L.). *Journal of Food Research*, 2(5): 10-23.

206. Wakshama, P.S., Akueshi, C.O and Ali, B.D. (2010). Comparative Studies on the Proximate Composition and Some Physical Characteristics of Dry Matter Samples of Fermented and Unfermented Groundnut (*Arachis hypogaea* L.) Seed, Pumpkin (*Curcubita pepo* L.) Seed and Pulp. Vol. 2

207. Waliyar, F., Reddy S.V., Lava Kumar P., Reddy, B.V.S., Rai, K.N., Ashok, S. and Ravinder, R.C. (2007). Surveillance for natural contamination of mycotoxins in sorghum and pearl millet grains in Andhra Pradesh and Maharashtra states, India. Paper presented (Abstract page 294) in 2nd Asian congress of mycology and plant pathology, 19-22 December 2007, Osmania University, Hyderabad, Andhra Pradesh, India.

208. Wang, Z. H., Li, S. X. and Malhi, S. S. (2008). Review effects of fertilisation and other agronomic measures on nutritional quality of crops. *Journal of the Science of Food and Agriculture, 88*: 7 - 23.

209. Williams, J.H., Phillips, T.D., Jolly, P.E., Stile, K.J., Jolly, C.M. and Aggarwal, D. (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure,

potential health consequences, and interventions. *American Journal of Clinical Nutrition*, 80(5): 1106-1122.

210. Wilson, D. M. and Payne, G. A. (1994). Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination in crops. In: *Toxicology of Aflatoxins: Human, Veterinary and Agricultural Significance.* Eayon, D. L. and Groopman, J. D. (Eds). San Diego, Califonia, Academic Press, Inc. 309-325.

211. Wilson, D.M., Walker, M.E. and Gascho, G.J. (1989). Some effects of mineral nutrition on aflatoxin contamination of corn and groundnuts. *In*. Soilborne Plant Pathogens: Management of diseases with macro- and microelements. Ed. A.W. Englehard, APS Press, St. Paul, MN 217 pp.

212. Wogan, G. N. and Busby, W. F. (1980). Naturally occurring carcinogens. In: *Toxic Constituents of Plant Foodstuffs*. Liener, I. E. (Ed.) Academic Press, Inc. London, United Kingdom, pp 329-369.

213. Woodroof, J.G. (1983).Groundnuts Production, Processing, Products. 3th ed. Avi Publishing Company Inc. Westport, Connecticut.

214. Zubair, A., Ud-Din, Z., Saleemullah, S. A. K., Hamid U. S., Barkat, A.K. and Ehsan, A. (2011). Fatty Acid Profile and Aflatoxin Contamination of Walnuts (*Juglans regia*). *ARPN Journal of Agricultural and Biological Science*, 6 (9): 1 – 8.