AFLATOXINS IN PEANUTS AND THE PREVALENCE OF AFLATOXIN INDUCED HEPATOCELLULAR CARCINOMA IN BUSIA AND KISII CENTRAL DISTRICTS, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award.

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DEDICATION

I dedicate this thesis to my supervisors, family members and friends.

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I thank God for granting me the strength to begin and complete this study. I acknowledge all the people who had anything to do with this work. The list is long and the gratitude immense. I would like to thank my hardworking supervisors; Dr. Margaret W. Muturi of the Department of Medical Laboratory Science and Dr. Lucy M. Kamau of the Department of Zoological Sciences, Kenyatta University for their encouragement, advice and support during the course of my work. I also thank Kenyatta University for the approval and Moi Teaching and Referral Hospital for permitting me to analyze their Hospital records for this study. May God bless the work of your hands. To my family and friends, I thank you for your support in every way. God bless you.

ABBREVIATIONS AND ACRONYMS

AFPA	*Aflatoxin flavus parasiticus* Agar
CDC	Center for Disease Control
CD4	Cluster of Differentiotion 4
CFU	Colony forming Units
CT scan	Computed Tomography Scan
ELISA	Enzyme-linked Immunosorbent assay
EU	European Union
FDA	Food and Drug Administration
HBV	Hepatitis B virus
HCC	Hepatocellular Carcinoma
HPLC	High Performance Liquid Chromatography
ICRISAT	International Crops Research for the Semi Arid Tropics
KEBS	Kenya Bureau of Standards
LD	Lethal Dose
MRI	Magnetic Resonance Imaging
Ppb	Parts per billion
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

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ABSTRACT

Aflatoxin is a carcinogenic toxin produced mainly by *Aspergillus flavus* and contaminates foods including peanuts. Aflatoxin is associated with liver failure, hepatocellular carcinoma (HCC) and death. Many people are exposed to chronic levels of aflatoxins through consumption of contaminated foods. In Kenya, most efforts have been focused on aflatoxin in maize while other highly predisposed foods such as peanuts have received little attention. Also limited studies have been done to link aflatoxin to HCC. This study identified aflatoxin producing *Aspergillus* species and the type and levels of aflatoxin contamination of various varieties of peanuts (*Arachis hypogaea L.*) in Busia and Kisii Central districts. It also determined the peanut producers' exposure to aflatoxins and the prevalence of aflatoxin induced HCC among patients from the study districts. Cross-sectional and retrospective study designs with systematic random sampling technique were adopted. One hundred and two (102) peanut and urine samples were collected from peanut growers in each district and transported to Cooppers Labs, Nairobi for analysis. *Aspergillus* species were identified using plate technique of serially diluted samples on modified Rose Bengal agar. Types and levels of aflatoxins were analyzed using high performance liquid chromatography (HPLC) technique while aflatoxin B (AFB) gual in urine was determined using fluorescence Spectrophotometer. Analysis of records for patients from Busia and Kisii Central districts who attended Moi Teaching and Referral Hospital in January 2010 to December 2012 was done to determine the prevalence of HCC among the study population. The diagnosis of HCC was confirmed by the presence of AFB1 guanine adducts in urine or AFB1 albumin adduct in blood. The levels of total aflatoxin ranges were 0.1 to 268µg/kg and 1.63 to 591.1µg/kg in peanuts from Busia and Kisii Central respectively. Majority of peanuts samples had levels within Kenya Bureau of Standards (KEBS) and European Union (EU) regulatory limits for total aflatoxins. Aflatoxin type B1 was the most dominant ($t = 12.4$, $df= 3$, $P = 0.034$). Overall, the occurrence of *Aspergillus flavus* L strain and *A. flavus* S strain were significantly higher than other species identified ($H = 15.55$, $df = 4$, $P = 0.004$) in peanuts from the two districts. However, *A. flavus* S-strain was the most dominant species ($F=3.15$, $df =25$, $P=0.031$) with an overall mean occurrence of 45.1%. Oil content in peanuts decreased with an increase in aflatoxin levels ($r = -0.496$, $P = 0.031$) except in peanuts of Uganda local red variety from Kisii Central. Overall, males in both districts had slightly higher incidences (9.8%) of exposure to urinary Aflatoxin B than females at 5.4%. The prevalence of aflatoxin induced HCC among the patients was 19.73%. There is need for urgent awareness through campaign among the peanuts producers on the aflatoxin levels and promote sound practices when handling peanuts. Continuous screening of patients with liver disorders should be done for early detection of HCC. The high levels of aflatoxins in peanuts suggest the necessity to screen other foods consumed in the study areas. The findings will form basis of policy development for aflatoxin contamination control and management of aflatoxin induced HCC in Kenya.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Aflatoxins are hepatotoxic and highly carcinogenic mycotoxins that are produced by *Aspergillus* species, specifically *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins contamination of foods including peanuts are major hazards to human health and has been associated with liver failure, stunted growth in children, hepatocellular carcinoma (HCC) and death (Khangwiset *et al.*, 2011). It has been estimated that more than 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Shephard, 2003; Stronsider *et al.*, 2006).

Aflatoxins are produced in food crops such as peanuts when they are poorly dried and stored (Fung, 2004). Aflatoxins can be produced at both the pre- and post-harvest stages (Waliyar *et al.*, 2008). The primary disease associated with aflatoxin intake is hepatocellular carcinoma (HCC) or liver cancer (Wu and Santella, 2012). This disease is the third leading cause of cancer death globally after lung and colon cancers (WHO, 2008); with about 550,000-600,000 new cases reported each year (Wu and Santella, 2012). Aflatoxin contaminations of foods play a causative role in 4.6 to 28.2 % of all global HCC cases (Liu and Wu, 2010). Eighty three percent of these aflatoxin induced HCC cases occur in Eastern Asia and sub-Saharan Africa (Strosnider *et al.*, 2006). In Kenya, a high incidence rate of liver cancer was

reported in the year 2008. The study reported that in every 100, 000 people suffering from liver cancer, 8.5 % were males while 4.9 % were females (Liu and Wu, 2010). Liver cancer has an increasing incidence that parallels the rise in chronic hepatitis B infection and among the potent hepatocarcinogenic agents known is aflatoxin (Wild and Gong, 2010).

Kenya has repeatedly experienced epidemics of acute aflatoxicosis especially in the Eastern province in the years 2001, 2004, 2005 and 2006 (WHO, 2008; Liu and Wu, 2010). The largest outbreak due to maize aflatoxin poisoning was reported in the year, 2004 where 125 people died out of the 317 reported cases (Lewis, 2005). Studies conducted in other developing countries established a relationship between aflatoxin exposure and the prevalence of hepatocellular carcinoma (Williams *et al.*, 2004). While this severe outbreak was devastating, more individuals still suffer from diseases associated with low chronic levels of aflatoxin consumption in crop produce (Liu and Wu, 2010).

In the western Kenya, peanuts play a key role in food security after maize due to its capability for production even during the dry seasons. Peanuts in the region are mainly used in relishes served with the staple maize porridge commonly known as ugali or ground and made into sauce (Mutegi *et al.*, 2009). Western Kenya has repeatedly reported high levels of stunting growth in children (Mutegi *et al.*, 2009), an aspect often positively correlated with long-term ingestion of sub-lethal

doses of aflatoxins (Gong *et al.*, 2002). In addition, studies carried out in the region have shown that aflatoxin levels in peanuts exceed the minimum acceptable limits of 10μg/kg total aflatoxins, according to KEBS (2007), results in outbreaks of aflatoxicosis (Mutegi *et al.*, 2013).

Currently, aflatoxin B1 which is the most carcinogenic has been linked to high incidence of liver cancer and high mortality rate (Gachomo *et al.*, 2004). However, data on characterization of the fungi producing the aflatoxins in Kenya is limited. According to Mutegi *et al.*, (2013), aflatoxin induced liver diseases which are linked to hepatitis B infection are on the rise in Kenya. However, there is limited published information on the levels and types of aflatoxins contaminating peanuts and the *Aspergillus* species producing the aflatoxins. Data on aflatoxin exposure to the population and the occurrence of HCC in Kisii Central and Busia districts which are within the highest peanuts production zones is also limited. This study was therefore designed to determine aflatoxins producing *Aspergillus* species in the different varieties of peanuts, types and levels of aflatoxins, peoples' exposure to aflatoxins and the prevalence of aflatoxin induced hepatocellular carcinoma in Busia and Kisii Central districts.

1.2 Statement of the Problem

Aflatoxin associated health risks in human such as liver failure, hepatocellular carcinoma and death have increased over the years (Wu *et al.*, 2011). Aflatoxin contamination in foods causes 4.6-28.2% of all global HCC cases (Liu and Wu, 2010). Kenya is one of the world's hotspots for aflatoxins, with the highest incidence of acute toxicity ever documented being in 2004 and 2010. This outbreak poisoned more than 300 people in the 2004 event alone and killed more than 100 of them (MacMillan, 2014).

Increased levels of aflatoxins contamination in highly predisposed foods such as peanuts and the prevalence of aflatoxins related HCC have been reported particularly in western Kenya (Mutegi *et al.*, 2013). Despite the increase in aflatoxin contamination of peanuts in western Kenya, there is limited documented information on the levels and types of aflatoxins, *Aspergillus* species producing the aflatoxins and the effects of peanut oil content in the production of aflatoxins by the species. Data on the peanuts growers' exposure to aflatoxins as well as the prevalence of aflatoxin induced hepatocellular carcinoma in Busia and Kisii Central districts is limited. This study identified the types and the contamination levels of aflatoxin, *Aspergillus* species producing aflatoxins in peanuts (*Arachis hypogaea L.*) produced in both Busia and Kisii Central districts. The study also determined exposure to aflatoxin and the prevalence of aflatoxin induced hepatocellular carcinoma among the people living in the two study districts.

1.3 Justification

The study areas, Busia and Kisii Central Districts are in western Kenya where peanuts are mainly grown and consumed in large amounts. This region has repeatedly reported high levels of stunting growth in children and an increase in HCC (Mutegi *et al.*, 2009); an aspect often positively associated with chronic exposure to aflatoxins. Although peanuts are largely produced and consumed in Busia and Kisii Central districts, there is limited documented information on the types and level of aflatoxins. The level of people's exposure to aflatoxins and the prevalence of aflatoxin induced HCC particularly among patients with liver disorders attending Moi Teaching and Referral hospital (main referral hospital) in western region also remain unclear. The *Aspergillus* species were analyzed and the types and the levels of aflatoxins in peanuts were determined in each of the two study districts. The peoples' exposure to aflatoxins and the prevalence of aflatoxin induced HCC in these major peanuts production districts were determined. The findings of this study could be applied to form policy in controlling aflatoxin contamination and management of aflatoxin induced HCC in Kenya.

1.4 Research Questions

i. What is the occurrence of aflatoxin in peanuts produced in Busia and Kisii Central districts?

ii. What are the types and levels of aflatoxins that contaminate different varieties of peanuts produced in Busia and Kisii Central districts?

iii. What are the *Aspergillus* species producing aflatoxins in the different varieties of peanuts produced in Busia and Kisii Central districts?

iv. Does aflatoxin productions vary with oil content of peanuts?

v. What is the level of exposure of peanut producers to aflatoxins in Busia and Kisii Central districts?

vi. What was the prevalence of aflatoxin induced hepatocellular carcinoma among patients from Busia and Kisii Central districts who attended Moi teaching and Referral hospital in the period of January 2010 to December 2012?

1.5 Hypotheses

Ho1. Peanuts produced and consumed in Busia and Kisii Central districts are not contaminated with aflatoxins.

Ho2. Aflatoxins that contaminate peanuts in Busia and Kisii Central districts are not produced by *Aspergillus* species.

Ho3. Peanut producers in Busia and Kisii Central districts are not exposed to aflatoxins through consumption of peanuts.

Ho4. Aflatoxin production by *Aspergillus* species does not vary with oil content of peanuts.

Ho5. Hepatocellular carcinoma in Busia and Kisii Central districts is not linked to aflatoxins contamination of peanuts.

1.6 Objectives

1.6.1 General Objective

To determine aflatoxins in peanuts and the prevalence of aflatoxin induced hepatocellular carcinoma in Busia and Kisii Central districts in Kenya.

1.6.2 Specific Objectives

i. To determine the occurrence of aflatoxin in peanuts produced in Busia and Kisii Central districts.

ii. To determine the types and levels of aflatoxins contaminating the different varieties of peanuts produced in Busia and Kisii Central districts.

iii. To characterize *Aspergillus* species producing aflatoxins in the different varieties of peanuts produced in Busia and Kisii Central districts.

iv. To determine the relationship between oil content and aflatoxin production in the different varieties of peanuts produced in Busia and Kisii Central districts.

v. To determine the peanut producers exposure to aflatoxins in Busia and Kisii Central districts.

vi. To determine the prevalence of aflatoxin induced hepatocellular carcinoma among residents of Busia and Kisii Central districts.

1.7 General Assumptions

Peanuts producers/farmers households were drawn from only two districts; Busia and Kisii Central districts participated in this study. The two districts were chosen due to high production and eating habits of peanuts that increases aflatoxin exposure. It was assumed that most of the aflatoxins that could have resulted to HCC apart from maize could be from peanuts. It was also assumed that the two districts were representative of Busia and Kisii Counties. In cases where households' heads were not available for personal interviews and sample collection, it was assumed that the information given by the family member over 18 years of age present at the households was credible.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aflatoxins

Aflatoxins are a group of chemically similar toxic fungal metabolites (mycotoxins) produced by some *Aspergillus* species most commonly *A. flavus* and *A. parasiticus* (Mutegi *et al.*, 2013). Other *Aspergillus* species that rarely produce aflatoxins are *A. nomius* and *A. niger.* Aflatoxins are highly toxic compounds and can cause both acute and chronic poisoning in humans and many other animals such as cows. Their importance was first established in 1960 when 100,000 turkeys and other poultry in the United Kingdom died in a single event (Pildain *et al.*, 2008). The cause of death was eventually traced to a toxic contaminant in groundnut meal in birds feed which was later named aflatoxin.

Four types of aflatoxins that are commonly produced in foods are aflatoxins B1, B2, G1 and G2. Aflatoxin B1 is produced by both *A. flavus* and *A. parasiticus* and it is the most common and toxic contaminant of food and feed (Pildain *et al.*, 2008). A common metabolite of aflatoxin B1 and B2 is the highly toxic M1 and M2 found in milk of animals which have consumed contaminated feed (Tara, 2005). While both *A. flavus* and *A. parasiticus* can produce aflatoxin B toxins, *A. parasiticus* exclusively produce the G1 and G2 aflatoxins (Fung, 2004). Aflatoxins B2 and G2 are the dihydro- derivatives of B1 and G1. They were so named because of their strong blue or greenish-yellow fluorescence in ultraviolet light.

These properties were used to facilitate rapid development of methods for monitoring food commodities such as grains for the presence of aflatoxins in the early 1960s (Kensler *et al.*, 2011). Chemically, aflatoxins are difurocoumarolactones (difurocoumarin derivatives). Their structure consists of a bifuran ring fused to a coumarin nucleus with a pentenone ring (in B and M aflatoxins) or a six membered lactone ring in G aflatoxins as shown in figure 2.1.

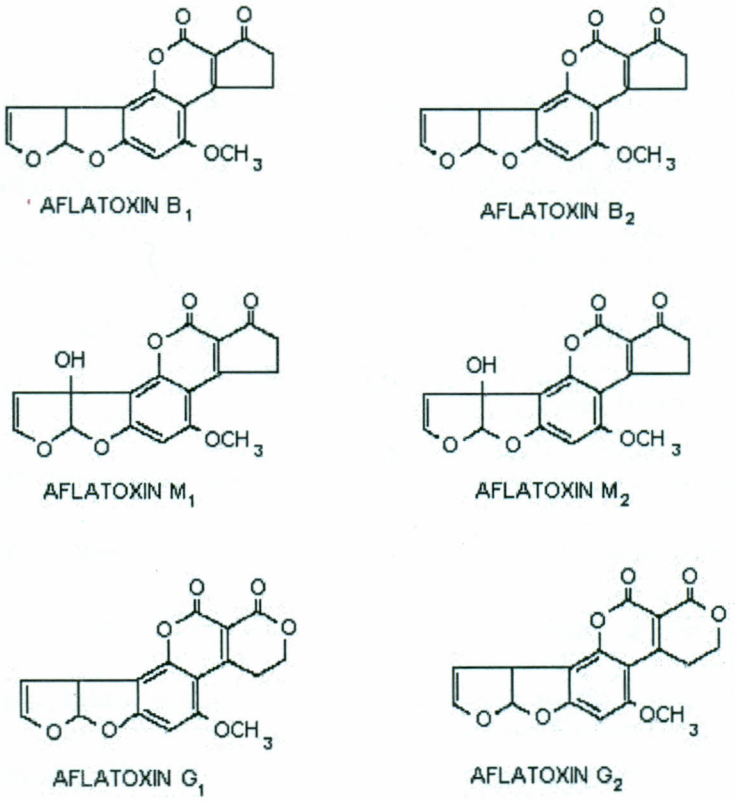


Figure 2.1: Structure of aflatoxins B and G metabolites (Source: Wild and Turner, 2002).

The aflatoxins occur mostly in developing countries in tropical and sub-tropical areas between 40° N latitude and 40° S latitude when the temperatures are between 24 and 35 °C with moisture content exceeding 7% (Williams *et al.*, 2004). They accumulate during the post-harvest and when storage is under conditions that promote fungal growth. Other aflatoxins that are rarely found in foods include sterigmatocystin, ochratoxins, cyclopiazonic acid and zearalenone (Youssef *et al.*, 2008).

2.1.1 Occurrence of aflatoxins in human food

Aflatoxins may be present in a wide range of food commodities, particularly cereals, oil seeds, spices and tree nuts. Maize, groundnuts (peanuts), pistachios, brazils, chillies, black pepper, dried fruit and figs are all known to be high risk foods for aflatoxin contamination, although the toxin is also detected in many other commodities as diverse as marijuana (Probst *et al.*, 2007; Fratamico, 2008). Milk, Cheese and other dairy products are also known to be at risk of contamination by aflatoxin M. The highest levels are usually found in commodities from warmer regions of the world where there is a great deal of climatic variation (Fratamico, 2008). It is important to note that, although it is primary food commodities that often get contaminated with aflatoxins by mould growth, these toxins are very stable in hot conditions. For this reason, aflatoxin contamination is a problem in various processed foods such as peanut butter, unrefined oils, and peanut snack foods and rejects nuts (Fratamico, 2008).

Contamination of peanuts with aflatoxins which are secondary metabolites produced by the fungi mainly *Aspergillus flavus* is a serious problem (Mutegi *et al.*, 2009). The fungi invade groundnuts seeds before harvest, during post- harvest drying/ curing and during storage (Fung, 2004). Optimum growth conditions for *A. flavus* during post harvest are between 25°C and 30°C and humidity levels of 0.99a_w, with production of aflatoxin occurring optimally at 25^{0}C and 0.99 a_w (Giorni *et al.*, 2009). Peanuts play an important role as a food and cash crop for smallholder farmers in Kenya. The crop is grown and managed mostly by resource-poor farmers especially women. Recent studies have shown that peanuts in Kenya are highly contaminated with aflatoxins (Mutegi *et al.*, 2013), but information gaps exist on the distribution of aflatoxin producing *Aspergillus* species. Further, few studies have been done to characterize the fungi and the aflatoxins types and people's exposure to the aflatoxins in Kenya. Therefore, there is need for determination of the *Aspergillus* species producing the aflatoxins and also the types and levels of aflatoxins contaminating peanuts in Kenya as well as the associated health risks such as HCC.

2.1.2 Aflatoxin exposure and health consequences

Humans are exposed to aflatoxins mainly by consumption of food commodities such as maize and peanuts contaminated during growth, harvest or storage. The levels of aflatoxin in the grain products vary from less than 1 µg/kg to greater than 12,000 µg/kg (Groopman *et al.*, 2008). In developed countries, harmful aflatoxin

exposure has been mostly eliminated due to strict regulatory limits on traded foods through food monitoring and optimal drying and storage practices (Stronsider *et al.*, 2006). However, it is difficult to apply these strategies in developing countries because of differences in food production, such as the prominence of subsistence farming in developing countries. In addition, these countries often lack systems necessary for routine food monitoring as well as optimal drying and storage practices (Lewis *et al.*, 2005) and they consume the moldy grains. In a recent outbreak of aflatoxicosis in Kenya, 55% of sampled maize products in local market had aflatoxin levels higher than the Kenya regulatory limit of 20 parts per billion (ppb), 35% had levels > 100 ppb, and 7% had levels > 1,000 ppb (Lewis *et al.*, 2005). Generally, diets may contain AFB1 and AFB2 in concentration ratios of 1.0 to 0.1. However, if all four aflatoxins occur, AFB1, AFB2, AFG1 and AFG2 present in concentration proportions of 1.0:0.1:0.3:0.03. Among the types of aflatoxins found in foods, aflatoxin B1 is the most abundant, toxic and most potent as a carcinogen (Groopman *et al.,* 2008).

Humans come into contact with aflatoxin through several ways such as direct ingestion of contaminated products (Wagacha and Muthomi, 2008), transmission through milk as M1 and M2 metabolites (Bahout and El-Abbassy, 2004), and through consuming the meat of animals fed on contaminated feed. Aflatoxin can also pass through human skin (Wagacha and Muthomi, 2008) through contact with contaminated produce. Aflatoxin B1 can permeate through the skin and dermal

exposure to this aflatoxin in hot and dry environmental conditions can lead to serious health problems (Boonen *et al.*, 2012). The toxin can also pass through the respiratory system, especially in people involved in peanut harvesting, shelling, storage, marketing and transportation (Mehan *et al.*, 1991). Populations with poor nutritional and health status are typically more vulnerable to aflatoxin poisoning (Gong *et al.*, 2002).

When aflatoxin is consumed, it can exert toxicity in several ways; it may alter intestinal integrity and consequentially its functions (Gong *et al.*, 2008) or modulate the expression of cytokines. This may result to stunted growth in children and/or immune suppression (Wu, 2010). In the liver, aflatoxins mainly AFB1 is metabolized by cytochrome P450, to AFB1-8, 9-exo-epoxide and AFB1-8, 9-endo-epoxide. AFB1-8,9-exo-epoxide is more toxic and active as it binds to DNA to form the predominant 8,9-dihydro-8-(N7-guanyl)-9-hydroxy AFB1 (AFB1-N7-Gua) adduct and induce hepatocellular carcinoma or liver cancer (Wu, 2010) or binds to liver proteins (serum albumin) to form long-5 lived lysine adducts (Wild and Turner, 2002) leading to their failure, potentially resulting in acute aflatoxicosis. The aflatoxin and disease pathways in humans are shown in figure 2.2.

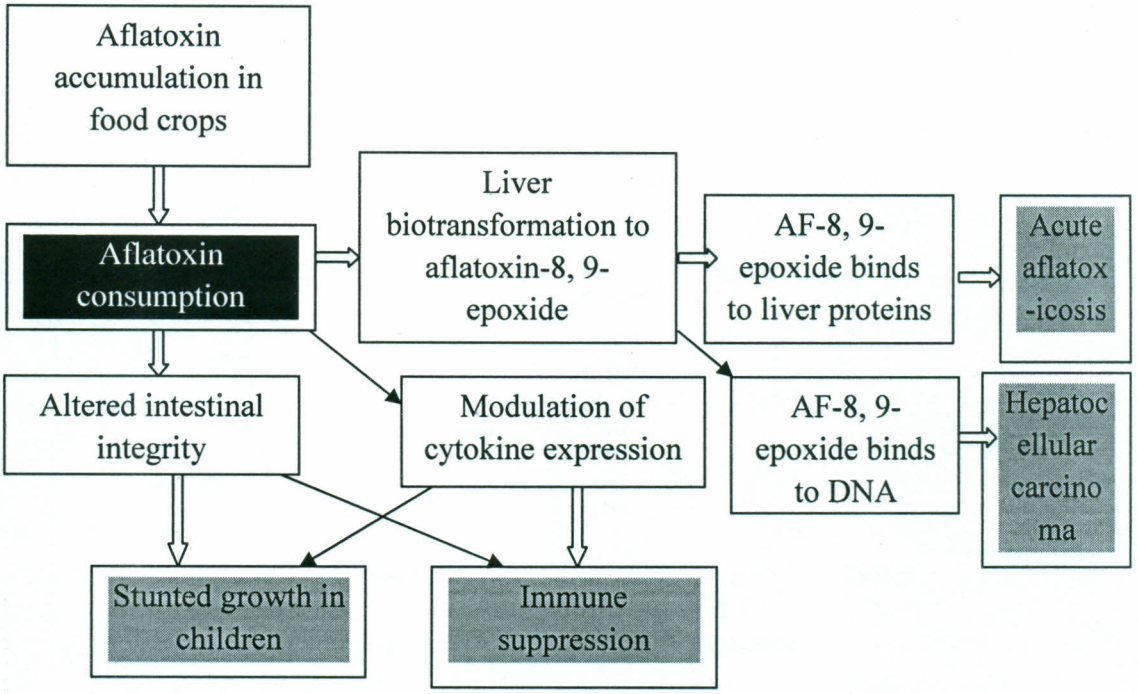


Figure 2.2: Aflatoxin and disease Pathways in humans (Source: Wu, 2010).

In addition, the epoxide is conjugated by glutathione S-transferases and then further metabolized to form aflatoxin-mercapturic acid detoxification product (Scholl *et al.*, 2008). Other metabolites formed from AFB1, include AFM1, AFQ1 and AFP1 (Wild and Turner, 2002). These metabolites and other naturally occurring aflatoxins, AFG1, AFG2 and AFB2, are less mutagenic, carcinogenic and toxic than AFB1, because they are poorer substrates for epoxidation and the AFB1-8, 9 exo-epoxide intercalates more readily into DNA (Wild and Turner, 2002). Figure 2.3 shows the metabolism of aflatoxin B1 leading to reactive metabolites and biomarkers.

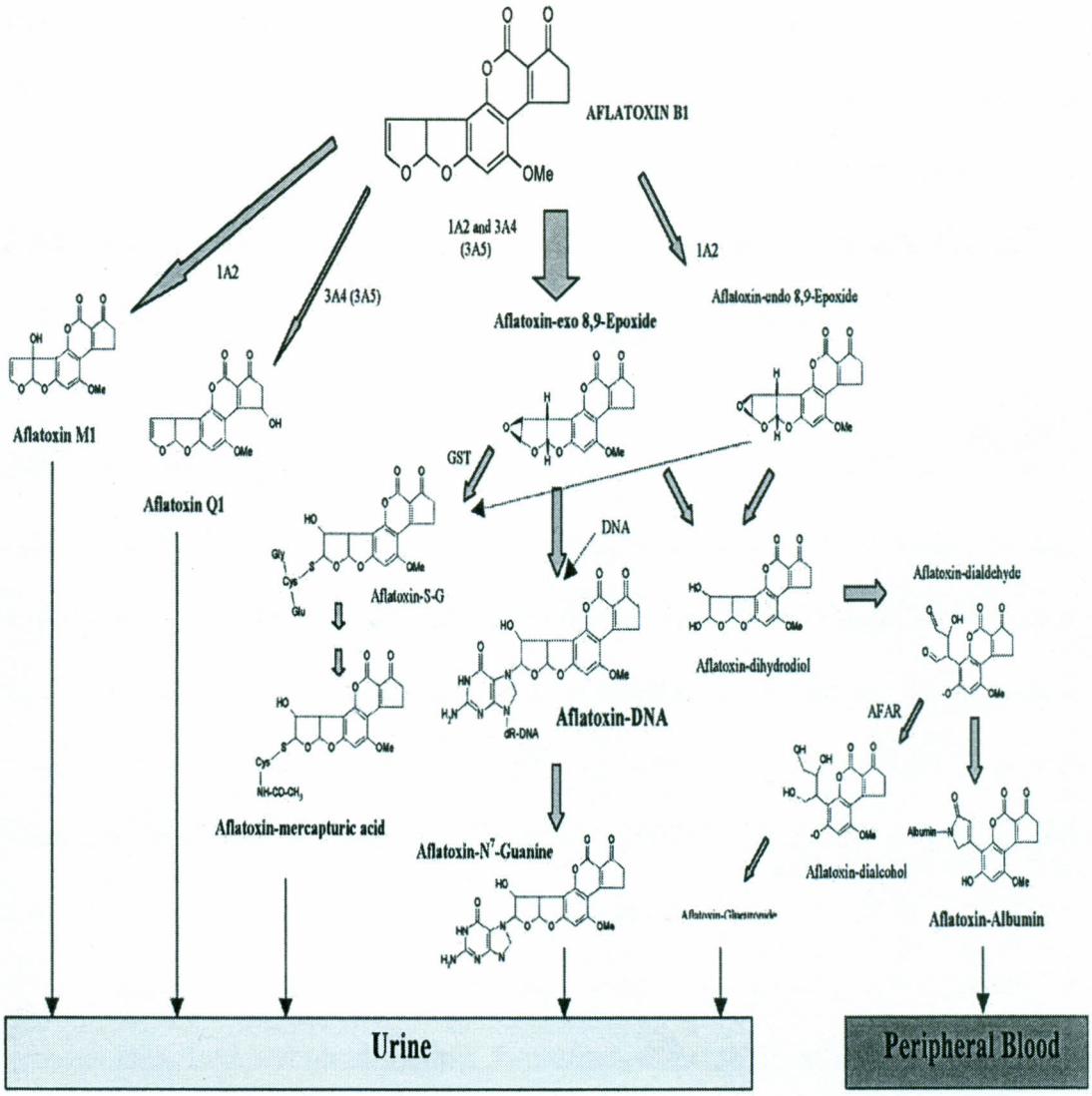


Figure 2.3: Principle metabolism of aflatoxin B1 leading to reactive metabolites and biomarkers (Source: Wild and Turner, 2002).

Epidemiological and clinical studies reveal that exposure to large doses (>6000mg) of aflatoxin may cause acute toxicity with lethal effect whereas exposure to small doses for prolonged periods is carcinogenic (Groopman *et al.*, 2008). The adverse health effects of aflatoxins on humans can be categorized into acute and chronic toxicity.

2.1.3 Acute exposure to aflatoxins

Acute aflatoxin poisoning has been reported in both humans and animals (Garland and Reagor, 2007) and has been linked to the various aflatoxin outbreaks reported in several parts of the world. The effect of aflatoxins on humans depends on a number of factors including species type, ingestion levels, susceptibility, age and aflatoxin concentration (Meissonnier *et al.*, 2005), in addition to gender and duration of exposure (Bunger, 2005). Studies reveal that exposure to large doses (>6000 μg/kg at once) of aflatoxin may cause acute toxicity accompanied by symptoms such as acute hepatitis, hemorrhage, acute liver damage, jaundice, edema, vomiting and sometimes death (Jolly *et al.*, 2007).

The conditions that increase the likelihood of acute aflatoxicosis in humans include limited availability of food, environmental conditions that favour fungal growth in crops and commodities, and lack of regulatory systems for aflatoxin monitoring and control (Unnevehr, 2003). There have been several reported cases of acute aflatoxicosis in Africa associated with consumption of contaminated

home-grown maize, including the outbreaks that occurred in Kenya in 1982, in which 12 people died, and in the year 2004 in which 317 people became ill and 125 died in the Eastern Province (Nyikal *et al.*, 2004; Lewis *et al.*, 2005; Strosnider *et al.*, 2006; Probst *et al.*, 2007).

2.1.4 Chronic exposure to aflatoxins

The long-term exposure to moderate or low aflatoxins concentration leads to chronic aflatoxin poisoning. Chronic exposure does not lead to symptoms as dramatic as acute aflatoxicosis but may result in carcinogenic and immunosuppressive effects. However, children are particularly affected leading to kwashiorkor, liver cirrhosis and delayed development (Abbas and Hamed, 2005).

Chronic exposure also leads to liver cancer or liver diseases (Collins *et al.*, 2010). The Center for Disease Control (2004) has estimated that more than 4.5 billion people in developing countries are chronically exposed to aflatoxins in their diet. Aflatoxin and immunosuppression in humans is relatively less well characterized, although it is likely to cause enormous significance in health globally (Williams *et al.*, 2004). Several studies in humans have shown evidence of immunomodulation (Turner *et al.*, 2003, Jiang *et al.*, 2008), although the actual outcomes of such immunomodulation have not yet been characterized in humans. However, aflatoxins immunotoxicity has been associated with stunted growth in children (Wu and Khlangwiset, 2010). The mechanism by which aflatoxin results in growth

impairment is not yet known; a possible explanation is altered intestinal integrity as a result of cell toxicity and/or immunomodulation (Gong *et al.*, 2008).

Studies have demonstrated that chronic exposure to aflatoxins in animals can cause growth inhibition and immune suppression (Khlangwiset *et al.*, 2011). Nursing animals can be affected and aflatoxin M1 may be excreted in the milk of cattle and other dairy animals. This in turn poses potential health risks to both animals and humans who consume the milk. Chronic aflatoxin exposure in animals is associated with impaired reproductive efficiency, reduced feed conversion efficiency, increased mortality rates, reduced weight gain, anaemia and jaundice. In laying hens, aflatoxicosis causes enlarged fatty liver and decreased egg production (Lubulwa and Davis, 1994).

2.1.5 Aflatoxins and children

Foetal and childhood environment as well as the nutritional status of the pregnant mother and the infant are critical for growth and risk of disease in early life. Malnourishment is one of the common problems in developing countries of which in most cases it is due to exposure to high levels of mycotoxins especially aflatoxins (Gong *et al.*, 2002). It has been proved that these aflatoxins are immunogenic, teratogenic, and retard growth of children. In the developing countries such as India where the environmental conditions favor aflatoxins production, high exposure to aflatoxins occurs throughout these regions. A study

in West Africa showed a significant correlation between aflatoxin exposure and stunted growth in children who are exposed to aflatoxin from neonatal stages as aflatoxins has the capacity to cross the placenta barrier. It can also cause genetic defects during foetal stages of life (Gong *et al.*, 2002).

Ingestion of aflatoxin, viral diseases, and genetic factors have been suggested as possible etiological agents of childhood liver cirrhosis. There are reports indicating that children exposed to aflatoxin in breast milk and dietary items such as unrefined groundnut oil, may develop cirrhosis (Gong *et al.*, 2002). Malnourished children are also prone to childhood cirrhosis due to consumption of contaminated food. Several investigators have indicated aflatoxin as an aetiological agent of Reye's syndrome in children in Thailand and in New Zealand though there was no conclusive evidence (Gong *et al.*, 2008). Epidemiological studies have shown the involvement of aflatoxins in Kwashiorkor mainly in malnourished children. The diagnostic features include edema, damage to liver tissues among others.

2.1.6 Aflatoxin exposure and hepatocellular carcinoma

Outbreaks of aflatoxicosis in humans have been attributed to ingestion of contaminated food such as maize and groundnuts (Wu and Khlangwiset, 2010). The link between aflatoxin exposure and acute aflatoxicosis and also with liver cancer are well documented (Azziz-Baumgartner *et al.*, 2005), whereas the

association of aflatoxins exposure with stunting in children (Gong *et al.*, 2002) and immunosuppression (Ferlay *et al.*, 2010) remains unclear.

Hepatocellular carcinoma is the third leading cause of cancer deaths worldwide with 749,000 cases and over 695,000 deaths annually with 85% of the cases occurring in developing countries (Ferlay *et al.*, 2010). Aflatoxin exposure plays a causative role in 4.6-28.2% of all global HCC cases (Liu and Wu, 2010). Most cases occur in sub-Saharan Africa, Southeast Asia, and China, where populations suffer from both high Hepatitis B Virus (HBV) prevalence and largely uncontrolled exposure to aflatoxin in foods (Wu and Santella, 2012). In these regions, the incidence of HCC ranges from 10 to 100 cases per 100,000 global cases reported per year (Ferlay *et al.*, 2010); while in developed countries, the rate of HCC incidence is much lower (1-10 cases/100,000/yr).

A study done in 1971 determined AFB1 levels in major diet components from different areas of Uganda over one year and found that the incidence of AFB1 contamination was particularly high in provinces with high HCC incidence (Alpert *et al.*, 1971). Similar associations were reported in Swaziland (Keen and Martin, 1971) and Thailand (Shank *et al.*, 1972). Studies that determined AFB1 ingestion levels by collecting "food from the plate" consistently found significant associations between calculated AFB1 ingested daily dose and adult male

incidence of HCC in different parts of Swaziland (Peers *et al.*, 1976) and other countries in Southeastern Africa (Van Rensburg *et al.*, 1985).

Data from Kenya quantifying daily intake of aflatoxins based on assumed intakes of AFB1 contaminated food found those with higher AFB1 intakes were residents in areas with an increased incidence of HCC (Peers and Linsell, 1973). Later, the first study measured urinary AFB1- DNA adducts in Kenya and found a degree of correlation between AFB1 exposure and HCC and no synergistic effect of exposure to AFB1 and HBV infection on HCC risk (Autrup *et al.*, 1987). Combining these reports together (Alpert *et al.*, 1971; Keen and Martin, 1971; Shank *et al.*, 1972; Peers and Linsell, 1973), confirmed higher HCC incidence with higher levels of AFB1 consumption (Wogan, 1975). Since the study done by Autrup *et al.*, (1987) in Kenya, there is no study that has determined the correlation between aflatoxin exposure and HCC infection particularly among the population in the study areas. The present study has established the association between aflatoxin exposure to peanuts farmers and the prevalence of HCC infection among people living in both Busia and Kisii Central districts.

2.1.7 Aflatoxins and hepatitis B and C infections

Aflatoxin B1 is the most toxic and liver carcinogen known compared to the other aflatoxins (Gachomo *et al.*, 2004). Aflatoxin, metabolized by enzymes in the liver, binds to proteins and causes acute toxicity (Wild and Turner, 2002). Aflatoxin exposure causes acute liver damage and liver cirrhosis, as well as development of tumors or other genetic effects (Wild and Turner, 2002). Liver cancer has increased in incidence and parallels chronic hepatitis B and hepatitis C infections (Groopman *et al.*, 2008). Studies have shown that persons with hepatitis B infection who live with chronic aflatoxin exposure have a risk of contracting liver cancer that is approximately 30 times greater than people who are hepatitis B-negative (Groopman *et al.*, 2008). The incidence of liver cancer in Country wide in Kenya in the year 2008 was high with 100,000 reported cases, 8.5% were males while 4.9% were females (Lu and Wu, 2010).

2.1.8 Aflatoxins and liver cirrhosis

The link between aflatoxin and liver cirrhosis is not as well documented as that with liver cancer. Some studies have indicated that there is sufficient evidence to associate aflatoxin with liver cirrhosis especially in persons with hepatitis C (Lu and Wu, 2010). A study on aflatoxin exposure and the cause of liver cirrhosis in Gambia found that chronic hepatitis B infection and aflatoxin exposure either separately or in synergy were the agents responsible for most cirrhosis cases in the West African populations (Kuniholm *et al.*, 2008). Sub-Saharan African and Asian

populations that have endemically high hepatitis B and C infections rates have a significantly increased disease burden for liver cancer. However, studies are needed to understand the mechanisms of aflatoxin exposure in conjunction with hepatitis B and C viruses (Levrero, 2006). In addition, other aflatoxin interactions are likely contributors to the disease burden, but still remain to be identified (Groopman *et al.*, 2008).

2.1.9 Aflatoxin biomarkers

Biomarkers of aflatoxins exposure are most commonly used for assessing exposure or intervention in epidemiological studies. A biomarker of exposure refers to measurement of the specific interactive products in human body compartment or fluid that indicates the presence and magnitude of current and past exposures. Biomarkers of aflatoxins reflect the presence and magnitude of a biological response to aflatoxin exposure (Kensler *et al.*, 2011). The knowledge of aflatoxin metabolism and carcinogenesis in humans provides the basis for the use of aflatoxins biomarkers to measure exposure and determine risk.

Urinary measures of AFB1-mercapturic acid and serum aflatoxin-albumin adducts are used as biomarkers of internal dose (Wild and Turner, 2002), while AFB1-N7-guanine in urine serves as a biomarker of biologically effective dose (Kirk *et al.*, 2005). The measurement of aflatoxin-DNA and protein adducts are of importance because they are derived from the carcinogenic metabolites aflatoxin-8, 9-exo-

epoxide (Kensler *et al.*, 2011). Measurements of aflatoxin biomarkers in urine reflect recent exposure, while aflatoxin-albumin adducts in serum reflect cumulative exposures over the past 2-3 months (Groopman *et al.*, 1992). Studies conducted in China and The Gambia reported high incidences of HCC and urinary aflatoxin biomarkers; aflatoxin M1 and aflatoxin-N7-guanine (Gong *et al.*, 2002). A similar association between dietary aflatoxin intake and aflatoxin-albumin levels was observed. In separate studies, a positive correlation was shown between population estimates of aflatoxin exposure and the proportion of HCC patients with TP53 249ser mutation (Kirk *et al.*, 2005). These findings suggested that the detection of TP53 249ser mutation may be used as a biomarker of an early neoplastic event and a chronic exposure to aflatoxin or a combination of both.

2.1.10 Diagnosis and treatment of aflatoxicosis

There are many signs and symptoms that are used to diagnose aflatoxicosis, depending on the level of exposure. The signs and symptoms include vomiting, abdominal pain and hemorrhaging, pulmonary edema, acute liver damage, loss of digestive tract function, convulsions, cerebral edema, and coma. Other symptoms are yellow eyes, vomiting, abdominal swelling, and water in the abdomen, leg swelling, general weakness, and drowsiness (Reddy and Raghavender, 2007). In humans, aflatoxins can be detected by measuring AFB1 guanine adducts in urine or AFB1 albumin adduct in serum (Bragulat *et al.*, 2001). The onset of symptoms is relatively slow, occurring about 8 hours after exposure to the toxin. In cases of

ingestion, feeding large quantities of an adsorbent, such as clay additives like NovaSil may be used to reduce the onset of symptoms (Reddy and Raghavender, 2007).

2.1.11 Prevention and control of aflatoxicosis

The measures used to prevent and /or control aflatoxicosis include education through awareness campaigns, advances in Biomarker Technology and organized surveillance systems. These measures have been shown to be effective in preventing and controlling the adverse health effects of aflatoxin. Proper drying and storage of food especially maize and peanuts.

2.1.11.1 Awareness campaigns

During the 2005 aflatoxin outbreak in Kenya, individuals received information on maize processing and storage through an awareness campaign run by the Food and Agricultural Organization (FAO) of the United Nations and Kenya's Ministries of Health and Agriculture. In a later study, it was shown that those individuals receiving this information had lower serum aflatoxin levels than those who did not receive this information (Liu and Wu, 2010). Awareness campaigns should use systems that are already in place for disseminating information to subsistence farmers, for example giving information to many organizations and using multiple means for spreading information to a broad range of people, given the diversity of cultures and the ruralness of villages (Strosnider *et al.*, 2006).

2.1.11.2 Advances in biomarker technology

Studies of how animals and humans metabolize aflatoxin have provided opportunities to develop chemoprevention approaches in human populations. The examination of biomarkers enables scientists to pinpoint areas where chemoprevention measures may be applied to crops, limiting the impact of aflatoxin contamination (Wild and Turner, 2002). Chemoprevention measures such as clinical interventions to control aflatoxin contamination can be considered a "secondary" intervention as it cannot reduce aflatoxin levels in food, but can reduce aflatoxin-related illness by reducing the bioavailability of either aflatoxin or its reactive oxygen species that binds to DNA to initiate cancer (Wu and Khlangwiset, 2010).

2.1.11.3 Formulation of policies using research evidences

Formulation of policies in the Sub-Saharan region to recognize the implementation of pre- and post-harvest aflatoxin control is useful to increase food production and ensure food safety for the protection of the health of people (Hell and Mutegi, 2010). Research on aflatoxins in Africa focusing on the priority areas including documenting the impact of aflatoxin on health would play a major role in the development of policies. It would also be useful to educate stakeholders and farmers especially in the rural areas on the dangers of selling and consuming moldy foods. Research would also recommend the development of infrastructure for strict surveillance on aflatoxins and other mycotoxins contamination.

Coordination of resources would also ensure the development of early warning mechanisms, especially in the highly prone areas, to avert the cases of acute aflatoxicosis that can lead to many deaths (Hell and Mutegi, 2010).

2.1.11.4 Management of aflatoxicosis

There is no specific cure for aflatoxicosis (Peterson *et al.*, 2006). Treatment is supportive and includes fluid therapy to correct dehydration and maintain the hydration status, anti-emetic medications such as metoclopramide or chlorpromazine to control vomiting and administration of antibiotics such as amoxicillin, amoxicillin/clavulinic acid or cephalexin to control bacterial co infections. In cases of chronic aflatoxicosis, management measures of chronic aflatoxicosis include administration of drugs such as famotidine, ranitidine or carafate and vitamin K1 that protects the gastrointestinal tract from destruction by the toxins (Peterson *et al.*, 2006).

2.2 *Aspergillus* species producing aflatoxins

Aspergillus is a filamentous, cosmopolitan and ubiquitous fungus found in nature (Pildain *et al.*, 2008). It is commonly isolated from soil, plant debris, and indoor air environment. The genus *Aspergillus* includes over 185 species. Around 20 species have so far been reported as causative agents of infections in humans. Among these, *Aspergillus flavus* is the most commonly isolated species, followed by *Aspergillus parasiticus* in the production of aflatoxins in foods. *Aspergillus*

flavus and *A. parasiticus* are the two most important species and are found in both the soil and the air (Klich, 2002). When conidia (spores) encounter a suitable nutrient source and favorable environmental conditions, the fungus rapidly colonizes, establishes and produced aflatoxin (Klich, 2002). In addition to producing aflatoxins, *A. flavus,* also produces cyclopiazonic acid (Dorner, 2008). *A. flavus* can infect and reproduce in peanuts at both pre- and post-harvest stages. Under the section *Flavi*, *Aspergillus flavus* is the dominant species that produces aflatoxins in foods including peanuts in Kenya (Mutungi *et al.*, 2008).

Aspergillus flavus isolates can be grouped into two phenotypes based on the size of the sclerotia as *S* strains which contain aflatoxin Q (aflQ) toxigenic gene with numerous small sclerotia (average diameter < 400 μm) and high levels of aflatoxin B1 and B2 production especially in wetter conditions. The *L* strain of *Aspergillus* species contains aflatoxin D (aflD) toxigenic genes which produce fewer, larger sclerotia and, generally, less aflatoxin in wetter conditions but high levels of aflatoxins in dry conditions (Gachomo *et al.*, 2004). Other effects of the *Aspergillus* fungi contamination in peanuts include pre-emergence and seedling rot caused by *A. niger*, *A. flavus*, *Rhizopus* species, *Penicillium* among others species.

A. nomius has a mycotoxin profile similar to *A. parasiticus* but morphologically resembles *A. flavus* (Peterson *et al.*, 2001). The species was considered rare, but recent studies indicate that *A. nomius* is widely distributed and might be of

economic importance (Ehrlich *et al.*, 2007). Other fungi known to produce aflatoxins but encountered less frequently in nature are *A. bombycis*, *A. ochraceoroseus, A. nidulans* and *A. pseudotamarii* (Peterson *et al.*, 2001). Previous studies have isolated fungi including *Aspergillus* species from peanuts in Eastern Africa (Ismail, 2001; Gachomo *et al.*, 2004). A high prevalence of *A. flavus*, *A. niger* and *Penicillium* species was reported on samples of peanut and desiccated coconut from Nairobi and Kampala (Ismail, 2001). Similarly, a study on peanut samples collected from markets in Nairobi, Kenya, found *A. parasiticus* and *A. flavus* among other fungi (Gachomo *et al.*, 2004). However, the *Aspergillus* species that produces aflatoxins in the present study areas could be different.

2.3 Peanut and its importance in food security

Peanut or groundnut is produced in China, India, the United States of America and many sub-Saharan African countries. Developing countries account for 92 per cent of total global groundnut production (Talawar *et al.*, 2005). The common varieties of peanuts include Spanish, Virginia and Valencia (Edinformatics, 2005). In Kenya, the common peanuts varieties grown especially in the western region of the country are Valencia red, Local red which have two bright red kernels, Homabay local with two to three large kernels, white in color and Uganda local red, which have two to three extra large kernel size which are red in color (Mutegi *et al.*, 2009).

In Kenya, the crop is mainly grown in parts of the Nyanza and Western provinces, and to a lesser extent in the Rift Valley, Coast and Eastern provinces (Shephard, 2003). In these regions, peanut is significant both as a cash and food crop, and has at least two harvest seasons per year. Peanuts play a significant role in nutrition in many developing countries due to its high protein, oil, fat and carbohydrates contents. It contains several minerals, including Na, K, Mg, Ca, and Zn among others, as well as vitamins E, K and B (Atasie *et al.*, 2009). Due to its high nutritional value, it has several uses such as weaning and therapeutic food, in confectionery, and as an animal feed (Atasie *et al.*, 2009). In western Kenya, other sources of protein, especially fish are expensive, and therefore, peanuts provide a less costly protein alternative (Mutegi *et al.*, 2009).

In the western region of Kenya, peanuts are mainly used in relishes served with maize porridge commonly referred to as *ugali;* boiled; ground and made into a sauce; and roasted or fried (Okoth and Ohingo, 2004). Therefore, efforts should ensure minimal losses from aflatoxins in terms of quality and quantity. It has been found that populations with poor nutritional and health status such as those in the study areas are typically more vulnerable to aflatoxin poisoning (Gong *et al.*, 2002). Despite this, most past efforts aimed at addressing food security in these areas have emphasized only on nutritional quality and food availability and ignored food safety improvements (Unnevehr, 2003). Health effects caused by

aflatoxins are varied and range from a minor irritation to death (Nyikal *et al.*, 2004).

2.3.1 Levels of aflatoxins in peanuts and feed stuffs

Aflatoxins are considered unavoidable contaminants of food and feed, even where good manufacturing practices have been followed. Establishing tolerance levels of aflatoxin in peanut and in other crop commodities has remained contentious resulting in different standards for the same commodity. For populations that depend on peanut as a source of food such as the one in the study areas, tolerance levels for aflatoxin have a direct impact on food safety. Strict standards are unlikely to improve health significantly as local produce is not necessarily subjected to inspection (Wu, 2004).

Studies have shown that the strict European Union standard would negatively affect export opportunities especially for African countries which are not able to meet these strict regulations (Dimanche, 2001). Otsuki *et al.* (2001) documented that the European Union (EU) regulation on aflatoxins resulted in reduced trade flow (63 per cent lower than when the *Codex Alimentarius* international standards were followed). Several factors including survey data, toxicology data, aflatoxin distribution and legislation played a role in establishing limits and regulations for peanuts and peanut products (ICRISAT, 2007). Inconsistencies in standards are indicated by the different tolerance levels in reference to the same commodity

across countries and economic commissions. The EU has one of the strictest standards, which specifies 2 µg/kg of Aflatoxin B1 and 4 µg/kg of total aflatoxins (Wu, 2004). India allows 30 µg/kg of total aflatoxins in their peanuts while for the US Food and Drug Administration, a safe limit for peanuts for human consumption is 20 µg/kg with the exception of milk that has an action level of 0.5 ppb for aflatoxin M1 (Kpodo and Bankole, 2008).

In Kenya, the safe limit for peanuts for total aflatoxin was set at 20 µg/kg, though it was recently changed to 10 µg/kg of total aflatoxin in peanuts or maize and 5 µg/kg aflatoxin B1 (Kenya Bureau of Standards, 2007), above which results to aflatoxicosis. Countries such as Cuba, Dominica, Malaysia and Portugal have zero tolerance to aflatoxin in peanuts (ICRISAT, 2007). Animal feed has higher tolerance levels for aflatoxin compared to peanuts for human consumption (Tara, 2005). The normal level for corn and other grains intended for breeding beef cattle and breeding swine is 100 ppb, while for corn and other grains intended for finishing (feed lot) for beef cattle is 200 ppb. For cotton seed meal intended for beef cattle, swine or poultry is 300 ppb (Tara, 2005).

2.3.2 Factors that facilitate aflatoxin production in peanuts

Contamination of peanut by aflatoxin producing fungi and subsequent production of aflatoxin can occur during pre- and post harvest periods (Donner *et al.*, 2010). Aflatoxin contamination of groundnut is widespread where the crop is grown

under rain fed conditions (Reddy *et al.*, 2003). Drought stress, erratic rains and elevated soil temperatures that are common in sub-Saharan Africa promote aflatoxin contamination. Attack of peanut pods by pests and diseases contribute to aflatoxin contamination (Bankole *et al.*, 2006) although some varieties are less susceptible than others, especially the local varieties which are more susceptible (Reddy *et al.*, 2003). Poor seed storage, poor or inadequate drying, pods damaging and poor transportation lead to conditions conducive to contamination (Waliyar *et al.*, 2005).

2.3.3 Effects of peanuts oils on growth of *Aspergillus* species and aflatoxin production.

A large number of compounds originated from plants including essential oils from foods and microorganisms have been proven as strong inhibitors of aflatoxin (AF) biosynthesis (Tsigarida *et al.,* 2000). Inhibition of growth and aflatoxin production by *A. flavus* and *A. parasiticus* by spice oils and their active components has been reported (Reddy *et al.,* 2002).

Studies towards identification of the target sites of these inhibitors have shown that they may act via interfering with the signal transduction regulatory networks involved in AF gene expression, blocking activities of AF biosynthetic cytosolic enzymes, down-regulating fungal genes of the oxidative stress defense system that combats metabolic and environmental stressors. The oils also inhibit fungal

pathogenesis factors, disrupting mitochondrial respiration, a critical process that provides acetyl-CoA for AF biosynthesis and they are also associated with morphological alterations in the mycelium, such as vacuolation of cytoplasm and attenuation of cell wall (Rasaninghe *et al.*, 2002). This suggests that the integrity of the cell barriers particularly that of the cell wall, may be crucial in the regulation of aflatoxin production and excretion (Rasaninghe *et al.*, 2002).

There are no studies documenting the effect of peanuts oil on growth of *A. flavus* and aflatoxin production in Kenya whereas such knowledge would be important in selection of peanut varieties for farming that are resistant to *Aspergillus* fungi that produces aflatoxin. The current study therefore evaluated the effect of peanut oils on growth of *Aspergillus* species and aflatoxin production in peanuts collected from Busia and Kisii Central Districts.

2.3.4 Management measures for aflatoxin in peanuts

The measures can either target pre or post-harvest stages. Growing peanut cultivars for aflatoxin resistance has been extensively researched on by ICRISAT, but still no peanut variety has yet been totally resistant to aflatoxin contamination. Use of biocontrol agents can be used as a better pre-harvest measure as use of fungicides or chemicals (ICRISAT, 2007). Bio-control agents have been reported to reduce contamination in the field by 77-98 per cent (Dorner, 2008). Use of non-

toxigenic strains of *A. flavus* and *A. parasiticus* (Dorner, 2008). *Streptomyces* spp. (strain ASBV-1) has also been shown to be inhibiting *A. parasiticus* in peanuts, reducing the viability of *A. parasiticus* spores by about 85 per cent (Zucchi *et al.*, 2008). *Trichoderma harzianum* and *Trichoderma viride* were found to effectively suppress the growth of peanut moulds and to significantly reduce Aflatoxin B1 and B2 (Gachomo *et al.*, 2004). Irrigation that eliminates drought stress (Craufurd *et al.*, 2006) can be used as a pre harvest measure of controlling aflatoxins contamination of peanuts. However, the suitability of irrigation in many African regions remains a challenge as most of the peanut is grown under rain-fed smallholder conditions.

Drying of pods, controlling storage pests, storing pods or kernels with less than 10 per cent moisture content are possible post-harvest control strategies (Waliyar *et al.*, 2008). Sorting which efficiently removes peanuts with certain discolorations, in-shell, split, over/undersized or misshaped nuts also reduces aflatoxin levels. Peanut processing methods such as roasting (Kaaya *et al.*, 2006) also reduce aflatoxin levels. Cleaning and separation procedures remove contaminated and damaged kernels and reduce aflatoxin levels by 40 to 80 per cent. Gamma irradiation reduced aflatoxin B1 in peanut kernels by up to 70 per cent in Brazil (Prado *et al.*, 2003). Gaseous ozonation has been proposed as a means of detoxifying peanuts (Proctor *et al.*, 2004). Aflatoxins continue to cause challenges especially in food security in the developing countries. The few studies conducted

in several parts of the Kenya have not established the magnitude of the aflatoxin problem in the Country. Data obtained from the current study will be important in making polices that would be used in control of aflatoxin contamination of peanuts and the management of aflatoxin induced hepatocellular carcinoma in Kenya.

2.4 Epidemiological aflatoxin monitoring systems

Recent exposure to aflatoxin is determined through excreted urine analysis, though only a small fraction of the dose is normally excreted in urine (Williams *et al.*, 2004). Previous aflatoxin outbreaks in Kenya have been identified by an increase in cases of jaundice, despite the lack of any organized or official reporting system (Azziz-Baumgartner *et al.*, 2005). Despite lack of national reporting systems for jaundice in developing countries, detecting cases of aflatoxicosis even through the possible causes are not known (Strosnider *et al.*, 2006). Given that aflatoxicosis confirmation tests by use of biological markers are limited, an organized reporting system for aflatoxin exposure cases may allow earlier detection of potential aflatoxicosis outbreaks.

As many diseases in the developing countries often go unreported, known aflatoxicosis outbreaks are likely to be underestimated. Furthermore, diseases attributable to chronic aflatoxin exposure such as liver cancer, impaired growth and immune suppression remain unclear (WHO and CDC, 2005). The recurrent outbreaks of aflatoxicosis emphasize the need to quantify and control aflatoxin

exposure in developing countries and report the potential role of public health in the control of aflatoxicosis. An early warning system to monitor aflatoxin levels in foods and food products, sources or individuals suffering from aflatoxicosis would prevent or reduce its adverse health effects. Monitoring aflatoxin levels in food or individuals is more difficult compared to monitoring incidence of aflatoxicosis; monitoring the rates of jaundice may identify susceptibility earlier and allow for a more timely intervention (Strosnider *et al.*, 2006).

Poor, rural agricultural communities in developing countries tend to use contaminated foods for human consumption, and the overall food security tends to be low (Cardwell and Henry, 2004). Foods which are at risk are heavily regulated in North America and Europe; many developing countries lack regulations and consistent aflatoxin monitoring systems (Williams *et al.*, 2004). Several key interventions include biocontrols and testing methods which have previously been inaccessible to farmers and regulators in developing countries. An ideal aflatoxin monitoring system such as that used in developed countries would be difficult to establish and sustain in poor countries. A targeted surveillance system for high risk areas or populations based on aflatoxin indicators in food, urine, or serum, would be appropriate for the country's capacity to collect such samples (Strosnider *et al.*, 2006). A combination of rapid field and laboratory confirmation tests analyzing aflatoxins in food, urine or serum would be important for an early warning monitoring system and can be applied in targeted surveillance for high risk areas

and populations. An early warning system must include a response protocol to prevent further aflatoxin exposure and associated health outcomes once a contaminated food source is identified (Williams *et al.*, 2004).

2.5 Laboratory methodologies for detection of aflatoxin in foods

There are various methods that are used for testing levels of aflatoxin in food samples and depend on factors such as cost effectiveness, precision, and number of samples being analyzed. Pascale and Visconti (2008) have summarized the various methodologies available for mycotoxin analysis such as Thin Layer Chromatography (TLC), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Liquid Chromatography/Mass Spectrometry (LC/MS), Enzyme-Linked Imunosorbent Assay (ELISA), and rapid tests. However, ELISA and HPLC are the most sensitive (ICRISAT, 2007). Enzyme-Linked Imunosorbent Assay procedures are the most widely used serological tests for aflatoxin analysis due to their simplicity, adaptability and sensitivity (ICRISAT, 2007). High Perfomance Liquid Chromatography has the advantage of being highly sensitive and has good selectivity, and is easily automated. However, HPLC's major challenge is its high cost, making it unsuitable for routine analysis (Pascale and Visconti, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Areas

The study was conducted in two districts in western Kenya namely Busia and Kisii Central which represented Busia and Kisii counties respectively. Western Kenya encompasses Nyanza and Western provinces, which are the main peanut producing areas in Kenya (Ogwang, 2006). Nyanza Province which encompasses Kisii Central district is the country's largest peanut producer with 14,723 hectares under production and has several peanut processors as well as a high demand for peanuts and their products. In the district, the leading peanuts producing divisions include Keumbu, Masimba, Suneka and Mosocho (Appendix i). Western Province with 2,667 hectares under peanut production ranks third after Eastern Province and most of the produce is traded in local markets (Ogwang, 2006).

Busia district is a major peanut producer and has several market outlets for peanuts. The district borders Uganda, another major peanut producer, which is characterized by a thriving cross border trade. The leading groundnut producing divisions in Busia district include Butula, Matayos, Funyula and Budalangi (Appendix ii). Peanuts are sold as raw kernels, roasted nuts, or processed into peanut butter. In western Kenya, there has been repeatedly reported high levels of stunting growth in children (Mutegi *et al.*, 2009), an aspect often positively associated with long-term ingestion of sub-lethal doses of aflatoxins (Gong *et al.*, 2002). In the

two districts of the present study, the prevalence of aflatoxin induced hepatocellular carcinoma is not known (Herman *et al.*, 1987). The population of Kisii Central district was 1,152, 282 with 48% male and 52% female. Busia district had a population of 743, 946 of which 47% were male and 53% female (Kenya Population and Housing Census 2009, 2010).

3.1.1 Medical facilities

Both Busia and Kisii Central districts are poorly served by medical facilities, with a few medical facilities including a district hospital in each district. The doctor to patient ratio is low in districts, at 1 doctor to 41, 200 patients in Busia district (Republic of Kenya, 2002) and 1 doctor to 34, 992 patients in Kisii Central district (Republic of Kenya, 2009). When patients with liver disorders turn to these facilities, they are referred to Moi Teaching and Referral hospital which serves as a referral hospital for the entire western region of Kenya.

3.2 Study population

The study comprised of peanuts farmers/producers in Busia and Kisii Central districts. The number of farmers/producers growing groundnuts varied between different agro-ecological zones (divisions) within each district. The agro-ecological zones were determined based on altitude, mean annual rainfall, temperature and probability of successful growing of peanuts in that zone (Mutegi

et al., 2009). The peanuts production statistics of farmers were obtained from the division's agricultural offices.

In Busia district, peanuts farmers in the leading groundnut producing divisions namely Butula, Matayos, Funyula and Budalangi participated in the study. The Township division had only small areas under groundnuts and was therefore not included in the study. In Kisii Central district, peanut farmers in Keumbu, Masimba, Suneka and Mosocho (the leading groundnut producing divisions) were recruited in the study. Farmers in Kisii Town and Marani divisions were excluded from the study due to small areas under groundnuts cultivation. The people in western Kenya use peanuts as an alternative to fish, which is more expensive, thereby playing a significant role in food security and there has been reportedly high levels of stunting growth in children (Mutegi *et al.*, 2009), which may be due to long-term exposure to aflatoxins (Gong *et al.*, 2002).

3.2.1 Inclusion criteria

i. Only peanut farmers from Busia and Kisii Central districts were included in this study.

ii. Peanuts producers or family members who were present at the households and were above 18 years were included in the study.

iii. Patients' records of people from Busia and Kisii Central districts who attended Moi Teaching and Referral hospital in the period of January 2010 to

December 2012 with liver disorders without hepatitis B and C were included in the study.

3.2.2 Exclusion criteria

i. Peanut farmers who were not from Busia and Kisii Central districts were excluded from the study.

ii. Peanuts farmers from the Township division of Busia district were excluded from the study.

iii. Peanuts producers in Kisii Town and Marani divisions in Kisii Central district were excluded from the study.

iv. Patients' records of people who attended Moi Teaching and Referral hospital in January 2010 to December 2012 who were not residents of Busia and Kisii Central districts were excluded from the study.

v. Peanuts farmers or family members who were present at the households and were aged below 18 years were also excluded from the study.

3.3 Study design

This study comprised of two distinct designs: A cross-sectional study was adopted among the peanuts farmers in Busia and Kisii Central districts while a retrospective study design was applied for patients with HCC. These patients were from Busia and Kisii Central districts who attended Moi Teaching and Referral hospital in the period of January 2010 to December 2012. The analysis of patient's

records in the hospital was carried out in order to determine the prevalence of aflatoxin induced hepatocellular carcinoma in the two districts.

3.4 Sample size determination

The minimum sample size was determined by the formula of Fisher *et al.*, (2003) using the peanuts aflatoxin contamination prevalence of 7.5% (Kasiulevičius *et al.*, 2006).

$N= Z^2 \times P\ (1-P)/ E^2$

Where:

N= Desired minimal sample size.

Z= Standard normal deviation = 1.96 (from the two tailed test in normal table).

P= Prevalence rate of peanuts aflatoxin contamination

E= The desired degree of accuracy at 95% confidence level= 0.05

$N= 1.96^2 \times 0.075\ (0.925)/0.05^2 = 102$ samples. Therefore, 102 samples of the different varieties of peanuts and 102 samples of urine from peanuts farmers were collected from each of the two districts.

3.5 Data collection

Peanut farmers were interviewed using a structured questionnaire (Appendix iv), to collect data on demographics characteristics, peanuts production and consumption, after giving written consent to participate in the study. After the interview, peanuts and urine samples were collected from the mother or father of the household or family member aged 18 years and above who were present at the household.

3.6 Sampling procedure

Peanuts farmers' households and peanuts were sampled in both Busia and Kisii Central districts. Hospital records of patients from Busia and Kisii Central district with HCC who attended Moi Teaching and Referral hospital in the period of January 2010 to December 2012 were also sampled.

3.6.1 Sampling of peanuts farmers' households

Peanuts farmers' households were sampled from the agro-ecological zones (divisions) and were identified by a list which was obtained from the divisions agricultural offices. In Busia, farmers in the leading groundnut producing divisions identified as Butula, Matayos, Funyula and Budalangi were purposively selected. The other division in Busia (Township) had only small areas under groundnuts cultivation and was therefore, not included in the study. In Kisii, farmers in the four leading peanut producing divisions; Keumbu, Masimba, Suneka and Mosocho

were also purposively sampled. The other divisions in the district (Kisii Town and Marani) were excluded from the study due to small areas under groundnuts production.

Within the divisions (agro-ecological zones) selected in Busia district, peanuts farmers households were randomly selected by staggering every fourth household within the division administrative boundary, the starting point being the fourth household from the division's agricultural office in the particular divisions. The sampling interval was obtained based on the approximate peanuts farmers' population of 408 in the study areas (Kipkoech *et al.*, 2007) divided by the sample size (102).

Within the divisions selected in Kisii Central district, peanuts farmers were also randomly sampled by staggering every third household within the division administrative boundary, the starting point being the third household from the division's agricultural office in the particular divisions. The approximate peanuts farmers' population of 300 in Kisii Central district (Okoko *et al.*, 2008) divided by the sample size (102) gave the sampling interval. A total of 102 peanuts farmer's households were sampled in each district.

3.6.2 Hospital patients records

All hospital records of patients with hapatocellular carcinoma without hepatitis B and hepatitis C from both Busia and Kisii Central districts who attended Moi Teaching and Referral hospital in the period of January 2010 to December 2012 were analyzed in the study.

3.7 Collection of peanut samples

Peanut samples from each household of the peanut farmer in the two districts were collected using the procedure of Whitaker (2006). Peanuts stored in sacks or boxes was sampled from different parts using a closed spear driven through the top and sides of each sack or box to obtain a total of 0.5 kg of sample. Each of the 0.5 kg samples of unsorted peanuts was put in clean polyethylene bags, sealed, labeled and transported in cool boxes. The peanut samples were taken to Bora Limited Laboratory, Nairobi and University of Nairobi, Department of Food Science, Nutrition and Technology and stored at $4^{o}C$ until the time for analysis. One sample of peanut was collected from each household. Therefore, one hundred and two (102) peanut samples were collected from each district.

3.8 Collection of urine samples

Twenty five (25) milliliters urine sample was collected from either the female or male head or any family member who was present in the household of the peanuts farmers using clean disposable containers. Each individual was sampled only once

and when the heads of the family were not available, any other family member who gave consent to participate were sampled to represent their households. A total of 204 urine samples from peanut farmers in both districts were analyzed for aflatoxins exposure. Sixty (60) urine samples were from male while 42 were from female peanut farmers in Busia district while in Kisii Central, 70 urine samples were from male and 32 were from female farmers. Immediately after collection, the samples were put on ice. At the end of the day, the samples were put in cold boxes and transported to Bora Limited Laboratory, Nairobi for analysis. The samples were refrigerated at 4°C until the time of processing which did not exceed 3 days from the time of collection (Bannet *et al.*, 1981).

3.9 Hospital patients records analysis

Analysis of hospital records of patients from Busia and Kisii Central districts who attended Moi Teaching and Referral hospital in the period, January 2010 to December 2012 was conducted to determine the prevalence of aflatoxin induced HCC in the two districts. The diagnosis of aflatoxin induced hepatocellular carcinoma was confirmed by laboratory records showing the presence of AFB1 guanine adduct in urine or AFB1 albumin adduct in blood and absence of hepatitis B and hepatitis C infections. Data collected was computed and population figures of the Kenya population and Housing Census data (Kenya Population and Housing Census 2009, 2010) was used as basis for the calculation of the prevalence of HCC in study areas.

3.10 Laboratory analysis

Peanuts samples were analyzed for *Aspergillus* species which produce aflatoxins and their types and levels in the peanuts. Urine samples were analyzed for AFB Gual to determine the farmers' exposure to aflatoxins (Bannet *et al.*, 1981).

3.10.1 *Aspergillus* species culture and identification

Twenty (20) grams of each peanut sample was mixed thoroughly by shaking and grounded using a dry mill kitchen grinder (Kanchan multipurpose Kitchen machine, Kanchan International Limited Mumbai, India). *Aspergillus* species were isolated from the peanut samples by using the dilution plate technique on modified Rose Bengal agar using the procedure of Probst *et al.*, (2007). Briefly, 10 grams of each grounded sample was added to 90 ml of 0.1% peptone dissolved in water. This mixture was shaken on a rotary shaker for approximately 15 minutes and diluted 10^2, 10^3 and 10^4. Aliquots of 0.1 ml of each dilution was spread (in triplicate) on the surface of the Dichloran Rose Bengal Chloramphenicol agar medium (King *et al.*, 1979). All plates were incubated for 3-7 days at 28°C in the dark and under room temperature. All the three sets of dilutions averaging between 10 and 60 colonies per petridish were counted and an average was obtained. The results were computed and expressed as colony forming units per g (cfu/g) of peanuts.

The colonies of *Aspergillus* species were sub-cultured on 9 cm diameter petridishes containing 20 ml of Malt Extract Agar (MEA) and Czapek-Dox agar (CZ), and then incubated for 7 days in the dark at 25°C. They were subsequentially examined for colony colour, presence and size of sclerotia, head seriation and conidial morphology. Identification was performed according to Klich (2002). All isolates were also cultured on *Aspergillus flavus parasiticus* agar (AFPA) for 3-5 days at 25°C in the dark to confirm group identification by colony reverse colour. All isolates were subsequently cultured on CZ agar at 42°C and colony diameters measured after 7 days of incubation (Ehrlich *et al.*, 2007). Identification of species isolates was done according to Klich (2002), and by comparison with reference strains obtained from Dr. Bruce Horn (USDA National Peanut Research Lab, Dawson, Georgia, United States of America).

3.10.1.1 *Aspergillus flavus* strains characterization

Phenotypical characterization of the identified *Aspergillus* section flavus isolates from the peanuts samples was determined using the procedure of Mellon and Cotty (2004). Briefly, up to 10 colonies of identified isolates of *Aspergillus flavus* were aseptically transferred onto 5/2 agar (5% V-8 juice and 2% agar, pH 5.2) and incubated for 5 - 7 days at 31 C. The isolates were then classified on the basis of colony characteristics and conidial morphology at x 400 magnification using a high resolution microscope. Colony radius was measured in millimeter (mm) and the colony color of isolates determined using Methuen color book (Kornerup and

Wanscher, 1978). Isolates with abundant small sclerotia (average diameter < 400mm) were classified as strain S of *A. flavus* (Cotty and Cardwell, 1999). Isolates with smooth conidia and large sclerotia (average diameter over 400mm) were classified as the L strain of *A. flavus* (Cotty and Cardwell, 1999).

3.10.2 Aflatoxin analysis

Each homogenized grounded peanut sample was triturated and analyzed to determine the types of aflatoxins and also the quantities of aflatoxin contaminants.

3.10.2.1 Extraction of aflatoxins from peanuts powder

Approximately fifty grams from each homogenized peanuts sample was triturated in a blender in 50 ml of 70% methanol (70 ml absolute methanol mixed in 30 ml distilled water) containing 0.5% potassium chloride until thoroughly homogenized. The extract was transferred to a conical flask and centrifuged at 300 rpm for 30 minutes to remove any particulates. The extract was filtered through Whatman No.4 filter paper and diluted 1:10 in phosphate buffered saline containing 500µl/L Tween-20 and analyzed for aflatoxins using High Performance Liquid Chromatography (HPLC).

3.10.2.2 Identification and quantification of aflatoxins

High Performance Liquid Chromatography (HPLC) was used to confirm identity of the aflatoxin and quantified using the method of Bragulat *et al.*, (2001). HPLC system consisted of a Hewlett Packard 1100 pump (Palo Atto, CA, USA) connected to an HP 1046A programmable fluorescence detector and quantification was done with an HP workstation. Twenty microliters (20 µl) of each extract was applied to HPLC and chromatographic separation was performed on a stainless steel C18 reversed phase analytical column (150×4.6 mm, 5µm particle size) preceded by a C18 pre-column (Ultrasep 104mm). Water: methanol: acetonitrile (4:1:1, v/v/v) was used as the mobile phase at a flow rate of 1.5 mL/min. Fluorescence of aflatoxin derivatives was recorded at excitation and emission wavelengths of 360 nm and 440 nm respectively. Aflatoxins were quantified based on HPLC fluorometric response compared with aflatoxin standards (Sigma chemical, St. Louis, MO, USA). The limit of detection was 0.005 µg/kg for AFB1 and AFG1, and 0.02 µg/kg for AFB2 and AFG2.

3.10.3 Determination of peanuts oil contents

Three samples of each of the four (4) varieties of peanuts obtained from Busia and 3 varieties from Kisii Central were analyzed and the average oil contents of the samples in each variety determined. The oil contents of peanuts were determined by a standard Soxtec extraction method using the procedure of Sundaram *et al.*, (2010). Five (5) grams of the homogenized ground peanut sample was accurately

weighed into an extraction thimble. The sample was then covered with cotton wool and the thimble was placed into the Soxtec extractor. A flat bottomed flask with 200 ml of petroleum was placed on a heating mantle and connected to the Soxtec extractor and the extraction started. The extraction of oil continued for about 8 hours. After the 8 hours, the solvent was evaporated in a rotary evaporator. The residue was then dried in an air oven at 105 ^{o} C for 1 hour. The crude oil content of the sample was then calculated as a percentage by dividing the weight of the crude oil by the weight of the sample (5 grams), multiplied by 100 %.

3.10.4 Analysis of urine for AFB Gual

The urine samples were analyzed for AFB Gual using the procedure of Bannet *et al.*, (1981). Samples were adjusted to pH 5 using 7 % methanol followed by centrifugation at 4 ^{o}C (1500g for 10 min) to remove any particulates. The supernatant was submitted for a cleanup by loading using a Sep cartridge rack onto C18 Sep-Pak cartridges that had previously been washed sequentially with 5 ml of 5% methanol: water and 80% methanol: water to remove any contaminant. The urine flowed at a maximum rate of 2 mL/min. The cartridge was washed with 5 ml of 10% methanol and 5 ml 7% acetonitrile before elution of the aflatoxin derivatives to remove urine compounds that may have comigrated with the aflatoxin derivatives.

The aflatoxin derivatives (AFB Gual) were eluted from the cartridge with 10 ml of 80% methanol and the eluate was concentrated to 0.5 ml by evaporation using a Speedvac evaporator. The residue was reconstituted in 0.3 ml of 0.1 N HCL with heating at 50 °C for 10 min to ensure that relatively insoluble aflatoxin DNA adducts was dissolved. After cooling at room temperature, 0.5 ml 1M ammonium formate, P^H 4.5 was added to precipitate the AFB Gual. Aflatoxin derivatives were then analyzed by fluorescence Spectrophotometer and the eluate was monitored at a wavelength of 365 nm. The identity of the AFB Gual was verified by fluorescence luminescence. A sample was considered positive for AFB Gual if it gave UV absorption at 365 nm and a characteristic synchronous fluorescent spectrum with a peak emission of 415 nm when exitation at 381 nm (Bannet *et al.*, 1981).

3.11 Ethical approval and permit

Ethical approval was obtained from Kenyatta University Ethics Review Committee (Appendix v). Research permit was obtained from the National Commission for Science, Technology and Innovation (NACOSTI) Kenya (Appendix vi). The study objectives were explained to the peanuts farmers and they were allowed to ask questions. After giving consent, the participants signed a consent form (Appendix iii) and both urine and peanuts samples were collected. Unique numbers were used to identify each participant and all information recorded in the study was treated with confidentiality. Permission to use the

hospital patients' records for analysis was sought from the Moi Teaching and Referral Hospital administration.

3.12 Data analysis

Data on the content of aflatoxin in all peanut samples were recorded and categorized into three: ≤4µg/kg, >4µg/kg to ≤10µg/kg and >10µg/kg. The ≤4µg/kg threshold represents the EU regulatory limit for total aflatoxin while the ≤10 µg/kg threshold represents the KEBS regulatory limit for total aflatoxin. The distribution of samples in the levels of aflatoxins were determined using Mann- Whitney U test and the most dominant aflatoxin type in peanuts was analyzed using t- test. Aflatoxin B1 levels in peanuts was categorized into two: ≤ 5µg/kg and > 5µg/kg. The ≤ 5µg/kg threshold represents the KEBS regulatory limit for aflatoxin B1 in foods intended for human consumption. The occurrences of *Aspergillus* species isolated in the two districts were analyzed using Kruskal-Wallis test. The distribution of *Aspergillus* species in the different peanut varieties was determined using One way Analysis of Variance (ANOVA) at P=0.05 value.

Correlation test was used to correlate AFB Gual positive and negative peanuts farmers in the two districts. The difference in oil contents of the different peanuts varieties from the two districts was also determined using Correlation test. The difference in the prevalence of HCC among the patients (gender and the district) who turned up for diagnosis at Moi Teaching and Referral Hospital during the

study period was determined using Correlation and Chi Square tests (P=0.05). Data on the demographic characteristics, peanuts production and consumption was analyzed using percentage frequencies and proportions. Statistical Packages for Social Sciences version 19.0 was used for the analysis of the data.

CHAPTER FOUR

4.0 RESULTS

4.1 Peanuts consumption by the respondents in the two study districts

A total of 98% of the respondents in both Busia and Kisii Central districts consumed peanuts on daily basis from their own harvest while 2% reported that they bought peanuts from the local market (Figure 4.1). On physical inspection, peanuts consumed in most of the households and especially the broken ones were contaminated with moulds. The respondents who suffered liver problems attended Busia district and Kisii Level 5 hospitals while others were referred to Moi Teaching and Referral Hospital.

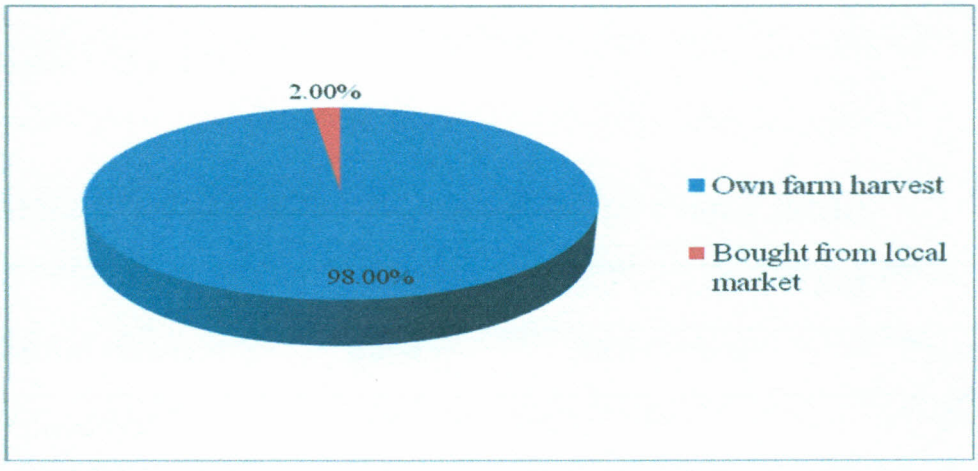


Figure 4.1: Sources of peanuts consumed by respondents in the two districts

4.2 Peanuts varieties from Busia and Kisii Central districts

A total of 204 peanut samples of different varieties were collected in the two districts. In Busia district, the 102 peanuts samples were of four different varieties; Valencia red, Uganda local, Homabay local and Local red. The 102 peanuts samples from Kisii Central district were of three different varieties; Valencia red, Uganda local and Homabay local. In both districts, Valencia red variety had the most number of the samples, 59 and 89 from Busia and Kisii Central districts respectively which were significantly different from the other varieties ($\chi^2 = 12.00$, df = 9, P = 0.02). There were more samples of Uganda local red (21) and Homabay local (20) varieties from Busia district compared to those from Kisii Central district. Local red variety had only 2 samples in Busia and none in Kisii Central (Table 4.1).

Table 4.1: Peanut varieties from Busia and Kisii Central districts

	Number of peanut samples collected		
Peanut variety	**Busia**	**Kisii Central**	**Total**
Valencia red [a]	59	89	148
Uganda local red	21	5	26
Homa Bay local	20	8	28
Local red	2	0	2
Total	**102**	**102**	**204**

a= peanut variety with a significantly higher number of samples in the study.

4.3 Incidence of aflatoxin in peanuts from Busia and Kisii Central districts

Aflatoxin was detected in almost all the peanut samples collected from both districts. All the peanuts samples from Kisii Central district and 99 (97.06%) samples from Busia district were contaminated with aflatoxins (Figure 4.2). However, aflatoxin was not detected in only 3(2.94%) peanuts samples from Busia district.

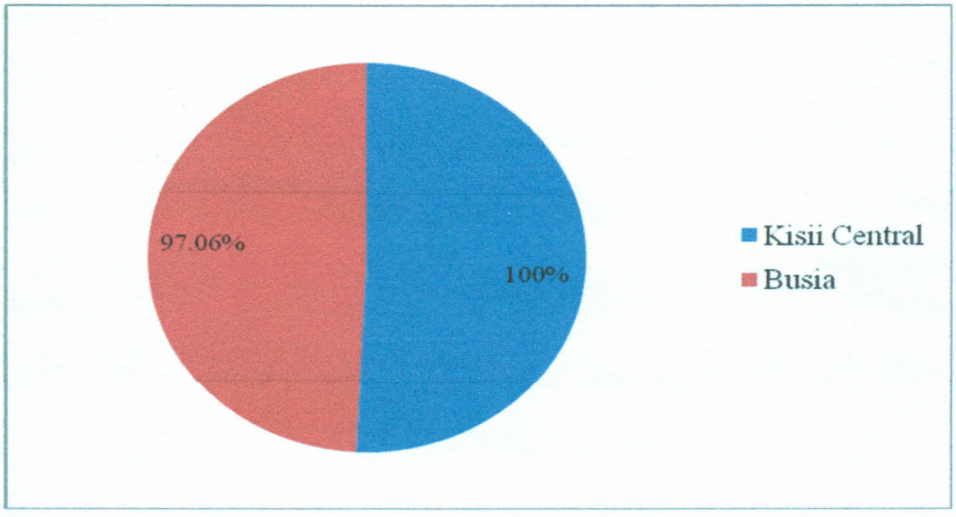


Figure 4.2: Peanut samples from the two districts contaminated with aflatoxin

4.4 Levels and types of aflatoxins contaminating the different varieties of peanut in Busia and Kisii Central districts

The levels and types of aflatoxins analyzed were compared with the Kenya Bureau of Standards (KEBS), European Union (EU) and United States Food and Drug Administration (USFDA) regulatory limits for total and types of aflatoxins in foods for human consumption.

4.4.1 Peanuts of different varieties with detectable levels of aflatoxins

Two hundred and one (201) samples of different peanuts varieties obtained from both Busia and Kisii Central districts had detectable levels of aflatoxins. Out of the 201 samples, 99 (49.3%) samples of different peanuts varieties were from Busia district while 102 (50.7%) were from Kisii Central district. The numbers of peanut samples with detectable levels of aflatoxin in the two districts were compared using a paired sample t-test which showed no significant difference in the number of peanut samples of known varieties with detectable levels of aflatoxin in the two districts ($t = 0.07$, $df = 1$, $P = 0.947$).

One-Way ANOVA test showed that Valencia red variety had a significantly higher rate of aflatoxin contamination than the other varieties, 89 (87.3%) and 58 (58.6%) for this variety of peanuts from Kisii Central and Busia respectively ($F = 13.15$, $df = 3$, $P = 0.015$). Peanuts of Homabay local and Uganda local red varieties from Busia had a significant number of peanuts contaminated with aflatoxins (Table 4.2) compared to those from Kisii Central ($\chi^2 = 5.12$, $df = 1$, $P = 0.017$). The 2 peanuts samples (2%) of Local red variety from Busia district had detectable levels of aflatoxins (Table 4.2).

Table 4.2: Peanuts samples of different varieties with detectable levels of aflatoxins

	Busia		Kisii Central	
Peanut variety	**N**	**%**	**N**	**%**
Valencia red	58[a]	58.6	89[b]	87.3
Uganda local red	19	19.2	5	4.9
Homabay local	20	20.2	8	7.8
Local red	2	2.0	0	0.0
Total	**99**	**100**	**102**	**100**

a and b = Peanuts samples of Valencia red variety had significantly higher rate of aflatoxin contamination in the two districts ($F = 13.15$, $df = 3$, $P = 0.015$).

4.4.2 Total aflatoxins in different varieties of peanuts from the two study districts

The levels of total aflatoxin were in the ranges of 0.1 to 268µg/kg and 1.63 to 591.1µg/kg in samples from Busia and Kisii Central districts respectively. Two samples of the Local red variety from Busia district had the highest level of aflatoxins at 267µg/kg and 268µg/kg. Four (4) samples of Valencia red variety had aflatoxin levels of between 252.8 – 262µg/kg while 4 samples of Uganda local red variety had aflatoxin levels in the range of 137.4µg/kg to 137.5µg/kg. Peanuts of Homabay local variety had lower levels of aflatoxins ranging from 1.1µg/kg to 133.4µg/kg.

Peanut samples of the different varieties were grouped into four based on threshold of the total aflatoxins (≤ 4 µg/kg, > 4 to ≤ 10 µg/kg, >10 to ≤ 20 µg/kg and > 20 µg/kg. The ≤ 4µg/kg is the limit set by European Union; ≤ 10µg/kg, Kenya Bureau of Standards while ≤ 20µg/kg is limit by the United States Food and Drug Administration regulatory requirement for total aflatoxins in foods including peanuts for human consumption (Table 4.3).

The two samples of Local red variety from Busia had total aflatoxins levels exceeding ≤ 4µg/kg and therefore unfit for human consumption based on the EU regulatory limits (Table 4.3). From Kisii Central district, all samples of Uganda local red and Homabay local peanut varieties had total aflatoxin levels exceeding the EU regulatory limits of ≤ 4 µg/kg. Only 5.6% samples of Valencia red from this district had total aflatoxins within the EU regulatory limit (Table 4.3). Mann-Whitney U two sample test showed that the number of peanut samples from Busia district in ≤ 4 µg/kg total aflatoxin category was significantly higher compared to Kisii Central ($W = 222.0$, $P = 0.036$).

Table 4.3: Association between levels of total aflatoxin categories and the varieties of peanut samples from the two study Districts

District	Peanut variety	n	Levels of total Aflatoxin categories (µg/kg)			
			≤ 4	> 4 to ≤10	>10 to≤20	>20
Busia	Valencia red	58	88.2%	5.1%	0.0%	6.7%
	Uganda local red	19	76.2%	4.8%	0.0%	19 %
	Homabay local	20	80.0%	20.0%	0.0%	0.0%
	Local red	2	0.0%	0.0%	0.0%	100%
Kisii Central	Valencia red	89	5.6%	4.5%	10.1%	79.8%
	Uganda local red	5	0.0%	0.0%	20.0%	80.0%
	Homabay local	8	0.0%	0.0%	12.5%	87.5%

n = Peanut samples of each variety from the two study districts in the EU, KEBS and USFDA regulatory limits for total aflatoxins.

Based on KEBS regulatory limits for total aflatoxins (≤ 10µg/kg), all peanuts samples, 20 (100%) of Homabay local variety from Busia district were within the KEBS regulatory limits. Two (100%) samples of local red variety from Busia had aflatoxin levels of above >20µg/kg. Ninety three (93%) and 81% of peanuts samples of Valencia red and Uganda local red varieties respectively from Busia were within the KEBS regulatory limits of ≤ 10µg/kg total aflatoxins. All the samples of Uganda local red and Homabay local varieties from Kisii Central had total aflatoxin levels exceeding >10 to ≤20µg/kg while only 4.5% samples of the Valencia red variety had total aflatoxins of ≤ 10µg/kg (Table 4.3). Kolomogorov-

Smirnoff (K-S) test showed that peanuts samples from Busia district within ≤ 10μg/kg total aflatoxin category was significantly higher compared to Kisii Central (K-S = 124, P= 0.033).

All peanut samples, 58 (100%) of the Valencia red variety from Busia district had total aflatoxins levels of ≤ 20μg/kg which is the USFDA regulatory limit (≤ 20μg/kg) of total aflatoxins in foods. Ninety three (93%) and 80% of samples of Valencia red and Uganda local red varieties respectively from Busia were within the USFDA regulatory limits of ≤ 20μg/kg (Table 4.3). Twenty (20%) percent of peanuts of Uganda local red variety from Kisii Central had total aflatoxin levels within the USFDA regulatory limit of ≤ 20μg/kg while 12.5% and 10.1% of Homabay local and Valencia red peanut varieties this district had total aflatoxin levels of ≤ 20μg/kg (Table 4.3). Mann-Whitney U two sample test showed that the distribution of samples in the levels of total aflatoxin categories from the two study districts were significantly different (W = 226.0, P = 0.038). However, the resulting distribution showed that in Busia district, the distribution was skewed to the left (≤ 4μg/kg category) while in Kisii Central district, the distribution was skewed to the right, > 20μg/kg category (Table 4.3).

4.4.3 Types of aflatoxins identified in the peanuts from the two study Districts

All the 201 peanut samples from both Busia and Kisii Central districts with detectable levels of aflatoxins were analyzed for the different types of aflatoxins. All the four aflatoxin types commonly found in food commodities (Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2) were found to be contaminating peanuts from the two districts and all the 4 peanut varieties analyzed. Overall, the four types of aflatoxins identified (B1, B2, G1 and G2) were at higher levels in peanuts from Kisii Central than those from Busia. The most common aflatoxin type found at the highest concentration in samples from both districts was Aflatoxin B1 at 0.0 - 115.15 µg/kg with a median value of 57.58 µg/kg and 1.30 – 510 µg/kg (median = 254.35) in Busia and Kisii Central respectively ($t = 12.4$, $P = 0.034$). Aflatoxin B2 levels in the peanuts from Busia and Kisii Central were at 0.0 - 46.23 µg/kg (median = 23.12 µg/kg) and 0.08 - 48.27 µg/kg, median = 24.10 µg/kg respectively (Table 4.4).

The levels of Aflatoxin type G1 were 0.0 - 34.90 µg/kg and 0.0 - 43.98 µg/kg in peanuts from Busia and Kisii Central respectively. Aflatoxin type G2 had the least levels at 0.01 – 15 µg/kg and 0.0 - 26.38 µg/kg in peanuts from Busia and Kisii Central districts respectively (Table 4.4). The levels of aflatoxin types B1, B2, G1 and G2 were higher in peanuts from Kisii Central than in Busia district (Table 4.4) although the difference between the levels of the toxin types in the two districts was not significant ($t = 1.08$, $P = 0.060$).

Table 4.4: Levels of the different types of aflatoxins identified in peanut samples from the two study districts

	Aflatoxin levels (µg/kg)			
Type of Aflatoxin	**Busia**		**Kisii Central**	
	Range	**Median**	**Range**	**Median**
Aflatoxin B1	0.0 – 115.15	57.58	1.30 – 510	254.35
Aflatoxin B2	0.0 – 46.23	23.12	0.08 – 48.27	24.10
Aflatoxin G1	0.0 – 34.90	17.45	0.0 – 43.98	21.99
Aflatoxin G2	0.01 – 15.0	7.50	0.0 – 26.38	13.19

The levels of Aflatoxins B1 in peanuts from the two districts were grouped into two categories (≤5 µg/kg, >5 µg/kg) based on KEBS Aflatoxin B1 regulatory limits of ≤ 5µg/kg. Out of a total of 201 peanuts samples from the two districts analyzed for aflatoxin B1, 103(51.2%) samples had levels within the KEBS accepted limits while 98 (48.8%) had levels above >5 µg/kg. Chi- Square test showed that most of peanuts (89.9%) from Busia had Aflatoxin B1 levels within the KEBS accepted regulatory limit compared to peanuts from Kisii Central with 14 (13.7%) samples ($\chi^2 = 5.23$, P= 0.016). Most of the samples from Kisii Central district (86.3%) had aflatoxin B1 levels above 5µg/kg and therefore were not safe for human consumption (Table 4.5).

Table 4.5: Aflatoxin B1 levels in peanuts samples collected from the two study areas

District	Type of Aflatoxin	Peanut samples having different levels of aflatoxin B1		
		≤ 5µg/kg [a]	> 5µg/kg	Total samples
Busia	Aflatoxin B1	89 (89.9%)	10 (10.1%)	99 (100%)
Kisii Central	Aflatoxin B1	14 (13.7%)	88 (86.3%)	102 (100%)
Total		**103(51.2%)**	**98 (48.8%)**	**201 (100%)**

a = Aflatoxin category with significant number of peanuts from Busia district in the KEBS accepted regulatory limit.

4.4.3.1 Association between Aflatoxin types B1, B2, G1 and G2 levels and categories of total aflatoxin in peanuts

Incidences of aflatoxin B1 and aflatoxin B2 in the total aflatoxin category of 0 ≤ 4µg/kg were higher in peanuts from Busia compared to peanuts from Kisii Central district (χ^2 = 13.01, P= 0.009). Approximately, 90% of peanuts of the different varieties from Busia had aflatoxin types B1, B2, G1 and G2 levels and were in the total aflatoxin category of 0 ≤ 4µg/kg. Peanuts from Kisii Central district (71.5%) had aflatoxin B1 levels in the total aflatoxin category of >20µg/kg. However, 58.9 % of peanuts from the same study district had aflatoxin type B2 levels in the total aflatoxin category of 0 ≤ 4µg/kg (Table 4.6). The incidences of aflatoxins type B1 and B2 in peanuts from the two study districts were significantly different (χ^2 = 14.82, P= 0.004). The incidences of aflatoxins type G1 and G2 were not significantly different when compared for the two districts (χ^2 = 19.01, P= 0.123). The levels of aflatoxins types G1 and G2 were however higher (> 81%) in peanuts

from both districts in total aflatoxins category, 0 ≤ 4µg/kg (Table 4.6). Lower incidences ranging from 0-14.7% of aflatoxins types G1 and G2 were obtained in peanuts in the total aflatoxin category of > 4 ≤ 10µg/kg in both districts (Table 4.6).

Table 4.6: Association between levels of aflatoxin types and the categories of total aflatoxin in peanuts from the two Districts

District	Type of Aflatoxin	Percentage (%) of peanuts samples with different levels of total Aflatoxin categories (µg/kg)			
		0 ≤ 4	> 4 ≤ 10	>10 ≤ 20	> 20
Busia	Aflatoxin B1	90.2%	0.0%	0.0%	9.8%
	Aflatoxin B2	90.2%	0.0%	0.0%	9.8%
	Aflatoxin G1	91.2%	4.9%	2.0%	1.9%
	Aflatoxin G2	90.0%	5.0%	3.0%	2.0%
Kisii Central	Aflatoxin B1	7.9%	13.8%	6.8%	71.5%
	Aflatoxin B2	58.9%	17.7%	20.6%	4.8%
	Aflatoxin G1	95.1%	3.9%	1.0%	0.0%
	Aflatoxin G2	81.4%	14.7%	2.9%	1.0%

It was noted that most of the peanuts (mean 75.61%) analyzed from the two study districts had aflatoxin levels within the total aflatoxin category of 0 ≤ 4 µg/kg compared to the other categories ($F = 23.61$, $df = 3$, $P = 0.001$), Table 4.7.

Table 4.7: Mean (%) of number of peanuts samples with different levels of total aflatoxins

	Aflatoxin levels (µg/kg)			
	0 ≤ 4	**> 4 ≤ 10**	**>10 ≤ 20**	**> 20**
Mean (%) peanut samples	75.61	7.50	4.54	12.60
Standard error	10.5	2.44	2.42	8.52

The mean incidences of Aflatoxin types G1 and G2 respectively were in the range of 0 ≤ 4µg/kg for 93.15% and 85.7% of peanuts samples from the two study districts. Peanuts samples which were in total aflatoxin category of > 4 to ≤ 10 µg/kg) had aflatoxin B2 levels within >10 ≤ 20 µg/kg and those which had Aflatoxin B1 were in total aflatoxin category, > 20 µg/kg (Table 4.8).

Table 4.8: Average in percentage of peanuts with Aflatoxin levels and types in Peanuts samples from the two study Districts

	Mean percentage (%) of peanuts samples with different levels of total Aflatoxin categories (µg/kg) [x]			
Type of Aflatoxin	**0 ≤ 4**	**> 4 ≤ 10**	**>10 ≤ 20**	**> 20**
Aflatoxin B1	49.05%	6.9%	3.4%	40.65%
Aflatoxin B2	74.55%	8.85%	10.3%	7.3%
Aflatoxin G1	93.15%	4.4%	1.5%	0.95%
Aflatoxin G2	85.7%	4.45%	2.95%	1.5%

[x] is based on a total number of 201 total samples with aflatoxins.

Kruskal-Wallis test revealed that the overall mean incidences of Aflatoxin B2 in samples from the two districts in the different total aflatoxin categories were different, 74.55%, 8.85%, 10.3% and 7.3% (H= 17.63, P = 0.03) for Aflatoxin categories; 0 to ≤ 4 µg/kg, > 4 to ≤ 10 µg/kg, 10 to ≤ 20 µg/kg and > 20 µg/kg respectively. Similarly, the incidence of aflatoxin G2 in samples was 85.7%, 4.45%, 2.95% and 1.5% (H= 16.50, P= 0.021) in Aflatoxin categories 0 to ≤ 4 µg/kg, > 4 to ≤ 10 µg/kg, 10 to ≤ 20 µg/kg and > 20 µg/kg respectively.

4.5 Aflatoxins producing *Aspergillus* species in peanuts from Busia and Kisii Central districts

The isolates in this study were identified as *Aspergillus flavus* (isolates with parrot green colony color in front and light greenish yellow in the reverse), *Aspergillus parasiticus* (isolates with white fluffy and yellow sporulation colony color in front and light yellow in the reverse) as outlined by Klich (2002). *Aspergillus niger* isolates were identified with olive green colony color in front and light greenish yellow in the reverse.

Five (5) *Aspergillus* species were identified as contaminants in peanuts analyzed in this study. They were *Aspergillus flavus* L- strain, *Aspergillus flavus* S- strain, *Aspergillus parasiticus*, *Aspergillus niger* and *Aspergillus tamari.* Overall, the occurrence of *Aspergillus flavus* L- strain and *A. flavus* S- strain were significantly higher than other species identified (H = 15.55, df = 4, P = 0.004) in peanuts from

the two districts. However, *A. flavus* S-strain was the most dominant species identified in the study with a mean occurrence of 45.1% (Table 4.9). *Aspergillus flavus* L- strain was the most common isolate (58.8%) in peanuts from Busia district while *A. flavus* S- strain was the most common strain (60.2%) in peanuts from Kisii Central district (Table 4.9).

Table 4.9: Occurrence of *Aspergillus* species isolated and identified in peanuts samples in the two study Districts

	Busia		Kisii Central	
***Aspergillus* species isolated**	**n**	**%**	**n**	**%**
A. flavus L- strain	60	58.8	22	21.8
A. flavus S- strain	30	29.4	62	60.2
A. parasiticus	7	6.9	12	12.0
A. niger	2	2.0	4	4.0
A. tamarii	0	0.0	2	2.0
Negative for *Aspergillus* species	3	2.9	0	0.0

Aspergillus parasiticus was the third most abundantly identified *Aspergillus* species in peanuts from both districts at 12% and 6.9% in peanuts from Kisii Central and Busia districts respectively. Other species including *Aspergillus niger* was isolated at 2% and 4% in peanuts from Busia and Kisii Central districts respectively. *Aspergillus tamarii* was the least occurring species at 2% in peanuts from Kisii Central district and none in peanuts from Busia district (Table 4.10). The mean occurrence for *Aspergillus tamarii* was 1% in both districts (Table

4.10). Only, 2.9% of peanut samples collected from Busia district were negative for *Aspergillus* species contamination while all peanut samples from Kisii Central district were contaminated with at least one aflatoxin producing species (Table 4.10).

Table 4.10: Mean occurrence of different *Aspergillus* species in peanuts from the two districts

***Aspergillus* species isolated**	**n**	**%**
A. flavus L- strain	82	40.2
A. flavus S- strain	92	45.1
A. parasiticus	19	9.3
A. niger	6	2.9
A. tamarii	2	1.0
Negative for *Aspergillus* species	3	1.5

4.5.1 *Aspergillus* species in the different varieties of peanuts

All the varieties of peanuts sampled from both Busia and Kisii Central districts were contaminated with at least one or more of *A. flavus L- strain, A. flavus S-strain, A. parasiticus, Aspergillus niger and A. tamarii* species. Overall, in establishing the most prevalent *Aspergillus* species isolated in peanut samples from Busia and Kisii Central districts, incidences of the five *Aspergillus* species were compared using One-way Analysis of Variance (ANOVA). The result showed that the incidence of *Aspergillus flavus* S-strain was significantly higher than other *Aspergillus* species identified ($F = 3.15$, $df = 25$, $P = 0.031$).

Aspergillus flavus L- strain was the most highly detected strain (60.6%) in all the peanut varieties from Busia district compared to the other *Aspergillus* species isolated (H = 10.03, df = 3, P = 0.018). The species was mostly found in Homabay local variety peanuts at 33.3% from Busia (Table 4.11). *Aspergillus flavus S-strain* was the most abundant species in peanuts of the Homabay local from Busia district at an incidence of 40%. *Aspergillus parasiticus* was also found to be contaminating all the peanut varieties from the study district but was isolated highly in peanuts of local red, Valencia red and Uganda local varieties at an incidence of 28.6%. *Aspergillus niger* was only detected in all the peanut samples of Local red variety while *A. tamarii* was not detected in any peanut varieties of peanuts from Busia district (Table 4.11).

In peanut samples from Kisii Central district, all the strains; *Aspergillus flavus* S-strain, *Aspergillus flavus* L- strain, *Aspergillus parasiticus, A. niger* except *A. tamarii* were isolated in all the varieties. However, *Aspergillus flavus S*-strain had higher occurrence at 60.8% compared to other species identified in peanuts from the district (H = 12.28, df= 4, P = 0.015). *Aspergillus flavus* S-strain was highly detected in samples of Valencia red variety with incidence of 79% compared to *Aspergillus flavus* L- strain at 54.6%. *Aspergillus parasiticus* species was found at an incidence of 41.7% in Homabay local variety while *Aspergillus tamarii* species was detected in Uganda local red and Homabay local peanut varieties at similar rates of 50% (Table 4.11).

Table 4.11: *Aspergillus* species isolated from the different varieties of peanuts

District	Peanut variety	*Aspergillus* species isolated				
		***A. flavus* L-strain**	***A. flavus* S-strain**	***A. parasiticus***	***A. niger***	***A. tamarii***
Busia	Valencia red	19 (31.7%)	7 (23.3%)	2 (28.6%)	0 (0.0%)	0 (0.0%)
	Uganda local red	19 (31.7%)	9 (30.0%)	2 (28.6%)	0 (0.0%)	0 (0.0%)
	Homabay local	20 (33.3%)	12 (40.0%)	1 (14.2%)	0 (0.0%)	0 (0.0%)
	Local red	2 (3.3%)	2 (6.7%)	2 (28.6%)	2 (100%)	0 (0.0%)
	Total	**60(60.6%)**	**30(30.3%)**	**7(7.1%)**	**2(2%)**	**0(0)**
Kisii Central	Valencia red	12 (54.6%)	49 (79.0%)	4 (33.3%)	1 (25.0%)	0 (0.0%)
	Uganda local red	5 (22.7%)	5 (8.1%)	3 (25.0%)	2 (50.0%)	1 (50.0%)
	Homabay local	5 (22.7%)	8 (12.9%)	5 (41.7%)	1 (25.0%)	1 (50.0%)
	Total	**22(21.6%)**	**62(60.8%)**	**12(11.7%)**	**4(3.9%)**	**2(2%)**

The rate in percentage of each species was calculated based on the total number of isolates of each species in each district of study.

4.6 Oil contents of the different varieties of peanuts from Busia and Kisii Central Districts

Using a paired T-test, peanuts from Busia district had significantly higher oil contents compared to those from Kisii Central ($t = 3.22$, $df = 6$, $P = 0.012$). Peanuts of Valencia red variety from both Busia and Kisii Central had higher oil content (mean 46.9), compared to other varieties (Table 4.13). In addition, Valencia red variety peanuts from Busia district had slightly higher oil content (47.2%) than peanuts of the same variety from Kisii Central which had 46.6% although the difference was not significant ($t = 1.08$, $df = 6$, $P = 0.394$). Peanuts of Local red variety from Busia district had the lowest oil content (42.7%) compared to other varieties from the same district ($t = 2.28$, $df = 6$, $P = 0.026$). Homabay local variety peanuts from Kisii Central had the lowest oil content (40.6%) among all the other peanuts varieties analyzed (Table 4.12).

Table 4.12: Oil contents of different varieties of peanuts from Busia and Kisii Central districts

Peanuts variety	Average (%)		Overall Mean (%)
	Busia	Kisii Central	
Valencia red	47.2	46.6	46.9
Uganda local red	46.7	45.7	46.2
Homabay local	43.2	40.6	41.9
Local red	42.7	-	42.7

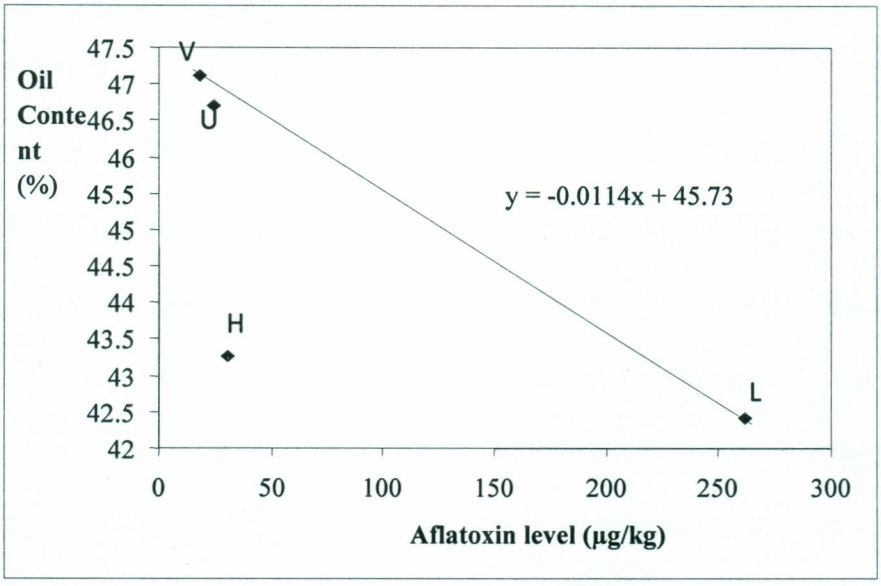
4.6.1 Relationship between oil contents and the levels of total aflatoxin in peanuts

Oil contents of peanuts from Busia and Kisii Central districts were compared with the total aflatoxins of the same samples. All the four peanuts varieties from Busia had lower levels of total aflatoxins except the Local red variety which had the highest total aflatoxins levels at 267µg/kg but with the lowest oil content average of 42.7% among peanuts varieties from Busia (Table 4.13). Peanuts varieties from Kisii Central had high levels of total aflatoxin but low oil contents. Peanuts of Uganda local red from Kisii Central had the highest total aflatoxin levels of 405µg/kg and average oil content of 45.7% (Table 4.13). Overall, there was an increase in oil content with decrease in total aflatoxin levels ($r = -0.496$, $P = 0.031$) except in peanuts of Uganda local red variety from Kisii Central district (Table 4.13).

Table 4.13: Oil contents in relation to total aflatoxins in peanuts analyzed

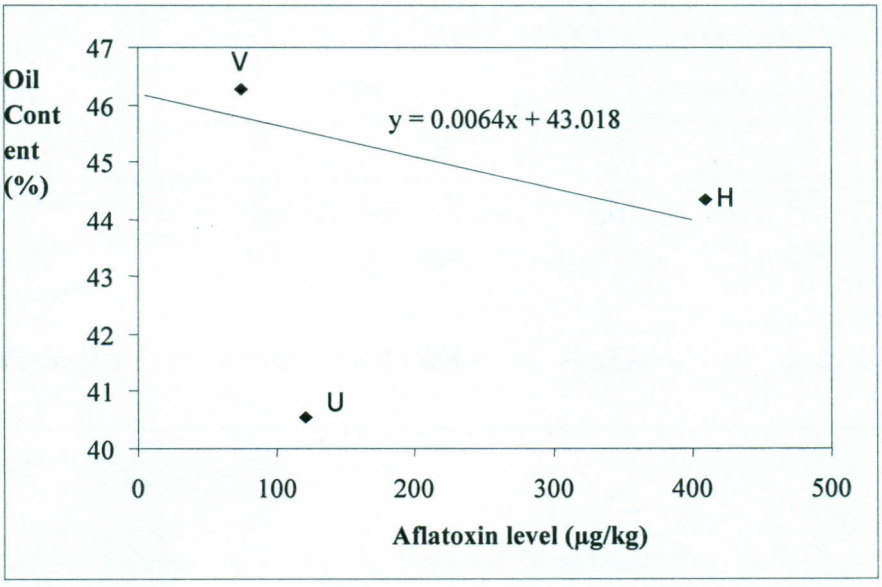
District	Peanut variety	Average oil content (%)	Total Aflatoxin level in the sample (µg/kg)
Busia	Valencia red	47.2	2.3
	Uganda local red	46.7	2.4
	Homabay local	43.2	2.8
	Local red	42.7	267
Kisii Central	Valencia red	46.6	93
	Uganda local	45.7	405
	Homabay local	40.6	101.5

Plotting oil content of peanut from Busia against aflatoxin levels showed a increase of oil content with decrease in aflatoxin levels ($r = - 0.0114$, $P = 0.051$) and those from Kisii Central also showed increase of oil contents with decrease in aflatoxin levels ($r = - 0.0064$, $P = 0.045$) except Homabay local variety as illustrated in figure 4.3 and 4.4 respectively.



V= Valencia red; U= Uganda local red; H= Homabay local; L= Local red

Figure 4.3: Relationship between Aflatoxin level with percentage oil content in peanuts from Busia District



V= Valencia red; U= Uganda local red; H= Homabay local

Figure 4.4: Relationship between Aflatoxin level with percentage oil content in peanuts from Kisii Central District

4.7 Aflatoxins exposure to peanuts farmers from Busia and Kisii Central Districts.

Out of the 204 urine samples, 31(15.2%) tested positive for AFB gual while 173 (84.8%) tested negative. From Kisii Central, 18 (8.8%) urine samples were positive for AFB gual while 13 (6.4%) samples from Busia were positive. Overall, male farmers had slightly higher incidences of exposure to aflatoxins (9.8%) compared to females, 5.4% (Table 4.14) though the difference was not statistically significant ($\chi^2 = 2.000$, $P = 0.157$). Similarly, comparing the aflatoxin exposure to the farmers in the two districts based on AFB positivity, revealed no significant difference between the two districts ($t = 1.50$, $P = 0.374$).

Table 4.14: Positive cases of peanuts farmers' exposure to aflatoxins in Busia and Kisii Central districts based on gender

	Urinary Aflatoxin B exposure positive cases.				
	Male		Female		
District	**N**	**%**	**n**	**%**	**Total (n)**
Busia	8	3.9	5	2.5	13 (6.4%)
Kisii Central	12	5.9	6	2.9	18 (8.8%)
Total (N)	**20**	**9.8**	**11**	**5.4**	**31 (15.2%)**

4.8 Prevalence of aflatoxin induced hepatocellular carcinoma in the study population from Busia and Kisii Central districts

Out of 517 patients from both Busia and Kisii Central districts who attended Moi Teaching and Referral hospital with liver disorders during the study period, 102

(19.73%) were diagnosed with aflatoxin induced hepatocellular carcinoma (HCC) while 415 (80.27%) tested negative for HCC. The results showed the prevalence of HCC among the peanut farmers in the study districts at 19.73%. However, the prevalence of aflatoxin induced hepatocellular carcinoma in the general population in Busia was 0.01 while that of Kisii Central district was 0.003%. This was calculated based on the 2009 Kenya Population and Housing Census results.

4.8.1 Aflatoxin induced hepatocellular carcinoma in the study population by gender of patients

Out of 102 patients (19.73%) who were positive for HCC, 61 (11.80%) were from Busia district while 41 (7.93%) were from Kisii Central (Table 4.15). Out of the 61 patients from Busia district who were positive for HCC, 39 (7.54%) were male while 22 (4.26%) were females. In Kisii Central district, 26 (5.03%) were male while 15 (2.90%) were female. Overall, more male patients (12.57%) had aflatoxin induced HCC compared to females (7.16%) in the two districts ($\chi^2 = 2.000$, $P = 0.047$) as shown in Table 4.15.

Table 4.15: Prevalence of aflatoxin induced hepatocellular carcinoma in the study population according to gender of patients

	No. of positive patients for HCC		
District	**N (HCC Positive)**	**Male**	**Female**
Busia	61(11.80%)	39 (7.54%)	22 (4.26%)
Kisii Central	41 (7.93%)	26 (5.03%)	15 (2.90%)
Total	**102 (19.73%)**	**65 (12.57%)**	**37 (7.16%)**

The prevalence of aflatoxin induced HCC in the study population was calculated based on the total number of patients (517) from both Busia and Kisii Central districts who attended Moi Teaching and Referral hospital during the study period with liver disorders.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Peanuts varieties from Busia and Kisii Central Districts

In both districts, peanuts of Valencia red variety had the highest number of samples, 59 and 89 from Busia and Kisii Central districts respectively which were significantly different from the other varieties. This could have been attributed by the fact that Valencia red variety is the most common improved variety of peanuts planted by farmers in the western region of Kenya (Mutegi *et al.*, 2009) compared to other varieties in the study areas. The variety tends to be preferred due to higher yield and resistance to diseases which may also reduce their susceptibility to infection by *Aspergillus* species compared to the local varieties (Mutegi *et al.*, 2009). The result of this study is in line with that of Mutegi *et al.*, (2010) who documented a higher number of peanuts of Valencia red variety than the other varieties in the study areas.

In this study, there were few samples of peanuts of local varieties; Uganda local red (26), Homabay local (28) and Local red (2) from the two study districts. It is possible that peanuts farmers avoid planting these varieties because they have low yields and are less resistant to diseases (Hell *et al.*, 2003). Moreover, local varieties of peanuts such as Uganda local red, Homabay local and Local red, have

been reported to be more susceptible to rosette virus and mould than improved varieties (Ogwang, 2006), and a positive correlation between the diseases and aflatoxin contamination of peanuts have been documented in other studies (Udoh *et al.*, 2000; Kasno, 2004; Robertson-Hoyt *et al.*, 2007). These findings are consistent with those reported in previous studies by Mutegi *et al.* (2010).

5.1.2 Occurrence of aflatoxin in peanuts from Busia and Kisii Central Districts

Aflatoxin was detected in almost all the peanut samples of the different varieties from Busia (97.06%) and Kisii Central districts (100%) as shown in Figure 4.2. This could have been attributed by the frequent rainfall (wetter) in Kisii Central compared to drier climatic conditions in Busia district. The wetter and more humid conditions tends to aggravate aflatoxins levels as it enhances the growth of *Aspergillus* species and production of aflatoxins in peanuts compared to drier climatic condition (Kaaya *et al.*, 2006). Previously, it was documented that significant correlations existed between weather conditions and aflatoxin levels, whereby a wet and humid climate tends to favor the growth of *Aspergillus* species and increase aflatoxin production in peanuts (Hell *et al.*, 2000; Mutegi *et al.*, 2010). Similar results were reported in previous studies in peanuts (Hell *et al.*, 2000; 2003; Mutegi *et al.,* 2009) and a in a survey of maize samples from Nigeria (Atehnkeng *et al.*, 2008).

5.1.3 Levels and types of aflatoxins contaminating the different varieties of peanuts in Busia and Kisii Central Districts

In this study, the levels and types of aflatoxins detected in peanuts from the two districts were compared with the Kenya Bureau of Standards (KEBS), European Union (EU) and United States Food and Drug Administration (USFDA) regulatory limits for total and types of aflatoxins in foods in order to determine whether they were safe for human consumption.

5.1.3.1 Peanuts of different varieties analyzed with detectable levels of aflatoxins

Most peanuts (50.7%) of different varieties from Kisii Central district were contaminated with aflatoxins compared to those from Busia (49.3%). The significantly higher number of peanuts samples from Kisii Central that were contaminated with aflatoxins compared to Busia could have been contributed to different climate conditions in the districts; wetter and humid conditions often occurring in Kisii Central and drier conditions in Busia (Mutegi *et al.*, 2009). Wet and humid conditions tend to aggravate growth of *Aspergillus* species especially *Aspergillus flavus* and *parasiticus* and therefore more production of aflatoxins compared to dry areas like Busia. This is probably because a high moisture condition does not allow sufficient drying of peanuts that are in most cases dried on bare ground or polythene sheets in homesteads and therefore facilitating the fungi contamination and hence higher production of aflatoxins. The results are in line with previous studies which documented a significant association between

climate and aflatoxin levels, whereby a wet and humid climate enhances production of aflatoxins in peanuts compared to drier areas (Mutegi *et al.*, 2009). Kaaya *et al.*, (2006), documented higher aflatoxin levels though in maize samples from humid areas compared to drier areas in Uganda.

Peanuts of Valencia red variety from both districts had a significantly higher number of samples with aflatoxin contamination than the other varieties, 89 (87.3%) and 58 (58.6%) from Kisii Central and Busia respectively ($F = 13.15$, $df = 3$, $P = 0.015$). This could probably be due to the fact that peanuts of Valencia red variety are the commonly grown in the study areas and therefore more samples were collected compared to other varieties. The results are consistent with previous study on peanuts which documented Valencia red variety peanuts as the mostly grown in western region and had the highest number of samples compared to the other varieties analyzed in the study contaminated with aflatoxins (Mutegi *et al.*, 2009). It is important to note that a higher proportion of peanuts of Valencia red variety from Kisii Central were contaminated with aflatoxins compared to those from Busia district. This could be due to the wet and humid climate in Kisii Central compared to Busia (dry) whereby the higher moisture contents facilitates the growth of *Aspergillus* species and therefore high Aflatoxin levels production. The result is in line with a report of previous study done on peanuts by Mutegi *et al.* (2009).

More peanuts samples of Uganda local red, Homabay local and Local red varieties from Kisii Central district had detectable levels of aflatoxins compared to those from Busia district (Table 4.2). This could probably due to higher number of samples of the peanut analyzed from Kisii Central district as compared to those from Busia. It might also have been contributed by more susceptibility to colonization by Aflatoxin producing *Aspergillus* species as they are local varieties. Moreover, the local peanut varieties; Homabay local, Uganda local red and Local red have been reported to be susceptible to stem rot, mould and rosette virus (Ogwang, 2006), and positive correlations between these diseases and aflatoxin contamination of peanuts have been documented in previous studies (Kasno, 2004; Robertson- Hoyt *et al.*, 2007).

5.1.3.2 Levels of total aflatoxins in different varieties of peanuts from the two study districts

In this study, the levels of aflatoxin in peanuts from the two study districts were investigated. The aflatoxin levels were identified and compared with KEBS, EU and USFDA regulatory limits of total aflatoxins in foods including peanuts. Overall, the levels of total aflatoxin ranges in peanuts from Kisii Central were higher (ranges were 1.63 to 591.1 µg/kg) compared to peanuts from Busia (ranged from 0.1 to 268 µg/kg). The higher aflatoxin levels in peanuts from Kisii Central district could have been attributed by high moisture contents in peanuts due to wet and humid climate in the area which increases colonization of *Aspergillus* species and therefore high aflatoxin production compared to Busia district with dry

weather conditions (Mutegi *et al.*, 2009). The study is in line with a previous study on peanuts (Gachomo *et al.*, 2004; Mutegi *et al.*, 2009). In another study, aflatoxins levels in maize samples were higher in wet areas compared to dry areas (Kaaya *et al.*, 2004). Similar results were obtained in a survey of peanut samples from Nigeria (Atehnkeng *et al.*, 2008).

In this study, the results showed that peanuts of local varieties and those from more humid areas were more contaminated with aflatoxins than those from improved varieties and less humid areas. Peanuts of Local red variety from Busia district had the highest level of aflatoxins at 268 µg/kg compared to other varieties analyzed while in Kisii Central district, peanuts of Valencia red variety had the highest aflatoxin levels at 591.07 µg/kg. This result could have been attributed by more susceptibility to aflatoxins contamination of peanuts of Local red variety in Busia district while in Kisii Central, the more humid and wetter climatic conditions may have contributed to increased moisture in peanuts which facilitated the growth of *Aspergillus* species and production of aflatoxins even in Valencia red, an improved peanuts variety. The results are in line with a previous study on peanuts by Mutegi *et al.* (2009) who reported high aflatoxins levels in peanuts of Local red and Valencia red varieties.

Most of peanuts from the different varieties in Busia except Local red were fit for human consumption based on the EU total aflatoxins levels regulatory limits (≤ 4μg/kg) compared to those in Kisii Central district. This is because 88.2 %, 76.2% and 80% of Valencia red, Uganda local red and Homabay local samples from Busia had total aflatoxin levels of ≤ 4μg/kg compared to only 5.6% and 0% of peanuts of Valencia red, Uganda local red and Homabay local respectively from Kisii Central district in the same category.

Based on the KEBS regulatory limit of ≤10μg/kg, the same trend was observed whereby over 81% of similar peanuts varieties from Busia had aflatoxin levels below the KEBS regulatory limit while only 10% of peanuts of Valencia red and none of the other varieties from Kisii Central had aflatoxin levels of ≤10μg/kg (Table 4.3). This was contributed by more aflatoxins contamination of peanuts in Kisii Central district compared to those from Busia district. The increase in aflatoxin contamination of peanuts in Kisii Central district compared to those from Busia district could have been contributed by the more wetter and humid climate condition than in Busia. This resulted in fewer peanuts samples in the KEBS regulatory limits and therefore most of the peanuts from Kisii Central were unfit for human consumption based on KEBS regulatory limit. The results are similar to reports in previous studies done on peanuts (Waliyar *et al.*, 2008; Mutegi *et al.*, 2009).

Based on the USFDA regulatory limit of >20 µg/kg, over 81% of Valencia red, Homabay local and Uganda local red peanuts varieties from Busia had aflatoxin levels of below the USFDA regulatory limit while only 11.2%, 12.5% and 20% of Valencia red, Homabay local and Uganda local red varieties respectively from Kisii Central had aflatoxin levels of below the USFDA regulatory limit (Table 4.3). The result was contributed by peanuts of all the varieties from Kisii Central district being more contaminated with aflatoxins compared to those from Busia. Therefore, most of peanuts from Busia district (over 81%) and 20% and below of peanuts from Kisii Central were fit for human consumption based on USFDA regulatory limit. The results are consistent with a previous study done by Mutegi *et al.*, (2010).

It was noted that all the samples of Local red variety which was sampled only from Busia district had total aflatoxin levels of >20 µg/kg. These peanuts were highly contaminated with aflatoxins and therefore unfit for human consumption based on USFDA regulatory limit. This could be attributed to the fact that local peanuts varieties such as Local red and Uganda red are reported to be more susceptible to moulds, stem rot and other diseases (Ogwang, 2006) of which the diseases correlate with growth of *Aspergillus* species and therefore more aflatoxin production. Similar results have been reported in previous studies (Kasno, 2004; Robertson-Hoyt *et al.*, 2007).

5.1.3.3 Types of aflatoxins identified in peanuts samples from the two study districts

In this study, the four types of aflatoxins identified (B1, B2, G1 and G2) were at higher levels in peanuts from Kisii Central than those from Busia. Perhaps due to wetter humid conditions in Kisii Central district compared to Busia. There were high incidences of Aflatoxin B1 and B2 types across the samples in both districts, and a significantly higher incidence of Aflatoxin type G1 in Kisii Central compared to Busia district. As expected, the high incidence of *Aspergillus flavus S-* strain reported in Kisii Central was associated with greater aflatoxin production. This particular strain has been documented to be responsible for production of aflatoxins and especially the more toxic B toxins particularly in more humid and wetter areas (Probst *et al.*, 2007). The high incidence of *Aspergillus flavus S-* strain identified in this study could therefore have been responsible for the high incidences of the Aflatoxin B1 and B2 types in the study districts. A similar trend of result has been reported in other studies, whereby the *Aspergillus flavus S-* strain is found to be the main source of aflatoxin in foods including peanuts in the United States (Abbas and Hemed, 2005) and in maize from Kenya (Probst *et al.*, 2007). The result of this study corresponds well with other studies that documented similar predominance in Aflatoxin B1 (Awuah and Kpodo, 1996; Mutegi *et al.*, 2007).

It was observed that as the total aflatoxin levels increased, the incidence of aflatoxin B1 generally increased which accords well with findings of Horn and Dorner (1999), who found a positive association between Aflatoxin B1 production and increased incidences of both *S*- and *L*- strains of *A. flavus*. Despite that majority of peanuts especially those from Busia being safe for human consumption compared to those from Kisii Central, according to EU and KEBS regulatory limits (Mutegi *et al.*, 2009), the high incidences of *Aspergillus flavus S*- strain and *Aspergillus flavus L*- strain reported in this study, implies a likelihood of increased aflatoxin levels if safe pre- and post- harvest aflatoxin contamination management measures are not adhered to.

In spite of the fact that there was low incidence of *Aspergillus parasiticus* (mean occurrence of 9.3%) in this study, it was the third most isolated *Aspergillus* species from *Aspergillus flavus S* and *Aspergillus flavus L*- strains, a fact that the species could have contributed to high levels of aflatoxins especially aflatoxin G1 type in Kisii Central District. The wetter and more humid growth conditions in Kisii Central compared to Busia could have contributed to increased production of aflatoxin G1 type by the *Aspergillus parasiticus* which produces exclusively toxin G1 and subsequently significantly higher aflatoxin level of the toxin type in Kisii Central. The result is in line with previous report on peanuts, which documented *Aspergillus parasiticus* as one of the key fungus that contributes to high production of aflatoxin G1 especially in wet areas (Mutegi *et al.*, 2009). In

addition to producing aflatoxin G1 and G2, *A. parasiticus* is also capable of producing of aflatoxin B1 and B2 (Waliyar *et al.*, 2008). This could have contributed to the high proportions of the two aflatoxins types in peanuts particularly those from Kisii Central District.

Most of peanuts samples (89.9%) of different varieties from Busia district had Aflatoxin B1 levels below ≤ 5µg/kg and were fit for human consumption based on KEBS Aflatoxin B1 regulatory limit of ≤ 5µg/kg. Only 13.7% of peanuts samples from Kisii Central had Aflatoxin B1 levels within the KEBS regulatory limit and therefore were fit for human consumption while 86.3% had levels exceeding ≤ 5µg/kg. This could be explained by the higher incidence of *Aspergillus flavus S-* strain (60.2%) in peanuts from Kisii Central compared to those from Busia (29.4%). The high incidence of this strain in the district may imply higher production of aflatoxin B1 in peanuts from the study area. Although there was no significant difference in incidences of *Aspergillus flavus S-* strain (60.2%) and *Aspergillus flavus L-* strain (58.8%) in peanuts from Busia district, most of the *L* strain *A. flavus* were atoxigenic which resulted to low production of aflatoxins including aflatoxin B1 in peanuts from Busia. The result corresponds with previous studies on peanuts by Mutegi *et al.* (2009).

5.1.4 Aflatoxins producing *Aspergillus* species in peanuts from Busia and Kisii Central Districts

This study identified the *Aspergillus* species in peanuts from Busia and Kisii Central that are involved in the production of aflatoxins in the peanuts. The predominant *Aspergillus* species across the districts with over 58% incidence were *A. flavus S*- strain and *Aspergillus flavus L*- strain, with an incidence of 60.2% and 58.8% respectively. *Aspergillus flavus* which includes *L* strain and *S* strain have been documented as the common species that grow and produce aflatoxins in foods including peanuts than other *Aspergillus* species (Mutungi *et al.*, 2008). These *Aspergillus* species have been isolated at slightly higher incidences in peanuts in a previous study (Mutegi *et al.*, 2010), *S* strain at 78% and *L* strain at 68%. The difference in incidences between the current and the previous studies could have been contributed by difference in sample sizes and the specific study Districts.

Aspergillus flavus L- strain was the most common isolate (58.8%) in peanuts from Busia district while *A. flavus* S- strain was the most common strain (60.2%) in peanuts from Kisii Central district. This might be contributed by difference in weather conditions between the two study districts. *Aspergillus flavus S* strain contains aflatoxin Q (aflQ) toxigenic genes which usually produces high aflatoxins in wet conditions while *Aspergillus flavus* L- strain (contains aflatoxin D toxigenic genes) that produces high aflatoxins in dry conditions (Gachomo *et al.*, 2004). It

was noted that the species was isolated at a high incidence in Kisii Central while *Aspergillus flavus* L- strain was isolated more in Busia district. The results are in line with other studies by Horn (2005) and Mutegi *et al.*, (2010). *Aspergillus parasiticus*, *Aspergillus niger* and *Aspergillus tamarii* were isolated in this study at overall mean occurrences of 9.3%, 2.9% and 1% respectively in peanuts from the two study districts. The low occurrences of these three *Aspergillus* species in the study areas is in line with the reports of Horn (2005), who documented these species in the United States of America and Mutegi *et al.*, (2010) in Kenya at comparable low occurrences.

The high incidence of *A. flavus* S- strain particularly in Kisii Central that produces aflatoxin (Gachomo *et al.*, 2004; Ehrlich *et al.*, 2007) and in particular, the most potent Aflatoxin B1, indicates a risk of aflatoxin contamination of peanuts in areas in the western Kenya with wet climatic conditions which enhances the growth and production of aflatoxins mainly by *Aspergillus flavus*. In as much as the occurrence of *Aspergillus flavus L*- strain was slightly low in peanuts from Busia (58.8%) compared to *A. flavus S*- strain in Kisii Central (60.2%), it did not result to higher level of aflatoxins in the peanuts compared to *A. flavus S*- strain and this could be attributed to the fact that most of the *L* - strains may be atoxigenic. This resulted to low levels of total aflatoxin in peanuts from Busia compared to those from Kisii Central. A similar trend has been found in other studies whereby

Aspergillus parasiticus has been found to be the main source of aflatoxin in foods including peanuts (Probst *et al.*, 2007).

Aspergillus parasiticus was the third most abundantly identified *Aspergillus* species in peanuts from both districts. The species was isolated in peanuts from Kisii Central district at 12% and 6.9% in peanuts from Busia district. *Aspergillus parasiticus* is known to be common in wet climatic conditions and such environmental conditions facilitates growth and aflatoxin production especially aflatoxin G1 by the species (Ehnlich *et al.*, 2007). This reason could explain to the high occurrence of *Aspergillus parasiticus* in Kisii Central district (wet condition) compared to Busia (dry condition). The results are consistent with previous study on peanuts by Mutegi *et al.* (2009) where *Aspergillus parasiticus* was documented as the third most common *Aspergillus* species after *A. flavus S-* strain and *Aspergillus flavus L-* strain in the production of aflatoxin in peanuts. The confirmation of occurrence of other species that produce toxins such as *A. niger* and *A. tamarii* which also produce cyclopiazonic acid (Bayman *et al.*, 2002), suggests the need to screen peanuts not just for aflatoxins but also for other carcinogenic mycotoxins.

5.1.4.1 Distribution of *Aspergillus* species in the different varieties of peanuts

The results of this study showed that all the varieties of peanuts sampled from both Busia and Kisii Central districts were contaminated with at least one or more of *A. flavus L-* strain, *A. flavus S-* strain, *A. parasiticus*, *Aspergillus niger and A. tamarii* species. All the *Aspergillus* species were isolated in all the peanuts varieties from Busia district except *A. niger* which was detected in peanuts of Local variety while *A. tamarii* was not detected at all (Table 4.11). Overall, *Aspergillus flavus* L- strain was the most highly detected strain (60.6%) in all the peanut varieties collected from Busia district ($H = 10.03$, $df = 3$, $P = 0.018$) followed by *Aspergillus flavus S- strain* at 30.3% occurrence. Previous studies have reported that *Aspergillus flavus L-* strain and *Aspergillus flavus S-* strain are the most common species involved in production of aflatoxins in foods including peanuts (Gachomo *et al.*, 2004) and *Aspergillus flavus L-* strain which contains aflD toxigenic genes produces aflatoxin more in dry weather conditions compared to *Aspergillus flavus S-* strain (Mutegi *et al.*, 2009).

Aspergillus flavus L- strain was found in peanuts of Homabay local variety at an occurrence of 33.3% while *Aspergillus flavus S-* strain had 40% in the same variety from Busia. This could probably have been contributed by high susceptibility of the local variety to crop diseases and pests, which result in plant stress thereby predisposing peanuts to the growth of *Aspergillus flavus* (Chapin *et al.*, 2004) particularly the most toxigenic *Aspergillus flavus S* strain. *Aspergillus*

parasiticus was also found to be contaminating all the peanut varieties from the study district but highly isolated in peanuts of Local red, Valencia red and Uganda local varieties at similar rates of 28.6%. This could be due to the fact that *Aspergillus parasiticus* grows and produce aflatoxins even in improved peanuts varieties such as Valencia red. *Aspergillus niger* was only detected in peanuts of Local red variety from Busia. This indicates that this peanut variety from the area is more highly susceptible to the growth of *Aspergillus* including the less common species. The results are in line with reports by Ogwang (2006) who documented that local peanuts varieties such as Local red, Homabay local and Uganda local red are more susceptible to diseases such as stem rot and mould which facilitates the growth of *Aspergillus* species.

In peanut varieties from Kisii Central district, all the strains; *Aspergillus flavus* S-strain, *Aspergillus flavus* L- strain, *Aspergillus parasiticus, A. niger* except *A. tamarii* were isolated in all the varieties. However, *Aspergillus flavus S*-strain had higher occurrence at 60.8% compared to other species identified from the district ($H = 12.28$, $df= 4$, $P = 0.015$). This is because the species contains an aflQ toxigenic gene which makes the species to grow in wet weather conditions compared to other *Aspergillus* species resulting to its high occurrence. This could have contributed to its high detection in peanuts of Valencia red variety (79%). *Aspergillus flavus L*- strain had low occurrence (21.6%) in all varieties from Kisii Central. However, it's important to note that it was also detected highly in peanuts

of Valencia red variety compared to other varieties. This suggests that the variety was more susceptible to *Aspergillus* species contamination than other varieties. This could probably be contributed by sowing of *Aspergillus* contaminated Valencia red variety seeds from the supplier in the district which resulted to contaminated harvests.

Aspergillus parasiticus species was found at higher incidence of 41.7% in Homabay local variety compared to other varieties. *Aspergillus niger* had higher occurrence in peanuts of Uganda local red while *Aspergillus tamarii* was detected in peanuts of Uganda local red and Homabay local varieties at similar rates of 50%. This could be probably due to higher susceptibility of local varieties to crop pests and diseases such as stem rot which facilitates *Aspergillus* species contamination including the less common species (Robertson-Hoyt *et al.*, 2007). The result is in line with the study of Mutegi *et al.* (2009) who showed that peanuts of local varieties have a higher likelihood of being contaminated with aflatoxin than improved varieties. Previous studies have documented higher susceptibility of local varieties peanuts to fungal contamination including *Aspergillus* species in the United Kingdom (Middleton *et al.*, 1994).

5.1.5 Oil contents of the different varieties of peanuts from Busia and Kisii Central districts

Essential oils from foods and microorganisms have been proven as strong inhibitors of aflatoxin biosynthesis (Tsigarida *et al.*, 2000). Inhibition of growth and aflatoxin production by *Aspergillus flavus* and *A. parasiticus* by spice oils has been reported (Reddy *et al.*, 2002). In the current study, peanuts from Busia district had significantly higher oil contents compared to those from Kisii Central district ($t = 3.22$, $df = 6$, $P = 0.012$). This could be because the climate in Busia is hot and dry which enhances formation of oils compared to Kisii Central (wet). Being the first study to be conducted in Kenya to determine oil contents in peanuts, there is no data to be compared with the results of the current study. However, the result is in line with a previous study on peanuts done in Turkey (Özcan and Serap, 2003).

Peanuts of Valencia red variety from both Busia and Kisii Central had higher oil content (mean 46.9), compared to other varieties analyzed in the study. This could probably be due to the fact that Valencia red is an improved peanut variety which takes shorter time to mature especially in dry climate and therefore very fast in the formation of oil compared to the other varieties analyzed. However, Local red variety peanuts from Busia and Homabay local from Kisii Central had the lowest oil content at 42.7% and 40.6% respectively. This could probably attribute to the

longer time the local varieties take to mature compared to the improved varieties and therefore low formation of oil.

5.1.5.1 Relationship between oil contents and the levels of total aflatoxin in the peanut samples

Oil contents of peanuts from Busia and Kisii Central districts were compared with the total aflatoxins of the same samples. Overall, there was increased oil content with decreasing total aflatoxin levels ($r = -0.496$, $P = 0.031$) except for Uganda local red variety from Kisii Central district. This is because low oil content in peanuts partially blocks activities of aflatoxin biosynthetic cytosolic enzymes hence increasing production of Aflatoxin production and vice versa (Reddy *et al.*, 2002). In addition, oils are known to disrupt fungal mitochondrial respiration that provides acetyl CoA for Aflatoxin biosynthesis and therefore the higher the oil content, the lower the Aflatoxin production by *Aspergillus* species (Reddy *et al.*, 2002). The results are consistent with a report of a study done by Reddy *et al.*, (2002) who reported an increased inhibition of growth and production of aflatoxin by *Aspergillus flavus* and *A. parasiticus* by increased concentration of spice oils and their active components. Therefore, the higher the oils contents, the higher the inhibition of growth and production of aflatoxins by *Aspergillus* species.

As stated earlier, the only exception in the study was observed in peanuts of Uganda local red variety from Kisii Central district which had the highest level of total aflatoxins (405µg/kg) compared with other varieties in the study. This variety also had high average oil content (45.7%). The result could be explained by other factors other than oil content such as higher susceptibility of local peanuts varieties to crop diseases and pests that result in plant stress predisposing them to aflatoxin contamination (Chapin *et al.*, 2004). Local peanuts varieties such as local red, Homabay local and Uganda local red have been reported to be susceptible to stem rot and mould (Ogwang, 2006), and a positive correlations between these diseases and aflatoxin contamination of peanuts have been reported (Robertson-Hoyt *et al.*, 2007). The result is in line with the study of Mutegi *et al.*, (2010) who reported high aflatoxin levels in peanuts of local varieties compared to the improved ones.

Samples of all peanuts varieties from Busia had lower levels of total aflatoxins with an increase of oil content except the Local red variety. This variety had significantly higher total aflatoxins levels at 267µg/kg and 42.7% average oil content. The result could probably be contributed by factors other than oil contents such as susceptibility to crop diseases and pests by local peanuts varieties that result in plant stress predisposing peanuts to growth and aflatoxin contamination by *Aspergillus* species. These results concur with the study of Hell *et al.*, (2003), who reported a positive correlation between the growing of local varieties and increased Aflatoxin levels in peanuts in Benin. Overall, peanuts from Busia district

had high levels of oil contents and low level of total aflatoxins compared to those from Kisii Central which had slightly lower oil content and significantly higher total aflatoxins levels.

5.1.6 Aflatoxin exposure to peanuts farmers from Busia and Kisii Central Districts

Detection of carcinogen AFB Gual in urine or blood samples from humans is indirect proof that genotoxic damage has occurred after exposure to aflatoxins. The presence of AFB Gual in the urine samples collected in various districts of Kenya is an indication that biological activation of the carcinogen form of Aflatoxin B has taken place and Aflatoxin B has reacted with cellular nucleic acids or their precursors. The AFB dual adduct is quite unstable and has been detected in urine collected from humans living in an area with high exposure to dietary Aflatoxin B (Autrup *et al.*, 1987). Although aflatoxin exposure is more commonly associated with maize and peanuts, aflatoxin has been detected in a wide range of dietary components (Liu and Wu, 2010). However, the aflatoxin level is not usually so high with natural contamination of food stuffs and peanuts appear to be a common substrate for extensive natural aflatoxin production by *Aspergillus flavus* (Fratamico, 2008).

The present study focused on Busia and Kisii Central districts in the western region of Kenya. The districts have different ethnic, cultural and social groups. Out of the 204 urine samples from the two districts analyzed for aflatoxin exposure, 15.2% turned positive for AFB while 84.8% turned negative. This indicates that some individuals are exposed to aflatoxins in the study districts. This could probably be due to improper storage of staple food including peanuts consumed by the population in the areas which results to high aflatoxin contamination. The results are comparable to another study by Autrup *et al.*, (1987) conducted in Central and Eastern regions of Kenya where 12.6% of urine samples analyzed for aflatoxin exposure were positive for AFB Gual.

The highest rate of aflatoxin exposure was observed among peanuts farmers from Kisii Central districts compared to those from Busia district where 8.8% and 6.4% of urine samples respectively turned positive for AFB Gual. This corresponds to high aflatoxin levels in peanuts from Kisii Central compared to those from Busia district and also high contamination of peanuts with *Aspergillus* species particularly *Aspergillus parasiticus* and *A. flavus* and therefore more aflatoxins are produced. Consumption of improperly dried and stored grains including peanuts by the population in Kisii Central district compared to Busia district could probably have contributed to the high exposure to aflatoxin. Exposure to aflatoxins through drinking of local alcohol beverages brewed from highly AFB contaminated grains favored by rainy weather conditions in Kisii Central

compared to Busia district could have contributed to the higher rate of aflatoxin exposure. The results are in line with a previous study by Autrup *et al.*, (1987) where he analyzed urine samples from Central, Western and Eastern regions and reported aflatoxin exposure of 8.1% in South Nyanza districts.

Overall, male peanuts farmers in this study were more exposed to aflatoxins than females. Approximately 9.8% of the urine samples collected from the peanuts farmers and were positive for AFB gual had been collected from males while 5.4% were collected from females in the two study districts. This could probably have been contributed by exposure through consumption of alcohol beverages brewed from AFB contaminated grains by males in the study areas. It might also have been attributed to higher consumption of porridge or sauce prepared from aflatoxin B contaminated grains including peanuts by males. Autrup *et al.*, (1987) reported a male to female ratio of greater than 1 on aflatoxin B exposure, meaning that males were more exposed to aflatoxins than females. However, male peanuts farmers from Kisii Central district were more exposed to aflatoxins (5.9%) compared to males from Busia district (3.9%). This could be due to more consumption of aflatoxins contaminated foods including maize and peanuts by the population in Kisii Central District where the peanuts were more contaminated with aflatoxin compared to the population in Busia District.

5.1.7 The prevalence of aflatoxin induced hepatocellular carcinoma in patients from Busia and Kisii Central Districts

Aflatoxin, a contaminant produced mainly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* in maize and peanuts have been classified as a potential human liver carcinogen (Liu and Wu, 2010). There has been observed a close link between Aflatoxin B and the incidence of hepatocellular carcinoma in several parts of the world including Kenya (Wu and Santella, 2012; Van Rensburg *et al.*, 1985). It was estimated that 4.5 billion people worldwide are exposed to uncontrolled aflatoxins from dietary food (Williams *et al.*, 2004). However, the burden of HCC cases in Kenya attributed to aflatoxin as the etiological agent remained undefined. In this study, hospital records of patients from Busia and Kisii Central districts attending the Moi Teaching and Referral hospital in the period, January 2010 to December 2012 were analyzed to determine the prevalence of hepatocellular carcinoma in the two districts.

In the present study, out of 517 patients with liver disorders from both Busia and Kisii Central districts who attended Moi Teaching and Referral hospital, 102 (19.73%) were diagnosed with aflatoxin induced hepatocellular carcinoma giving the prevalence of aflatoxin induced HCC among the study population in the two districts as 19.73%. This prevalence was comparable to that reported by Autrup *et al.*, (1987), who reported a prevalence of 19.95% for liver cancer after analysis of medical records of patients attending the liver clinic at Kenyatta National Hospital

over a period of 5 years (1978-1982). However, the prevalence of 19.95% for liver cancer may have included all the etiological agents including aflatoxins. The prevalence of aflatoxin induced HCC reported in the present study could have been contributed by more exposure to aflatoxins of the population in the study districts and may reflect the country wide picture.

In this study, the prevalence of aflatoxin induced hepatocellular carcinoma among the study population was higher among patients from Busia district (11.80%) than those from Kisii Central district (7.93%). This could be due to shorter distance from Busia district to the hospital compared to the distance from Kisii Central district to Moi Teaching and Referral Hospital. The shorter distance could have resulted to more patients with liver disorders attending the facility and therefore higher prevalence of aflatoxin induced HCC reported in this study.

The finding could also be contributed to good local hospital facilities in Kisii Central district compared to those in Busia district where more patients with liver disorders may seek treatment in the local hospitals and less number from the district attending the referral hospital. Also the prevalence of aflatoxin induced hepatocellular carcinoma among the population in Busia district was also higher (0.01) than that of Kisii Cental district (0.003). The difference of the population size in the study districts probably contributed to the difference in the prevalence rates. This study being the first one to be carried in Kenya to determine the

prevalence of aflatoxin induced hepatocellular carcinoma, the results could not be compared to other results in any other study in Kenya.

5.1.7.1 The prevalence of aflatoxin induced hepatocellular carcinoma in the study population according to gender

In the present study, aflatoxin induced hepatocellular carcinoma in the two study districts was more predominant in males (12.57%) than in females (7.16%). The findings indicate that males are more exposed to aflatoxins than females. Despite the fact that diet is the main source of aflatoxin B exposure in the Kenyan population (Mutegi *et al.*, 2013; Pears *et al.*, 1976), local alcohol beverages brewed from aflatoxin contaminated grains may also be a potential source of aflatoxin exposure. Therefore males may be more exposed to aflatoxins through taking such alcohol which results to higher prevalence of aflatoxin induced hepatocellular carcinoma in males than in females. It could also have been contributed by higher body mass index (BMI) in males than in females which leads to fatty liver diseases and therefore higher risks of getting HCC (Autrup *et al.*, 1987). Increased iron stores in the liver leading to cirrhosis and liver cancer could probably have contributed to the higher prevalence of aflatoxin HCC in males than in females in the study. The results of this study are in line with Autrup *et al.*, (1987) who reported a ratio of 3:1 of male to female in the incidence of liver cancer (liver cancer) caused by various factors including aflatoxins among patients attending the Kenyatta National Hospital liver clinic.

5.2 CONCLUSIONS

i. Almost all respondents (98%) in both Busia and Kisii Central districts consumed peanuts on daily basis in their households from their own harvest.

ii. Aflatoxin was detected in all peanuts from Kisii Central and 97.06% in those from Busia.

iii. The level of aflatoxin varied considerably among samples although majority of peanuts samples of the different varieties had aflatoxin levels within the Kenya Bureau of Standards and European Union regulatory limits for total aflatoxins for human consumption.

iv. Improved variety (Valencia red) had significantly lower aflatoxin contamination compared to local varieties (Uganda local red, Homabay local and Local red).

v. Aflatoxins B1, B2, G1 and G2 were found in peanuts; B1 was the most predominant in both districts.

vi. The predominant *Aspergillus* species were *A. flavus S*-strain and *A. flavus L*-strain. However, *A. flavus S*-strain was highly isolated in peanuts from Kisii Central while *A. flavus L*-strain was mostly detected from Busia.

vii. Oil content in different varieties of peanuts from Busia district was slightly higher than those from Kisii Central. However, the oil contents in peanuts decreased with an increase in aflatoxin levels ($r = -0.496$, $P = 0.031$) except peanuts of Uganda local red variety from Kisii Central.

viii. Peanuts producers from Kisii Central district were significantly exposed to urinary aflatoxin B compared to those in Busia district. However, male farmers in both districts of study were more exposed than females.

ix. The prevalence of aflatoxin induced HCC among the study population was high (19.73%) with more patients from Busia Districts compared to those from Kisii Central.

5.3 RECOMMENDATIONS

i. The presences of high levels of aflatoxins in peanuts suggest the need for urgent awareness on the aflatoxin levels through campaign among peanuts producers and promote sound practices when handling peanuts. This will help in prevention, control and mitigation of aflatoxin contamination.

ii. An assessment on the levels of aflatoxins should also be done by the relevant stakeholders in other foods for example maize which is a key staple food in the Districts.

iii. Frequent aflatoxin screening of peanuts from the Districts particularly aflatoxin type B should be done.

iv. Peanuts producers in the western region and other parts of the country ought to be encouraged to grow improved varieties of peanuts such as Valencia red which was least contaminated with aflatoxin and had higher oil content.

v. The confirmation of occurrence of other species that produce toxins such as *A. niger* and *A. tamarii* which also produces cyclopiazonic acid suggests the need to screen peanuts for other carcinogenic mycotoxins.

vi. Frequent screening for aflatoxin induced HCC of people particularly those with liver disorders by health workers is recommended for early detection and proper management of the disease.

5.4 SUGGESTIONS FOR FURTHER STUDIES

i. Further studies on aflatoxin contamination of peanuts products particularly peanut butter which is consumed in Kenya should be carried out.

ii. Peanuts should be screened for other carcinogenic mycotoxins other than aflatoxin.

iii. More studies on the prevalence of aflatoxin induced HCC should be done in other areas in Kenya where peanuts are consumed as the prevalence of the disease in the general population remains unclear.

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APPENDICES

APPENDIX I: Map showing Kisii Central Districts, Kenya

Source: Mutegi *et al.* (2013).

APPENDIX II: Map showing Busia Districts, Kenya

Source: Mutegi *et al.* (2013).

APPENDIX III: Consent Form

Title of Study: Aflatoxins in Peanuts and the Prevalence of Aflatoxin Induced Hepatocellular Carcinoma in Busia and Kisii Central Districts, Kenya.

Institutions: Kenyatta University and the Government Chemist, Kenya

Principle Investigator: Mr. Nelson Menza, Department of Medical Laboratory Sciences, Kenyatta University, Kenya.

1st Co principal Investigator: Dr. Margaret Muturi, Department of Medical Laboratory Sciences, Kenyatta University, Kenya.

2st Co principal Investigator: Dr. Lucy Kamau, Department of Zoological Sciences, Kenyatta University, Kenya

Investigators Statement

This is an educational research study that will be carried out by a researcher from Kenyatta University. This consent form will provide for you information that you will need to help you decide whether to participate in the study or not. The researcher will administer it to you. You may ask any question concerning the purpose of the research, procedures that will be followed, your rights as a participant in the study, risks and benefits of the study.

Purpose of the Study

The purpose of the study is to determine the incidence of aflatoxins on peanuts and the individual exposure to aflatoxins. Therefore, peanuts samples will be collected for the aflatoxin analysis and urine samples will be collected to determine the individuals' exposure to aflatoxins after administration of a questionnaire.

Procedures:

Questionnaire

This questionnaire intends to find out practices that are directly or indirectly related to mould or aflatoxin contamination of peanuts. If you agree to take part in the study, the age, gender, education level, amounts of peanuts produced, consumed, form of peanuts consumed and the source of the peanuts will be recorded. Peanuts and urine samples will be collected and taken to The Government Chemists Laboratories, Nairobi for analysis.

Risks

There are no direct or indirect risks in this study.

Benefits

There is no direct benefit to you apart from the satisfaction that your participation will help better understand the incidence of aflatoxins on peanuts and the HCC incidence in the region.

Assurance of Confidentiality

Any information relating to your participation in this study will remain private. Your name will not be used in any report resulting from this study. The consent form will be safely kept and laboratory specimens will have only a study number not your name. If you have more questions regarding the study, feel free to contact Kenyatta University Ethics Review Committee on the emails: kuerc.chairman@ku.ac.ke or kuerc.secretary@ku.ac.ke or ercku2008@gmail.com.

Subject Statement and Signature

The study has been explained to me. I volunteer to take part in this study.

Name of the participant.....

Signature or fingerprint of participant.....

APPENDIX IV: Questionnaire

Title of Study: Aflatoxins in Peanuts and the Prevalence of Aflatoxin Induced Hepatocellular Carcinoma in Busia and Kisii Central Districts, Kenya.

Introduction

I am Nelson Menza, a PhD student in the Department of Medical Laboratory, School of Health Sciences at the Kenyatta University. I am investigating the Aflatoxins in Peanuts and the Prevalence of Aflatoxin Induced Hepatocellular Carcinoma in Busia and Kisii Central Districtss in western Kenya. I kindly request that you participate in this study by providing the information to the best of your knowledge as outlined in this questionnaire

Serial No: ---------- Date: ----------

QUESTIONNAIRE PART I (Demographic Information)

1. Age in years

(i) <25 ( ) (ii) 25-30 ( ) (iii) 31- 40 ( ) (iv) 41- 50 ( ) (v) > 50

2. Area of Residence: ----------

3. Religion/Denomination: (i) Christian ( ) (ii) Muslim ( ) (iii) None ( )

(iv) Others (specify)_____

4. Marital status :(i) Married ( ) (ii) Widow ( ) (ii) Single ( ) (iii) Separate ( )

(iv) Divorce ( )

5. Nationality: _____

6. Highest Educational Level: (i) Primary ( ) (ii) Secondary ( ) (iii) post-secondary ( ) (iv) None ( )

7. Occupation: _____

QUESTIONNAIRE PART II

1. What is the approximate amount of peanuts do you produce per harvest? (in tonnes)

i) < 1 ( ) (ii) 2 to 4 ( ) (iii) 4 to 6 ( ) (iv) More than 10

2. What form of the peanuts do you or your household consume?

(i) Podded raw (ii) Shelled raw (iii) Roasted (iv) Fried

3. How many times do you or your household take peanuts per day?

(i) 1 (ii) 2 (iii) 3 (iii) 3 (iv) 4 (v) more than 4

4. What is the approximate amount of the peanuts do you or your household take per day?

(i) Less than 1 kg (ii) 2 kgs (iii) 3 kg (iv) more than 3 kgs

5. Do the peanuts you consume have moulds?

(i) Yes (ii) No

6. What is the source of the peanuts you consume?

(i) Own harvest (ii) Bought locally (iii) Bought from neignbouring Countries

7. Do you pack your peanuts before or after drying?

(i) Before (ii) After

8. How do you pack your peanuts?

(i) Using boxes (ii) Using sacks (iii) Using basins (iv) Using polythene bags (v) Other, specify.....

9. How long do you store your peanuts before consumption ?

(i) 1 week or less (ii) 2 weeks (iii) 1 month (iv) more than 1month (specify).....

10. Have you or your household suffered from liver diseases after consuming peanuts?

(i) Yes (ii) No

11. If yes when was your last time to suffer from the disease?

(i) Last 1 month (ii) Last 2 months (iii) Last 6 months (iv) Last 1 year

12.Did you or you household seek medical attention?

(i) Yes (ii) No

13. If yes, where?

(i) Local dispensary (ii) District hospital (iii) Provisional hospital (iv) Refferal hospital

14. If referral hospital, which one?

(i) Kenyatta National Hospital (ii) Moi Teaching and Refferal hospital (iii) Others (State)

APPENDIX V: Ethical Approval

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
kuerc.secretary@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844
Nairobi, 00100
Tel: 8710901/12
Tel: 8710901/12

Our Ref: KU/R/COMM/51/243

Date: 23rd October, 2013

Nelson Chengo Menza
Kenyatta University
P.O. Box 43844 - 00100 Nairobi

Dear Mr. Chengo

APPLICATION NUMBER **PKU/161/I 141 – "DETERMINATION OF AFLATOXINS IN PEANUTS AND THE ASSOCIATED HEPATOCELLULAR CARCINOMA IN BUSIA AND KISII DISTRICTS, KENYA" – Version 2**

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic "Determination of aflatoxins in peanuts and the associated hepatocellular carcinoma in Busia and Kisii districts, Kenya" dated 23rd October, 2013.

2. APPLICANT

Nelson Chengo Menza
Kenyatta University
P.O. Box 43844 - 00100 Nairobi

3. SITE

Busia and Kisii County, Kenya

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines, and is of the view that against the following elements of review,

(i) Scientific design and conduct of study,
(ii) Recruitment of research participant,
(iii) Care and protection of research participants,
(iv) Protection of research participant's confidentiality,
(v) Informed consent process,
(vi) Community considerations.

AND APPROVED that the research may proceed for a period of ONE year from 23rd October, 2013

ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.

iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

KENYATTA UNIVERSITY
OFFICE OF THE CHAIRMAN
23 OCT 2013
ETHICS REVIEW COMMITTEE
P.O. BOX 43844 - 00100 NAIROBI

PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I NELSON CHENGO MENZA ..... accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of.....22./10./..... 2013.

cc. Vice-Chancellor
Director: Institute for Research Science and Technology

APPENDIX VI: Research Permit

THIS IS TO CERTIFY THAT:
MR. NELSON CHENGO MENZA
of KENYATTA UNIVERSITY, 0-100
nairobi,has been permitted to conduct
research in *Busia , Kisii Counties*

on the topic: *DETERMINATION OF AFLATOXINS IN PEANUTS AND THE ASSOCIATED HEPATOCELLULAR CARCINOMA IN BUSIA AND KISII DISTRICTS, KENYA*

for the period ending:
31st August,2014

.....
Applicant's Signature

Permit No : NACOSTI/P/13/0768/385
Date Of Issue : 13th January,2014
Fee Recieved : Kshs khs2000.00

.....
for Secretary
National Commission for Science, Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A 835

CONDITIONS: see back page

APPENDIX VII: Hospital permission

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575
Email: kuerc.chairman@ku.ac.ke
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(iv) Protection of research participant's confidentiality,
(v) Informed consent process,
(vi) Community considerations.

AND APPROVED that the research may proceed for a period of ONE year from 23rd October, 2013

ADVICE/CONDITIONS

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ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.

iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.

If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

KENYATTA UNIVERSITY
OFFICE OF THE CHAIRMAN
23 OCT 2013
ETHICS REVIEW COMMITTEE
P.O. BOX 43844 - 00100 NAIROBI

PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I NELSON CHENGO MENZA ..... accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of.....23/10/..... 2013.

cc. Vice-Chancellor
Director: Institute for Research Science and Technology

LAB MANAGER

Allow the student to collect data from your department.

Catherine Chepkwony
MTRH Administrator
14/1/2014