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THE EXTENT OF AFLATOXIN AND *ASPERGILLUS* SECTION *FLAVI*, *PENICILLIUM* SPP. AND *RHIZOPUS* SPP. CONTAMINATION OF PEANUTS FROM HOUSEHOLDS IN WESTERN KENYA AND THE CAUSATIVE FACTORS OF CONTAMINATION

Charity Kawira Mutegi

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Doctor of Philosophy (Food Security),
African Centre for Food Security,
School of Agricultural Sciences and Agribusiness,
Faculty of Science and Agriculture,
University of KwaZulu-Natal,
Pietermaritzburg.

ABSTRACT

Peanuts contribute significantly to food security in western Kenya due to their high nutritional value and cash crop potential. However, the crop is highly susceptible to aflatoxin contamination. Yet little information is available on the extent of contamination in the region. This study explores the level and extent of contamination of peanuts by aflatoxins, *Aspergillus* section *Flavi*, *Rhizopus* and *Penicillium* spp. in western Kenya.

A survey of 769 households was carried out in the Busia and Homa bay districts of Kenya. Information on peanut pre- and post-harvest practices was collected through person-to-person interviews. Aflatoxin levels of samples collected from each household were determined by indirect competitive ELISA method. Isolation of *Aspergillus* section *Flavi*, *Penicillium* and *Rhizopus* spp. was done on Modified Dichloran Rose Bengal (MDRB) agar, while identification of specific fungal species was done on Czapek yeast extract agar (CYA). Screening isolates of *A. flavus* and *A. parasiticus* for aflatoxin production was done in high sucrose yeast extract (YES) liquid medium, and the aflatoxin types identified on TLC plates, using analytical grades of aflatoxin B_1, B_2, G_1 and G_2 as reference standards.

Common household preparation techniques (roasting, making peanut paste and boiling peanuts) were evaluated for effectiveness in reducing aflatoxin levels in peanuts. The boiling procedure was modified to test the effect of *magadi* (locally available salt used mainly to soften legumes, vegetables or maize while cooking), ammonium persulphate and sodium hypochlorite during soaking. M*agadi*, sodium bicarbonate and locally prepared ash was subsequently used to boil the nuts after soaking.

Aflatoxin levels ranged from zero to 7525 µg/kg. Most samples were safe to consume, based on the European Union and Kenya Bureau of Standards tolerance levels, with 63.7 per cent of all samples having undetectable levels, and only 7.54 per cent being contaminated based on KEBS standards. Peanuts from the Busia district, which has more of Lower Midland 1 (mean annual rainfall of 1600-1800 mm) and Lower Midland 2 (mean annual rainfall of 1300-1700 mm) agro-ecological zones had significantly (χ^2=14.172; *P*=0.0002) higher levels of aflatoxin compared to the Homa bay district, that has more of the drier Lower Midland 3 agro-ecological zone (mean annual rainfall of 900-1500mm). Improved cultivars had significantly (χ^2=9.748; *P*=0.0018) lower levels of aflatoxin compared to local cultivars. Over 60 per cent

of all samples had *A. flavus* S-strain, *A. flavus* L-strain and *A. niger*. *A. flavus* S-strain was positively correlated with aflatoxin levels. As expected, grading of peanuts post-harvest significantly reduced the incidence of *A. flavus* S- and L-strains, while peanuts collected from farmers who belonged to producer marketing groups had a significantly lower incidence of *A. flavus* S- and L-strains, *A. niger* and *Rhizopus* spp. The incidence of *A. flavus* L-strain, *A. niger* and *Rhizopus* spp. was significantly higher in local landraces compared to the improved cultivars. Over 60 per cent of isolates produced Aflatoxin B_1.

Intermediate processes such as sorting and dehusking led to a significant decline in levels of aflatoxin. Soaking peanuts in water, magadi, NaOCl and ammonium persulphate significantly reduced aflatoxin levels by 27.7, 18.4, 18.3 and 1.6 per cent respectively; while boiling the peanuts in magadi, local ash, baking powder and water reduced aflatoxin levels by 43.8, 41.8, 28.9 and 11.7 per cent respectively. Using *magadi* during boiling increased the acceptability of the boiled peanuts while reducing the aflatoxin levels.

The impact of aflatoxin levels in peanuts studied in this research is within safe limits except a few samples, and therefore aflatoxin contamination of peanuts at household level is not a serious threat. Contamination by aflatoxin and post-harvest fungi can be reduced by focusing on improved control strategies for wetter and more humid zones such as planting improved peanut cultivars and controlling pre-harvest pest damage. Conventional household peanut preparation techniques should be explored as possible aflatoxin management strategies in Kenya. The aflatoxin binding properties of locally available salts such as *magadi* and locally prepared ash should be further investigated.

DECLARATION

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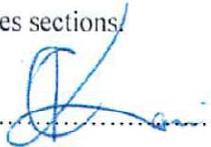
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DEDICATION

Dedicated to Imani, for your endurance and giving me a reason to carry on.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: STATEMENT OF THE PROBLEM

1.1 Introduction

Peanuts (*Arachis hypogaea* L.) are a profitable and reliable -owing to their capability to produce even during drought seasons- crop in western Kenya's Nyanza and Western provinces, and are planted in both the short and long rainy seasons. In this region, peanuts are mainly used in relishes served with the staple stiff maize porridge commonly referred to as *ugali;* boiled; ground and made into a sauce; and roasted or fried (Anonymous, 1992; Anonymous, 2005). Peanuts are sold as raw kernels, roasted nuts, or processed into peanut butter. The nuts are rich in protein (Mehan et al., 1991) and are an ideal alternative to fish, which is more expensive in western Kenya, thereby playing a significant role in food security.

Peanuts, maize (*Zea mays* L.) and tree nuts are common substrates for aflatoxin contamination (Lisker et al., 1993; Richard and Abbas, 2008). The fungi responsible for the production of the toxins are mainly *Aspergillus flavus* and *Aspergillus parasiticus*, and to a lesser extent *Aspergillus nomius.* In peanuts, aflatoxins can be produced at both the pre- and post- harvest stages (Waliyar et al., 2008). Due to the adverse effects associated with aflatoxin contamination especially in maize and peanuts, many countries have strict regulatory control measures, especially with regard to tolerance levels in food and fodder. Many governments, for example Kenya and Malawi, have recently scaled up awareness raising campaigns regarding aflatoxin contamination.

In Kenya, awareness raising campaigns have been tied to acute cases of aflatoxin outbreaks, mainly from maize (the staple food) or its products (Shepard, 2003). Other potential food substrates for aflatoxin, such as peanuts - which are equally important as a food crop in some regions and seasons in Kenya, are often overlooked. Acute outbreaks in the country have overshadowed chronic (and often sub-clinical) incidences of aflatoxin poisoning, which are more pervasive and have adverse effects on human health (Marasas et al., 2008; Wild and Turner, 2002). Reporting of toxicity in Kenya has also not been systematic and only incidences of high mortality are reported (Ngindu et al., 1982; Nyikal et al., 2004), as chronic incidences are usually attributed to other causes, in addition to there being no monitoring system.

Erratic rainfall, high temperatures, high humidity and smallholder production conditions are considered to be conducive to high levels of aflatoxin production. Damage of pods and kernels during weeding, harvesting, drying and transportation can lead to contamination. Most peanuts produced in western Kenya are sold through informal marketing systems whose environmental conditions (open stalls exposed to the weather) favour fungal development, making monitoring and enforcement of safety standards impractical.

Local varieties planted in the area are susceptible to diseases and pests that result in plant stress, predisposing the peanuts to aflatoxin contamination (Hell et al., 2000; Chapin et al., 2004). In spite of this, peanut production in western Kenya is increasing due to initiatives by the International Crops Research Institute for the Semi Arid Tropics (ICRISAT). ICRISAT has introduced improved varieties bred for disease resistance, and seed bulking programs to meet the increasing demand for high quality seed in the region. It is yet unknown if the new varieties offer greater control of aflatoxin contamination.

Extensive research on aflatoxin contamination and the prevalence of *Aspergillus* section *Flavi* has been conducted in West Africa (Kpodo et al., 1996; Cardwell and Cotty, 2002; Bankole and Adebanjo, 2003; Atehnkeng, 2008), but the same level of research has not been conducted in East Africa. In particular, very few studies have been conducted in Kenya (Gachomo et al., 2004). Due to its importance as a staple as well as incidences of acute poisoning involving many fatalities, most aflatoxin research in East Africa has focused on maize, with very little attention on peanut, despite being an important food crop and potential export crop. Therefore, baseline contamination levels, and the evidence of aflatoxin contamination is not known. Quantitative information on the risk of exposure to aflatoxin is necessary for decision-making and policy decisions.

1.2   Problem statement

This study was designed to establish the extent of aflatoxin contamination and the incidence of *Aspergillus* section *Flavi*, *Penicillium* spp. and *Rhizopus* spp. in peanuts sampled in households in western Kenya, and to identify factors associated with contamination of peanuts with aflatoxin or aflatoxin producing fungi.

1.3   Sub-problems

To address the problem above, the study explored four sub-problems.

Sub-problem 1: To determine the prevalence and factors associated with aflatoxin contamination of peanut samples from households in western Kenya.

Sub-problem 2: To assess the incidence of *Aspergillus* section *Flavi, Penicillium*, and *Rhizopus* species in peanut samples from households in western Kenya and the factors associated with their incidence.

Sub-problem 3: To establish the incidence of Aflatoxin B_1, B_2, G_1 and G_2 produced by *A. flavus* and *A. parasiticus* isolated from peanut samples from households in western Kenya.

Sub-problem 4: To evaluate common household preparation practices used in western Kenya and their effectiveness in reducing levels of aflatoxin.

1.4   Study limits and general assumptions

Households from two districts, i.e. Busia and Homa bay, participated in this study. More districts could not be accommodated due to financial constraints. The two districts were also chosen based on differences in production systems and eating habits that could have affected peanut production and consumption practices. It was assumed that the two districts were representative of the western region of Kenya. In cases where household heads were not available for personal interviews, it was assumed that the information given by the party present was credible. While Busia district is predominantly of the Luhyia ethnic community, Homa bay district is predominantly of the Luo ethnic community. The findings resulting from variations in cultural practices may not necessarily be inferred for other areas with similar agro-ecological zones.

1.5   Outline of the thesis

Chapter one sets out the background to the problem, presents the sub-problems, assumptions and study limits. Chapter two presents a general literature review on the topic and discusses the importance of peanuts in Kenyan diets, the role of peanuts with regard to food security, factors that predispose peanut crops to aflatoxin contamination and the effect of contamination on health and trade. The chapter also addresses tolerance levels for aflatoxin contamination in peanuts and discusses the various available standards. Control strategies for aflatoxin contamination are presented. Chapter three discusses the characteristics of the study area and includes socio-economic indicators and land use patterns.

The three subsequent chapters are presented as a series of papers that address the four sub-problems of the study. Chapter four addresses aflatoxin prevalence and factors associated with aflatoxin contamination of peanuts from western Kenya. Chapter five addresses sub-problem two and three, evaluating the incidence of *Aspergillus* section *Flavi*, *Penicillium* spp. and *Rhizopus* spp. in western Kenya and factors that affect the incidences and the presence of Aflatoxin B_1, B_2, G_1 and G_2 in *A. flavus* and *A. parasiticus* isolated from samples. Chapter six discusses the common household processing techniques used to prepare peanuts in western Kenya and their effectiveness in reducing aflatoxin levels. Chapter seven summarises the key findings of the three research chapters and presents the overall conclusions and recommendations of the study.

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CHAPTER 2: LITERATURE REVIEW

2.1   The peanut crop and its role in food security

Peanut (*Arachis hypogaea* L*.*) or groundnut, is a four-foliate legume of the family *Fabaceae*. Native to South America, peanut 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; ICRISAT, 2005). The four common market types are: i) Spanish-small kernels with reddish-brown skins-, ii) Runner-have a consistent medium size-, iii) Virginia-have an extra large kernel size- and iv) Valencia-have three or more kernels to a shell and are bright red- (Edinformatics, 2005).

Peanut is high in protein (26 to 39 per cent), fat (47 to 59 per cent) and carbohydrates (11 per cent) (Nelson and Carlos, 1995; Atasie, Akinhanmi and Ojiodu, 2009). It contains several minerals, including Na (42.0 mg/100g), K (705.11 mg/100g), Mg (3.98 mg/100g), Ca (2.28 mg/100g), Fe (6.97 mg/100g), Zn (3.2 mg/100g) and P (10.55 mg/100g) (Atasie et al., 2009), as well as vitamins E, K and B (Technical Advisory Committee, 1997). Due to its high nutritional value, it has several uses such as weaning and therapeutic food, in confectionery, and as an animal feed.

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 (Anonymous, 2004). In these regions, peanut is significant both as a cash and food crop, and has at least two harvest seasons per year. Value addition techniques are rudimentary at farm level and the nuts are most commonly sold as whole kernels. Its use for oil has not been fully exploited in Kenya, owing to a lack of processing equipment. However, initiatives to introduce oil presses and shellers by ICRISAT in collaboration with non-governmental organizations, such as Compatible Technology International (CTI), are gaining momentum.

Most commercially available peanuts are processed by small and micro-enterprises, a sector that contributes approximately 18 per cent of Kenya's Gross Domestic Product (Mitullah, 2003). Many traders operate in the informal markets where produce is not subject to the scrutiny of regulatory agencies. Some farmers have organised themselves into producer marketing groups (PMG) through public-private sector initiatives, to exploit economies of

scale, facilitating access to improved seeds, markets and better bargaining power (Anonymous, 2009). Such groups are able to pay for regular inspections by the Kenyan Bureau of Standards (KEBS). However, inspections are not widespread and effort and resources are required to scale up such initiatives.

2.2 Aflatoxins and their occurrence in peanuts

Aflatoxins are secondary metabolites produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Kurtzman et al., 1987; Klitch and Pitt, 1988). Optimum growth conditions for *A. flavus* during post harvest are between 25^0C and 30^0C and humidity levels of $0.99a_w$, with production of aflatoxin occurring optimally at 25^0C and 0.99 a_w. (Giorni et al., 2009). Several types of aflatoxins exist, but the four main types are Aflatoxin B_1, B_2, G_1 and G_2, with Aflatoxin B_1 being the most toxic (Olaru et al., 2008). A common metabolite of Aflatoxin B_1 and B_2 is Aflatoxin M_1 and M_2 found in the milk of animals that have consumed contaminated feed (Bahout and El-Abbassy, 2004). While both *A. flavus* and *A. parasiticus* can produce the B toxins, *A. parasiticus* (more prevalent in peanuts than in other crops) also produces the G toxins (Diener et al., 1987; Klitch and Pitt, 1988). *A. nomius* produces both B and G toxins and is morphologically similar to *A. flavus* (Vaamonde et al., 2003).

Aflatoxin is found in many food commodities, but common substrates are maize and peanuts (Lisker et al., 1993). Contamination is found in various products and at all points in the value chain including in peanut butter, unrefined oil, peanut snack foods and reject nuts (Mehan et al., 1991). Fungal species and different mycotoxins coexist. For example, *Rhizopus stolonifer*, *Fusarium spp.*, *Penicillium* spp., *Eurotium repens*, among others, have been isolated from samples of stored peanuts in Kenya (Gachomo et al., 2004). Youssef et al. (2008) found aflatoxins, sterigmatocystin, ochratoxins and zearalenone coexisting in Egyptian peanut kernels. Similarly, more than one mycotoxin can be produced by the same fungus. For example, *A. flavus* produces aflatoxin and cyclopiazonic acid (Lisker et al., 1993; Vaamonde et al., 2003).

Humans and animals come into contact with aflatoxin through several channels such as direct ingestion of contaminated products (Wagacha and Muthomi, 2008), transmission through milk as M_1 and M_2 metabolites (Bahout and El-Abbassy, 2004), and through consuming the

meat of animals reared on contaminated feed. The toxin can also pass through human skin (Wagacha and Muthomi, 2008) through direct contact with contaminated produce. Aflatoxin can also pass through the respiratory system, especially in people engaged 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 (Hendrickse, 1984; Gong et al., 2002; Anonymous, 1984).

2.3   Status of baseline data on aflatoxin and *Aspergillus* section *Flavi* contamination in peanuts

Although not much work has been done in Kenya, several studies in key producer countries have been conducted to establish baseline data on aflatoxin contamination in peanuts. In the United Stated for example, Toyofuku et al. (2009) studied the distribution of aflatoxin in non-irrigated peanuts and in particular, that of Aflatoxin B_1 and total aflatoxin. All three lots showed evidence in the single kernel probability density of peaks at about concentrations of 10^5 and $5x10^3$ ng/g, and a partial peak at a concentration of $<5x10^2$ ng/g. Horn (2007), has shown a high genetic diversity in populations of aflatoxigenic fungi in *Aspergillus* section *Flavi* in the United States. A study by Okano et al. (2008), of Aflatoxin B and Aflatoxin G contamination in peanuts imported into Japan from various countries, including isolates of *Aspergillus,* found that aflatoxin contamination in imported peanuts from China was mainly as a result of *A. parasiticus,* while contamination by Aflatoxin B and Aflatoxin G by peanuts from South Africa was as a result of both *A. parasiticus* and *A. flavus*. Contamination in peanut-based animal feed has also been studied, with several studies conducted in India. The extensive research in India is due to the importance of peanuts in region, with peanuts being a major component of the country's poultry and livestock feed. For example, Ahamad et al. (2009) found high concentrations of Aflatoxin B_1 in broiler finisher mash and groundnut oil cake from samples collected in Namakkal area of Tamil Nadu. Prevalence of Aflatoxin B_1 in peanuts has also been shown by FengQuin et al. (2009) in Chinese peanut butter and sesame paste samples.

A survey by Elzupir et al. (2009) on aflatoxin contamination in animal feeds in Khartoum State in Sudan, showed aflatoxin contamination levels of over 64 per cent of all samples analysed. Over 80 per cent of manufactured rations were contaminated with between 54.41

and 579.87 µg/kg aflatoxin. Aflatoxin B_1 was the most common contaminant in the samples. Another study by Odoemelam and Osu (2009) in Nigeria investigated contamination of edible grains marketed in the Niger Delta region by Aflatoxin B_1 and found that peanuts had the highest levels of Aflatoxin B_1.

Studies on mycobiodata have shown regular contamination of *A. flavus* in peanuts. Youssef et al. (2008) found *A. flavus, A. niger, A. ficuum, Penicilliums* spp. *and Fusarium* spp. in Egyptian peanut kernels. Soil samples in major peanut growing areas of Gujarat in India showed predominance *A. flavus* (Kumar et al., 2008), with a positive correlation between *A. flavus* soil population and aflatoxin contamination in peanut kernels. Gonzalez et al. (2008), also found *A. flavus*, *Rhizopus* spp. and *Fusarium* spp. as the prevalent fungi in peanut hulls from Sao Paulo state in Brazil.

2.4   Effects of aflatoxins in peanuts on health

Peanuts have a high protein and oil content, and play a significant role in nutrition in many developing countries. In western Kenya, other sources of protein, especially fish, are expensive, and so peanuts remain a less costly protein alternative. 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 the one in the study area, are typically more vulnerable to aflatoxin poisoning (Hendrickse, 1984; Gong et al., 2002; Anonymous, 1984). Despite this, most past efforts aimed at addressing food security in these areas have laid emphasis only on nutritional quality and food availability and ignored food safety improvements for public health (Unnevehr, 2003).

Contamination of peanuts by aflatoxins can occur during production, storage, transportation and marketing (Nigam et al., 2009). Health effects are varied and range from a minor irritation to death. Acute effects have been reported in both humans and animals (Ngindu et al., 1982; Nyikal et al., 2004; Garland and Reagor, 2007) and can be linked to the various aflatoxin outbreaks reported in several parts of the world. The effect on humans and animals depends on a number of factors, including species type, ingestion levels, susceptibility (Hussein and Brasel, 2001), age (Meissonnier et al., 2005), aflatoxin concentration (Meissonnier et al., 2005), gender, and duration of exposure (Bunger, 2005). Among animals,

ruminants are more resistant to the adverse effects of aflatoxin ingestion compared to monogastric species (Hussein and Brasel, 2001).

Epidemiological, clinical and experimental studies reveal that exposure to large doses (>6000 ng at once) of aflatoxin may cause acute toxicity accompanied by symptoms such as acute hepatitis, jaundice, oedema, vomiting and sometimes death (Jolly et al., 2007; Nyikal et al., 2004). Chronic effects are as a result of exposure to lower doses for prolonged periods and may result in carcinogenic and immunosuppressive effects and stunted growth in children (Gong et al., 2002, Hendrickse, 1997), liver cirrhosis and reproductive problems (Cousin et al., 2005). Williams (2004), has shown that concurrent infection with hepatitis B virus during aflatoxin exposure increases the risk of primary hepatocellular carcinoma. Both aflatoxins and hepatitis B virus act synergistically in the aetiology of liver cancer (Montesano et al., 1997, Groopman et al., 1996).

2.5   Factors that influence fungal colonisation and aflatoxin production

Contamination of peanut by aflatoxin producing fungi and subsequent toxin production can occur at pre- and post- harvest (Dorner, 2008; Holmes et al., 2008). Several factors therefore influence fungal colonisation and toxin production.

Aflatoxin contamination of groundnut is widespread where the crop is grown under rain fed conditions (Reddy et al., 2003). End-season drought stress and elevated soil temperatures common in Sub-Saharan Africa promote aflatoxin contamination (Bankole et al., 2006; Rachaputi et al., 2002). Attack of peanut pods by pests and diseases contribute to aflatoxin contamination (Mehan et al., 1991; Waliyar et al., 2003). Some varieties are less susceptible than other varieties (Kasno, 2004; Reddy et al., 2003). Poor seed storage, mechanical damage during harvesting, poor or inadequate drying, and poor transportation lead to conditions conducive to contamination (Waliyar et al., 2005; Jones and Duncan, 1981; Bilgrami and Choudhary, 1990).

2.6   Tolerance limits established for aflatoxin in peanuts and peanut products

Establishing tolerance levels of aflatoxin in peanut products - and indeed in other crop commodities - has remained contentious resulting in different standards for the same commodity. Efforts have been made to harmonise standards, but no common standards have been agreed upon, partly due to competing trade interests (Egmond, 2000; Kendra and Dyer, 2007). For populations that rely on peanut as a source of food, tolerance levels for aflatoxin have a direct impact on food availability and safety. Stricter standards are unlikely to improve health significantly as local produce is not necessarily subjected to inspection (Wu, 2004).

Dimanche (2001) has shown that the strict European Union standard would negatively affect export opportunities especially for African countries not able to meet these strict regulations. Otsuki et al. (2001) illustrate that the European Union regulation on aflatoxins resulted in reduced trade flow (63 per cent lower than when the *Codex Alimentarius* international standards were followed). Several factors have played a role in establishing limits and regulations for peanuts and peanut products. These include survey data, toxicological data, method of analysis, aflatoxin distribution, and legislation (ICRISAT, 2007).

Inconsistencies in standards are shown by the different tolerance levels in reference to the same commodity across countries and economic commissions. According to the Codex Alimentarius, tolerance levels for aflatoxin in peanuts intended for further processing is 15μg/kg (Codex Alimentarius, 1995). The EU has one of the strictest standards, that specifies 2 μg/kg Aflatoxin B_1 and 4 μg/kg total aflatoxins (Wu, 2004). India allows 30 μg/kg of total aflatoxin in their peanuts while for the US Food and Drug Administration, a safe limit for peanuts for human consumption is 20 μg/kg (Kpodo and Bankole, 2008). According to the Uganda Bureau of Standards, tolerance levels for aflatoxin in peanuts are 10 μg/kg *(personal communication by David Eboku, Uganda National Bureau of Standards)*. In Kenya, the safe limit for peanuts and corn for total aflatoxin was 20 μg/kg but, this has recently changed to 10 μg/kg of total aflatoxin in peanuts or maize (Kenya Bureau of Standards, 2007). 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 as compared to peanuts for human consumption (Odoemelam and Osu, 2009). According to the Codex Alimentarius Aflatoxin

M_1 in milk is 500 ng/l (Rahimi et al., 2009). Several European countries have put tolerance levels for Aflatoxin M_1 as 50 ng/l (Rahimi et al., 2009).

2.7 Methodologies for aflatoxin determination

Various methods are suggested for testing levels of aflatoxin and depend on factors such as cost effectiveness, precision, and number of samples being analysed. Equally important is the sampling strategy as this significantly affects the margin of error in analysis of results (ICRISAT, 2007). Pascale and Visconti (2008) have summarized the various methodologies available for mycotoxin analysis as including 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. ELISA procedures are the most widely used serological tests for aflatoxin analysis due to their simplicity, adaptability and sensitivity (ICRISAT, 2007). ELISA procedures allow for analysis of multiple samples which is ideal for screening purposes. HPLC 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.

Emerging technologies for mycotoxins analysis include lateral flow devices (LFDs), Fluorescence Polarization Immunoassay (FPIA), Infrared Spectroscopy, capillary electrophoresis, fibre-optic immunosensors and molecularly imprinted polymers (Pascale and Visconti, 2008). Whichever method that is used should enable detection of tolerance levels, to facilitate monitoring programs and ensure international trade safety (Pascale and Visconti, 2008).

2.8 Management strategies for aflatoxin in peanuts

Management includes strategies that either prevent fungi contamination or aflatoxin production. Such strategies can either target pre- or post-harvest stages. Breeding peanut cultivars for resistance has been extensively researched by ICRISAT, even though no variety has yet been suggested as totally resistant to aflatoxin contamination (Pettit, 1986; Waliyar et al., 1994). Use of bio-control agents is proposed as a better pre-harvest tool as use of

fungicides or chemicals can add to production costs. Proponents of bio-control agents also suggest that breeding for disease resistant crops is time consuming and does not address the problem of emerging virulent fungal species (Rajasekaran et al., 2009). Bio-control agents have been shown to reduce contamination in field by 77-98 per cent (Horn and Dorner, 2009). A possible bio-control agent is use of non-toxigenic strains of *A. flavus* and *A. parasiticus* (Horn and Dorner, 2009; Dorner, 2009). *Streptomyces* spp. (strain ASBV-1) has also been shown to be a promising bio-control agent for inhibiting *A. parasiticus* in peanuts, reducing the viability of *A. parasiticus* spores by as much as 85 per cent (Zucchi et al., 2008). Another possible bio-control agent that has been investigated is *Trichoderma harzianum* and *Trichoderma viride* that were found to effectively suppress the growth of peanut moulds and to significantly reduce Aflatoxin B_1 and B_2 (Gachomo and Kotchoni, 2008).

Another effective control measure for pre-harvest aflatoxin contamination in peanuts is irrigation that eliminates drought stress (Craufurd et al., 2006; Reddy et al., 2003; Sudhakar et al., 2007). However, the suitability of irrigation in many African regions remains uncertain as most of the peanut is grown under rain-fed smallholder conditions.

Soil treatments such as application of lime (0.5 t/ha), manure (10 t/ha) and cereal crop residue (5 t/ha) at the time of sowing have also been effective in *reducing A .flavus* seed infection and aflatoxin contamination in peanuts by 50-90 per cent in studies conducted at ICRISAT research stations in Niger and Mali (Waliyar et al., 2008). Waliyar et al. (2008) have also suggested other cultural practices such as summer ploughing, selecting planting dates to take advantage of periods of higher rainfall, maintaining good plant density in the fields, removing prematurely dead plants, managing pests and diseases, timely harvesting and excluding damaged and immature pods, as control strategies for aflatoxin contamination.

Drying of pods quickly, controlling storage pests, storing pods or kernels with less than 10 per cent moisture content and use of mechanical threshers, are possible post-harvest control strategies (Waliyar et al., 2008). Even though these methods are cost effective for small-scale peanut farmers, adoption has mainly been hindered by socio-economic constraints including farmers' attention to other revenue generating activities (Waliyar et al., 2008). Sorting also reduces aflatoxin levels. This includes either manual sorting (Awuah et al., 2009; Kaaya et al., 2006; Dorner, 2008) or sorting at a commercial level using electronic sorting machines (Whitaker et al., 2005; Pitt, 2003; Dorner, 2008).

Peanut processing methods such as roasting (Kaaya et al., 2006; Ogunsanwo et al., 2004) also reduce aflatoxin levels. Using machinery such as threshers, shellers and hermetic packaging protects peanuts from mold and reduces aflatoxin in peanuts (Pramawati et al., 2006). Physical cleaning and separation procedures remove contaminated and physically damaged kernels and can reduce aflatoxin levels by 40 to 80 per cent (Park, 2000). Gamma irradiation reduced Aflatoxin B_1 in peanut kernels by up to 70 per cent in Brazil (Prado et al., 2003). A 10 per cent H_2O_2 treatment of peanuts reduced aflatoxin levels in peanuts in the laboratory (Conzane et al., 2002), while gaseous ozonation has been proposed as a means of detoxifying peanuts (Proctor et al., 2004). ICRISAT has proposed that integrated approaches including a combination of host resistance, soil amendments with lime, organic supplements to enhance water holding capacity, use of antagonistic bio-control agents, and awareness raising campaigns could be most effective in reducing aflatoxin levels (Waliyar et al., 2008).

Aflatoxins continue to pose challenges with regard to food security, especially in the developing world. Paucity of data in several parts of the region makes it difficult to establish facts about the extent of the problem in Kenya. This study seeks to establish baseline information on aflatoxin contamination of peanut samples from western Kenya. This information will be important in influencing policy and strengthening monitoring systems.

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CHAPTER 3: DESCRIPTION OF THE STUDY AREA AND PRODUCER MARKETING GROUPS

3.1   Socio economic infrastructure, population and geography, based on the Republic of Kenya's district development plans for the period 2002-2008

Busia District is one of the districts of the Western Province covering an area of 1,261.3 km^2, 137 km^2 of which is part of Lake Victoria. The district is divided into six administrative divisions, namely Nambale, Butula, Funyula, Budalangi, Township and Matayos. These divisions are further divided into 30 locations and 99 sub-locations (Republic of Kenya, 2002*a*). Homa bay District is one of the twelve districts of the Nyanza province and covers an area of 1,160.4 km,^{2} of which 29.5km^2 is water. The district is divided into six administrative divisions namely Rangwe, Asego, Ndhiwa, Nyarongi, Riana and Kobama divisions. Kobama division was originally part of Riana division. The divisions are further sub-divided into 26 locations and 63 sub-locations (Republic of Kenya, 2002*b*).[1]

Of the total area, Busia has 924 km^2 of arable land while Homa bay has 977km^2. The Busia District has a higher female population compared to Homa bay, with female: male ratios of 100:89 (Republic of Kenya, 2002*a*) and 100:110 (Republic of Kenya, 2002*b*), respectively. Both districts have a relatively high infant mortality rate of 75 deaths in every 1000 live births (Republic of Kenya, 2002*a*) and 137 deaths in every 1000 live births (Republic of Kenya, 2002*b*) in Busia and Homa bay respectively. According to statistics from antenatal clinics, the HIV prevalence rate in Homabay is currently 24 per cent (Republic of Kenya, 2002*b*), and 33 per cent in Busia district (Republic of Kenya, 2002*a*). This is much higher than the national prevalence of 6.7 percent and Nyanza province's prevalence of 15.1 percent (Central Bureau of Statistics, 2004). Despite the relatively high awareness levels in both districts, cultural practices conducive to the spread of HIV are still prevalent and have led to decreased agricultural productivity. In both districts, there is a high proportion of youths and high dependency ratios, mainly due to unemployment and the adverse effects of HIV (Republic of Kenya, 2002*a*; Republic of Kenya, 2002*b*).

In both districts, agriculture is a major contributor to household income. In Busia, 35.4 per cent of household income comes from agriculture (Republic of Kenya, 2002*a*) and 52 per

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[1] The administrative boundaries are discussed as they were in 2006, the year which the study was carried out.

cent in the Homa bay District (Republic of Kenya, 2002*b*). Average farm sizes in both districts are small, with an average of 2.5 ha in Busia and 2.0 ha in Homa bay. Main food crops include maize, cassava and sorghum. Cash crops include sugar cane, peanuts, cotton and rice (Republic of Kenya, 2002*a*; Republic of Kenya, 2002*b*). While sugar cane is grown at a larger scale in the two regions, peanut is mainly grown under smallholder conditions. Figure 3.1 illustrates land use patterns in the Busia and Homa bay districts.
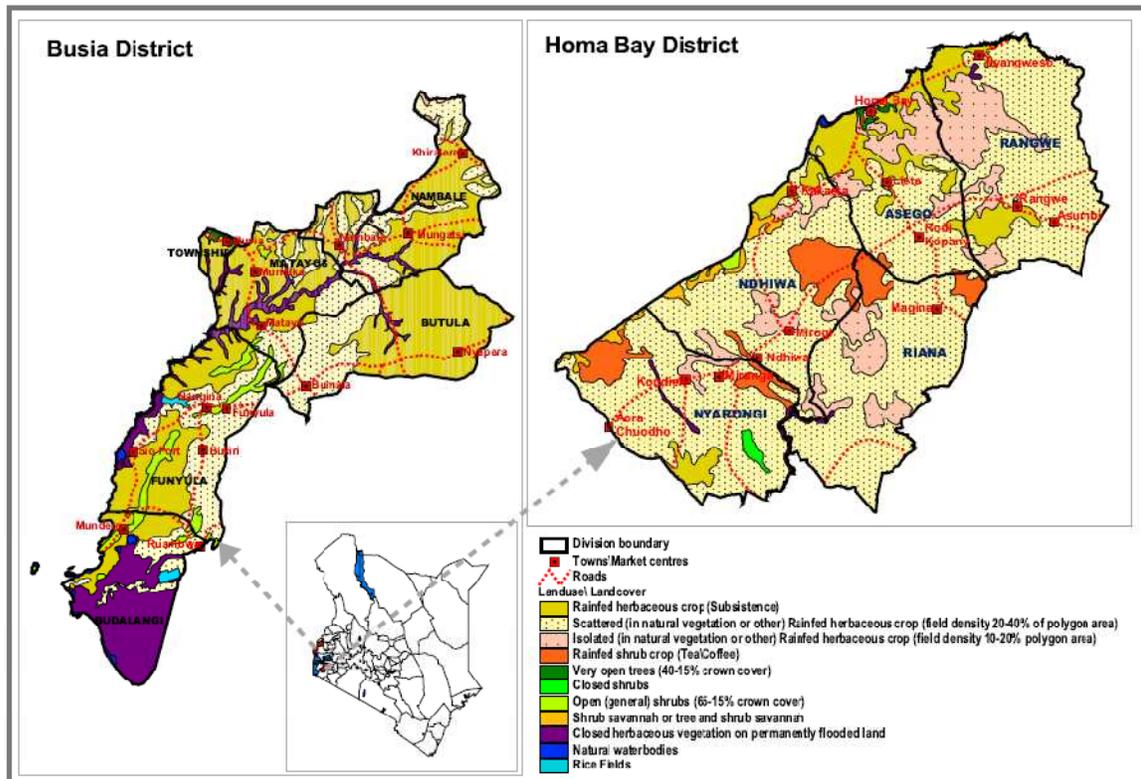
Figure 3.1: Land use patterns in the Busia and Homa bay Districts of Kenya (FAO-Africover, 2002).

Absolute poverty levels in both districts are high, with an average of 66 per cent in Busia and 73.3 per cent in Homa bay. Figure 3.2 shows the density of poor people (persons living on less than a United States dollar per person per day) in Busia and Homa bay. Even if the districts have fairly well distributed road networks, a small proportion of these are tarmac, restricting movement of farm produce to main market centres (Figure 3.2). Less than 12 per cent of all roads in the districts are bitumen and the majority of roads become impassable

26

during the rainy seasons. Both districts have an area of Lake Victoria, making fishing a significant source of livelihood. However, a water transport system is not well developed.

Basic educational facilities (up to primary level) exist in both districts. However, secondary level and tertiary educational institutions are scarce, accompanied by high school dropout levels. Adult literacy is higher in men than women. For example, in Busia, the literacy level among adult males is 76 per cent but only 55.3 per cent for women (Republic of Kenya, 2002*a*).
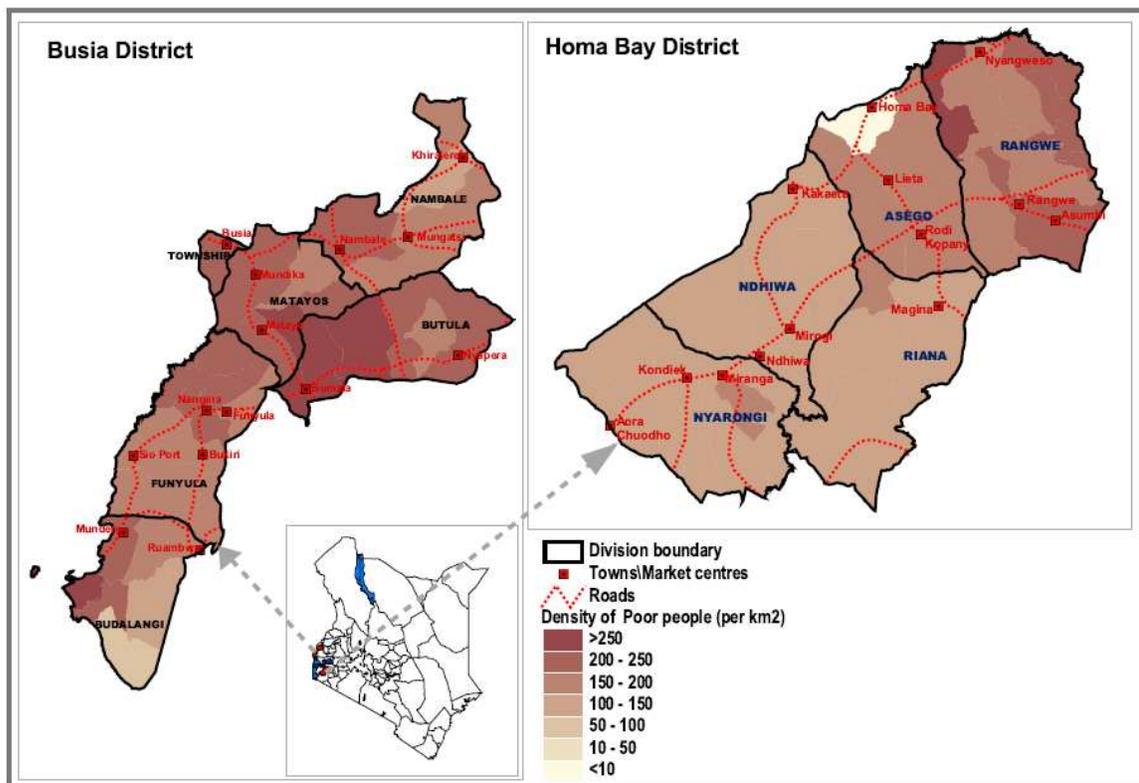
Figure 3.2: Density of poor people-persons living below one dollar per day-in Busia and Homa bay Districts, Kenya (Thornton et al., 2002).

Both districts are poorly served by medical facilities, with few medical centres, a lack of nursing staff and a shortage of medicines being major challenges. The doctor to patient ratio is dismal in both districts, with a ratio of 1 doctor to 41,200 patients in Busia (Republic of Kenya, 2002*a*) and 38, 707 patients in Homa bay (Republic of Kenya, 2002*b*).

Unlike Homa bay, Busia has benefited from a rural electrification programme, and the district has most of the high potential regions served with electricity. This has led to emergence of several service industries, such as restaurants, barbershops, bars and bakeries.

3.2   Producer marketing groups (PMG's)

Producer marketing groups (PMGs) were initiated in 2003 under the Technical Assistance for Rural Growth and Economic Transformation (TARGET) project with ICRISAT as lead agent, and Catholic Relief Services (CRS) and TechnoServe Kenya as collaborating agencies. The groups were set up to boost the livelihoods of peanut, pigeonpea and chickpea farmers in Kenya, Tanzania and Uganda. The main objectives of the project were to promote market-demanded legume varieties; strengthen seed marketing systems; improve rural grain marketing businesses; and enhance linkages between producers and markets (ICRISAT, 2003). The PMGs were seen as a vehicle through which specific challenges facing peanut farmers could be addressed including lack of improved seeds; diseases (especially leaf spot and rosette virus); poor agronomic practices; aflatoxin contamination; labour intensive shelling practices; varying marketing needs; the need for different varieties; and inadequate marketing information.

Producer marketing groups targeting peanuts in the region were established in the main peanut producing areas including the Homa bay, Busia and Siaya districts. PMGs consist of about 30 members each, with a gender balance. Members within the groups in Kenya have benefited from new peanut varieties introduced by ICRISAT; improved peanut agronomic practices, improved post-harvest handling practices, reduced shelling labour due to machines, and training on management of aflatoxin contamination. In the long term, members have benefited from improved local capacity to produce and market peanuts through better coordination of production and marketing resulting in increased household incomes, better food security and nutrition, and a sustainable groundnut seed system.

Producer marketing groups offer an opportunity for awareness creation about the management of aflatoxins in peanuts. The socio-economic indicators discussed earlier in this chapter indicate a need to raise the incomes of farmers through agriculture that contributes to

livelihoods of people in the region. Improving profitability of the peanut crop could benefit farmers by increasing incomes from sales and improving their health status.

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CHAPTER 4: PREVALENCE AND FACTORS ASSOCIATED WITH AFLATOXIN CONTAMINATION OF PEANUTS FROM WESTERN KENYA[2]

4.1 Introduction

Peanut is an important crop in terms of nutrition and income in western Kenya. The nuts contain high levels of protein (Desai et al., 1999) and are relatively affordable compared to other sources of protein (Mayatepek et al., 1992). In western Kenya, peanut has the added appeal in that two crops can be harvested in a year. Western Kenya encompasses Nyanza and Western provinces, which are the main peanut producing areas in the country, according to the Crop Development Division Annual Report of 2004 (Anonymous, 2004). Nyanza Province is the country's largest peanut producer with 14,723 hectares under production while Western Province with 2,667 ha ranks third after Eastern Province (Anonymous, 2004). Most of the produce is traded in local markets (Ogwang, 2006).

Aflatoxin contamination of peanuts poses a risk to human health and is a major constraint to trade in Africa (Lubulwa and Davis, 1994). Little is known about the prevalence or levels of aflatoxins in peanuts harvested in western Kenya. However, several indicators and anecdotal evidence suggests possible human exposure to aflatoxins. First, western Kenya has repeatedly recorded high levels of stunting in children (Central Bureau of Statistics, 2003), an aspect often positively correlated with long-term ingestion of sub-lethal doses of aflatoxins (Gong et al., 2002; Bhat and Vasanthi, 2003). Second, erratic rainfall, high temperatures and high humidity prevalent in the major production areas favour peanut infection and development of aflatoxin. Wet and humid areas have been linked to higher levels of aflatoxin-producing fungi in other parts of Eastern Africa (Udoh et al., 2000; Kaaya and Kyamuhangire, 2006) and Nigeria (Atehnkeng et al., 2008). Third, peanuts in Kenya are produced under small holder conditions, characterised by mechanical damage to pods, poor harvesting, drying and storage methods, linked to aflatoxin contamination of peanut elsewhere in sub-Saharan Africa (Jones and Duncan, 1981; Bilgrami and Choudhary, 1990; Waliyar et al., 2005*a*). Fourth, many farmers plant local varieties that are susceptible to diseases such as rosette virus, mould

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[2] This paper was published as: Mutegi CK; Ngugi HK; Hendriks, SL; Jones RB (2009). Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. International Journal of Food Microbiology: 130: 27–34.

infestation and leaf spot (Ogwang, 2006). While diseases and pests of peanuts are common in western Kenya smallholder cultivation that uses minimal investment in inputs precludes the use of modern management tactics such as chemical pesticides; stress from diseases can predispose peanut plants to aflatoxin contamination (Hell et al., 2000; Chapin et al., 2004; Timper et al., 2004; Kaaya et al., 2005).

In spite of the paucity of data on aflatoxin, production of peanuts in western Kenya is on the increase due to recent initiatives. For example, the International Crops Research Institute for the Semi Arid Tropics (ICRISAT) has introduced improved varieties and seed bulking programs to meet increasing demand for high quality seed. Increased production will require peanut traders in the region to seek external markets that impose strict safety standards. These stringent measures are mainly driven by the health implications of aflatoxins, which are both carcinogenic and immunosuppressive (Fooladi and Farahnaky, 2003), and the common presence of this mycotoxins in peanut and maize products (Council for Agricultural Sciences and Technology, 1989). For example, the European Union (EU) market has a tolerance level of 2 μg/kg for Aflatoxin B_1 and 4 μg/kg for total aflatoxin for peanut kernels imported into the EU (Sobolev, 2007).

To ensure aflatoxin requirements for external markets are attained, there is a need to develop sampling procedures suited to local production systems and identify factors associated with high levels of aflatoxin contamination. Information on factors that influence the level of aflatoxin is critical to developing mitigating strategies appropriate for the region. This study was undertaken to establish baseline levels of aflatoxin in peanuts harvested in western Kenya, identify factors associated with high levels of aflatoxin and to model the relationship between these factors and the likelihood of a peanut sample from western Kenya exceeding the national aflatoxin regulatory threshold.

4.2   Methods

4.2.1   Survey and peanut sample collection

A household survey was carried out in Busia and Homa bay districts in western Kenya (Figure 4.1). These districts were chosen based on their significance in terms of peanut

production, and because they offered contrasting environments, under which peanuts are cultivated. In Busia district, peanuts are mainly grown in the wetter and more humid Lower Midlands (LM) agro ecological zone (AEZ), otherwise referred to as LM1. In contrast, in the Homa bay district the crop is mainly produced in the drier LM3 zone.
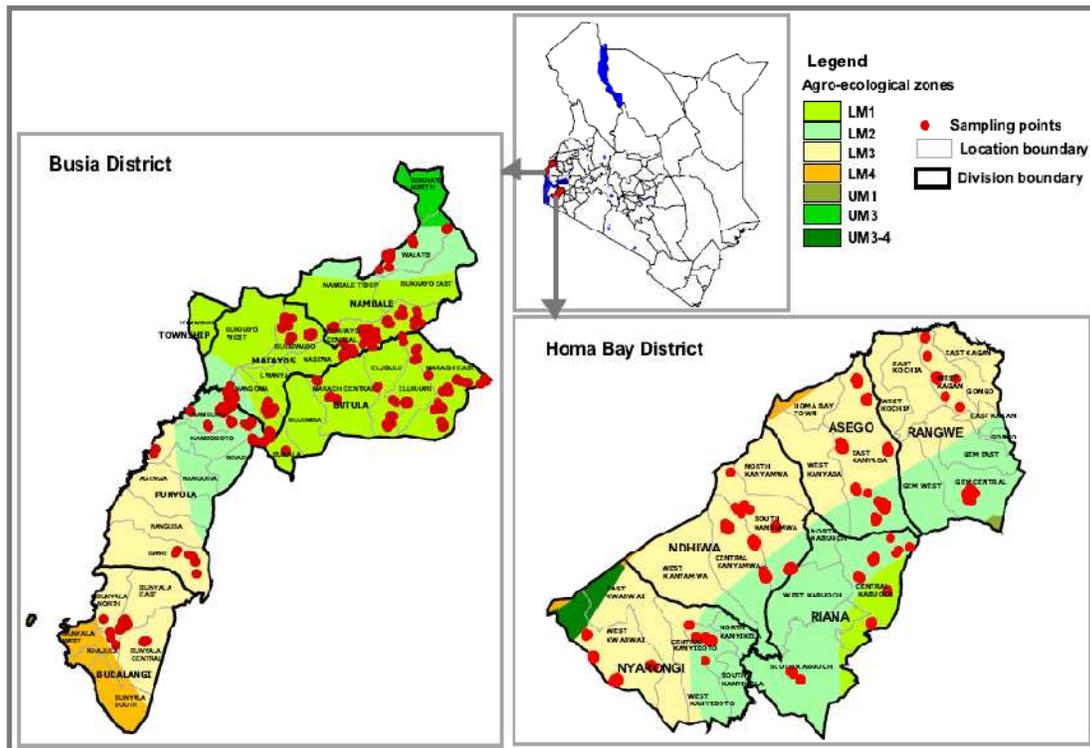
Figure 4.1: Sampling areas within the Busia and Homa bay districts, stratified based on agro-ecological zones. Some sampling points may overlap on the map

The survey was based on a total of 769 peanut-growing households, with 384 and 385 respondents from Busia and Homa bay districts, respectively. Information was collected through personal interviews using a pre-tested questionnaire, which was developed after conducting focus group discussions involving 40 and 44 participants from Busia and Homa bay districts, respectively. The participants were drawn from peanut farmers, village elders, community leaders and provincial administration staff. Thereafter 40 randomly selected households were used to pre-test the developed questionnaire, 20 from Asego division of Homa bay district and 20 from Butula division of Busia district. Each of the 40 households was selected by staggering every fourth household within the location administrative

boundary, the starting point being the fourth household from the division's agricultural office, from where activities for the day commenced.

For the purposes of sampling, the district was stratified into Agro-Ecological Zones (AEZs), namely LM1, LM2 and LM3, where peanut is commonly grown. The AEZs are determined based on altitude, mean annual rainfall, temperature, evapotranspiration and the probability of successfully growing the main crops of that zone (Jaetzold and Schmidt, 1982; Ngugi et al., 2002). The sample size for each AEZ was proportionate to acreage under peanut production (Table 4.1). Production statistics were obtained from the Ministry of Agriculture, while updated information on the AEZ mapping was acquired from the Geographic Information Systems Centre at the World Agro Forestry Centre, Nairobi, Kenya. Within the AEZs, farmers were randomly selected at village level from a list compiled by the extension staff of the Kenya Ministry of Agriculture (MOA).

Table 4.1: Number[x] of peanut samples obtained from households in different agro-ecological zones (AEZ) within each of the two districts surveyed in western Kenya, August 2006

	District		
AEZ	Busia	Homabay	Total
Lower Midland1	193 (221)	32 (487)	225
Lower Midland 2	152 (174)	161 (2455)	313
Lower Midland 3	39 (45)	192 (2930)	231
Total (n)	384	385	769

[x]Values in parenthesis represent peanut production in hectares.

Data collected through the survey included:
- farm size
- whether or not respondents practiced crop rotation
- number of times a crop was weeded per season

- fertiliser use (whether commercial fertilisers, organic fertilisers or no fertiliser was used on the crop)

- pest and disease management practices (commercial pesticides, organic pesticides, cultural methods, and no control method at all)

- whether or not respondents perceived drought, erratic rainfall, damage by moles and/or rats as production problems

- type of cultivar(s) planted (whether improved or local landrace)

- aspects of peanut utilisation (e.g., methods of food preparation and whether crop was sold)

- extent of awareness about aflatoxin.

Farmers were also asked whether they belonged to a Producer Marketing Group (PMG). A PMG is a group of local peanut farmers brought together for the purposes of sourcing markets and to facilitate technology transfer (Mutegi et al., 2007). A one kilogram peanut sample was obtained from each interviewed household for aflatoxin testing. The sample was drawn from different parts of the farmer's storage container and thoroughly mixed. The samples were assayed for levels of aflatoxin as described below.

4.2.2   Determination of levels of aflatoxin

A 200 g sub-sample was drawn from each one kilogram sample and ground into a fine powder using a dry mill kitchen grinder (Kanchan Multipurpose Kitchen Machine, Kanchan International Limited, Mumbai, India). The ground sample was then sub-divided into two equal portions. The powder was triturated in 70 per cent methanol (v/v 70 ml absolute methanol in 30 ml distilled water) containing 0.5 per cent w/v potassium chloride in a blender, until thoroughly mixed. The extract was transferred to a conical flask and shaken for 30 min at 300 rpm. The extract was then filtered through Whatman No.41 filter paper and diluted 1:10 in phosphate buffered saline containing 500 µℓ/ℓ Tween-20 (PBS-Tween) and analyzed for aflatoxin with an indirect competitive ELISA (Waliyar et al., 2005*b*) by preparing an aflatoxin-bovine serum albumin conjugate in carbonate coating buffer at 100 ng/mℓ concentration and dispensing 150 µℓ in each well of the Nunc-MaxiSorp® ELISA plates [3].

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[3] Nunc A/S, Kamstrupvej 90, P.O.Box 280, DK-4000, Roskilde, Denmark

The plates were incubated at 37 °C for one hour before the toxin solution was collected and stored in a large glass bottle for disposal. The plates were washed in three changes of PBS-Tween, allowing a holding time of three minutes per wash. The plates were blocked with a 200 µℓ per well solution of 0.2 per cent bovine serum albumin (BSA) in PBS-Tween and incubated at 37 °C for one hour. The blocked plates were then washed in three changes of PBS-Tween allowing three minutes for each wash. To the washed plates, 100 µl of peanut kernel extract was added followed by 50 µℓ of antiserum. Instead of the peanut kernel extract, 100 µl aliquots of different concentrations of Aflatoxin B_1 (25 ng to 100 pg) were added into the first 20 wells (two rows of 10 wells each) to serve as a standard. The plates were then incubated for one hour at 37 °C to facilitate reaction between the toxins and the antibody.

The plates were subsequently washed in three changes of PBS-Tween, allowing three minutes for each wash. A dilution of 1:1000 goat anti-rabbit IgG labelled with alkaline phosphatase was prepared in PBS-Tween containing 0.2 per cent BSA. A 150 µℓ aliquot was added to each well, and incubated for one hour at 37 °C. The plates were washed in three changes of PBS-Tween, added a 150 µℓ per well of substrate solution (p-nitro phenyl phosphate prepared in 10 per cent diethanolamine buffer, pH 9.8) and incubated for about one hour at room temperature. Absorbance was measured at 405 nm in an ELISA plate reader (Multiskan Plus, Labsystems Company, Helsinki, Finland).

4.2.3   Statistical analyses

In order to characterise the distribution of aflatoxin levels, samples were grouped into categories with established economic (levels used to impose trade restrictions) or biological relevance (based on LD_{50} of various animal species), based on their aflatoxin content (Table 4.2). For each district, the percentage of samples in each category was calculated and plotted against median values for the categories to obtain frequency distribution histograms. To test if the resulting frequency distributions were similar for the two districts, the data were subjected to Kolmogorov-Smirnoff and the Mann-Whitney U two samples tests (Sprent and Smeeton, 2001). Several probability distribution models (negative binomial, gamma and lognormal distributions) were also evaluated for their ability to describe the frequency

distributions. Goodness of fit for the probability distribution models was assessed by analysis of deviance using GenStat Ver 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station).

To identify factors associated with different levels of aflatoxin, the samples were grouped into three categories based on their aflatoxin content: samples with <4 µg/kg; ≥4 µg/kg to ≤20 µg/kg, and >20 µg/kg. The <4 µg/kg category represents the EU regulatory limit for total aflatoxins (Felicia, 2004); peanuts in the second group would be rejected in the EU but accepted under the Kenya Bureau of Standards (KEBS) limits (Felicia, 2004), while nuts in the third category would be rejected under the KEBS and EU standards. Categorical data analysis by means of contingency tables was used to assess for association between these aflatoxin categories and descriptive variables.

Table 4.2: Amounts of aflatoxin with biological and/ or economic relevance used to establish frequency distributions of aflatoxin levels in the Busia and Homa bay Districts

Category mid points plotted (µg/kg)	Category based on biological and/ or economic relevance (µg/kg)	Description of economic or biological relevance
0	0	Undetectable levels
2	> 0- ≤ 4	Permissible levels for total aflatoxins under the EU regulations (Sobolev, 2007)
12	<20	Permissible levels for total aflatoxins according to KEBS (Mehan et al., 1991)
60	20-100	Not safe for human consumption under KEBS standards, but safe for animal feed (Mehan et al., 1991)
550	100-1000	Based on LD_{50} of various animal species (Mehan et al., 1991)
1500	1000-2000	Based on LD_{50} of various animal species (Mehan et al., 1991)
4000	2000-6000	Manifestation of sickness symptoms-nausea, headaches, rash (Biological Safety Working Group, undated)

The relationship between factors identified as significantly associated with levels of aflatoxin and the likelihood of a sample exceeding Kenya's regulatory limit of 20 µg/kg (hereafter considered contaminated) was modelled using a logistic regression approach. The binary response variable was the sample level of aflatoxin (AL) whereby 1= >20 µg/kg and 0 = <20 µg/kg. All variables with a significant association ($P < 0.05$) were tested as explanatory variables and those found to be insignificant were dropped to obtain the most parsimonious model. Categorical data analyses (Stokes et al., 2000) were carried out using SAS Ver. 9.1 (SAS Institute, Carry, NC).

4.3   Results

4.3.1   Levels of aflatoxin in peanut samples from the Busia and Homa bay districts

The levels of aflatoxin ranged from 0 to 2,687.6 µg/kg and from 0 to 7,525.0 µg/kg in samples from Busia and Homa bay districts, respectively. Overall, 63.7 per cent of all samples had undetectable levels of aflatoxin while 7.54 per cent were contaminated based on KEBS standards; 2.1 per cent of the samples were unsuitable even for animal feed (i.e., exceeded 100µg/kg) based on FDA action levels. Kolmogorov-Smirnoff (K-S) and the Mann-Whitney U two samples tests showed that the frequency distributions of aflatoxin levels in samples from the two districts were significantly different (K-S $P = 0.325$; Man-Whitney U-test: $P = 0.798$ for equal distribution). For both districts, however, the resulting distributions were highly skewed to the left indicating that most of the samples were safe, based on the KEBS and EU regulatory limits (Figure 4.2) (In Figure 4.2, fitted values are frequencies obtained from fitting the gamma probability distribution function to the observed values)**.**

The distributions were generally well fitted by gamma, negative binomial and lognormal distributions, with the gamma distribution providing the best fit for samples from the two districts (e.g., deviance values for the three models were = 17.94, 22.72 and 36.13, respectively, for samples from Busia district).
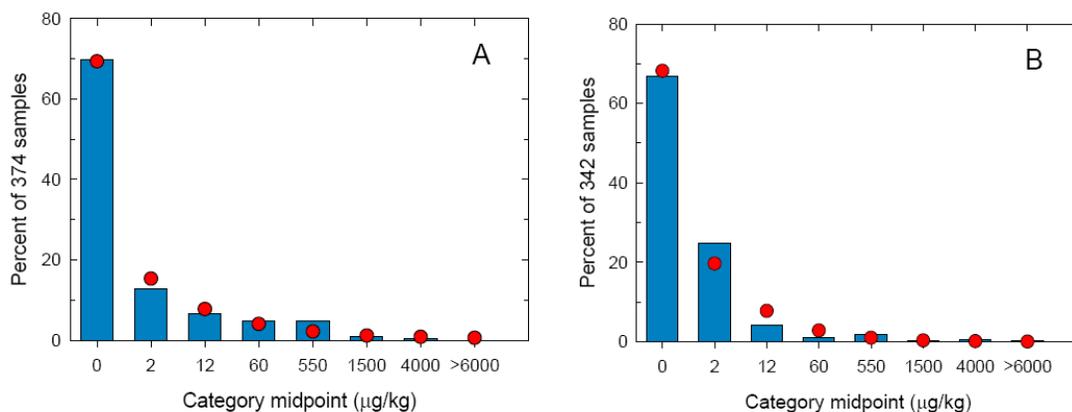
Figure 4.2: Frequency distribution of levels of aflatoxin in peanuts from Busia (A) and Homa bay (B) based on mid-points of aflatoxin categories with biological and/or economic importance.

4.3.2   Factors associated with levels of aflatoxin

Figure 4.3A shows percentage of samples in each of the three categories of aflatoxin levels plotted against the district of origin, i.e., Busia or Homa bay. A highly significant association (χ^2 = 14.172; *P* = 0.0002) was found between district of sample origin and aflatoxin levels. The percentage of safe samples according to KEBS standards was lower in Busia district (82.62 per cent) compared to Homa bay district (91.81 per cent). While 10.70 per cent of samples from Busia district had aflatoxin levels >20 µg/kg, only 4.09 per cent of samples from Homa bay were in this category.

There was a highly significant (χ^2 =11.983; *P* = 0.0005) association between AEZ and aflatoxin levels. A pattern was also noted whereby, the percentage of contaminated samples declined with decreasing precipitation across the region (i.e. from the wet LM1 AEZ to the drier LM3 AEZ; Figure 4.3B). The frequency of samples containing <4 µg/kg of aflatoxin was 81.78 per cent in LM1, 86.06 per cent in LM2 and 93.49 per cent in LM3. Conversely, samples with aflatoxin levels of ≥20 µg/kg were 10.28 per cent in LM1, 8.71 per cent in LM2 and 3.26 per cent in LM3. Samples that would have otherwise been accepted under the KEBS regulations but rejected under the EU regulations were 7.94 per cent, 5.23 per cent and 3.26 per cent for LM1, LM2 and LM3, respectively.
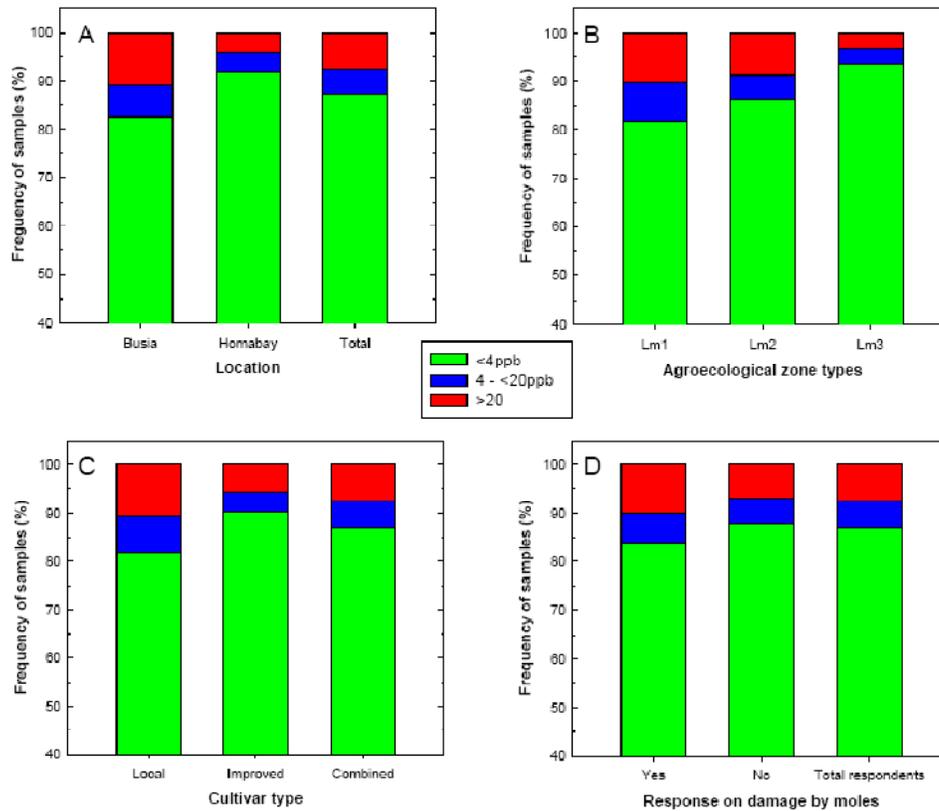
Figure 4.3: Percentage of samples in the three categories of levels of aflatoxin plotted against district of origin of the sample (A), agro-ecological zones (B), cultivar type (C), and farmer response to whether or not the crop was damaged by moles (D).

A strong association was noted between levels of aflatoxin and cultivar improvement status (improved versus local landrace) whereby improved cultivars had significantly lower percentages of contaminated samples (χ^2 = 9.748; P = 0.0018 as shown in Figure 4.3C). Indeed, for cultivars with a sufficient sample size (n > 45) a significant association (χ^2 = 4.27; P=0.0388) between individual cultivars and levels of aflatoxin was also noted, with more samples from the improved cultivars having lower levels of aflatoxin compared to the local cultivars (Table 4.3). For example, while improved cultivars ICGV 12988 and ICGV 12991 had 92.75 per cent and 95.56 per cent of their samples, respectively, below 4 µg/kg, Homabay Local and Local Red had 87.16 and 77.78 per cent in the same category. On the other hand, ICGV 12988 and ICGV 12991 had 5.80 per cent and 4.44 per cent of the respective samples with aflatoxin levels ≥20 µg/kg, while Homabay Local and Local Red had 4.59 per cent and 15.97 per cent of the samples in this category.

A significantly higher proportion of samples obtained from farmers who reported damage from moles as a problem had higher levels of aflatoxin compared with those from farmers who had not experienced rodent damage. While 83 per cent of peanut samples from farmers reporting moles as a problem had less that 4 µg/kg per sample, over 88 per cent of the samples from farmers who had no problem with this pest were in this category of less than 4 µg/kg ($\chi^2 = 4.449$; $P = 0.0349$; Figure 4.3D).

Although the proportion of samples with <20 µg/kg of aflatoxin was numerically higher for farmers belonging to PMGs (which, among other activities train farmers on methods to mitigate aflatoxin contamination) than non-PMG members, the association was not statistically significant ($\chi^2 = 3.61$; $P = 0.0573$). No significant association was detected between levels of aflatoxin and use of fertilizers; number of times the crop was weeded; application of crop rotation; disease and pest control or drought during the cropping season.

Table 4.3: Association between levels of aflatoxin and cultivars commonly grown in Busia and Homa bay districts of western Kenya, August 2006

Cultivar	Status of cultivar[y]	n	Percent of samples with different levels of aflatoxin		
			<4 µg/kg	≤4 - <20 µg/kg	≥20µg/kg
CG7	I	74	89.19	8.11	2.70
Homabay local	L	109	87.16	8.26	4.59
ICGV88	I	69	92.75	1.45	5.80
ICGV 12991	I	135	95.56	0.00	4.44
Local red	L	144	77.78	6.25	15.97
Uganda red	I	100	81	9.00	10.00
Valencia red	I	47	91.49	2.13	6.38

[y]Local landrace = L; improved variety = I

$\chi^2 = 4.27$; $P=0.0388$.

4.3.3   Relationship between factors associated with high levels of aflatoxin and the likelihood of finding a contaminated sample

Logistic regression analysis indicated that aflatoxin level (AL) was only significantly affected by district of sample origin (DT), and cultivar improvement status (CIP), but with no significant interaction between the two (Table 4.4). The negative value of the estimate CIP indicates that samples obtained from improved cultivars were less likely to exceed the regulatory limit. The fitted model describing this relationship was: logit (AL) = -2.306 + 0.051 DT – 0.594 CIP. The test for goodness-of-fit (D=0.968; P = 0.325) indicated that the model fitted the data well. Including AEZ as an explanatory variable did not significantly improve the fit of the model. Based on this model, the odds of a sample from Busia exceeding Kenya's regulatory limit (20 µg/kg) were 2.65 times higher (Wald χ^2 = 9.183; P = 0.0024) than those for a sample from Homa bay district. The odds for a sample from an improved cultivar exceeding this threshold were half (odds ratio = 0.552) of those for a sample obtained from a local landrace.

Table 4.4: Parameter estimates from a logistic regression relating levels of aflatoxin with district of sample origin and cultivar improvement status

Parameter	DF	Estimate	SE	Wald χ^2	$P > \chi^2$
Intercept	1	-2.306	0.215	115.11	<0.0001
District	1	0.488	0.161	9.18	<0.0024
Cultivar improvement	1	-0.594	0.288	4.27	0.0387

4.4   Discussion

In this study, the prevalence and levels of aflatoxin in peanuts from western Kenya were investigated. The factors associated with high levels of aflatoxin were identified, and the risk of a peanut sample from the region exceeding the national regulatory threshold of 20 µg/kg determined. The levels of aflatoxin ranged from zero to >7525 µg/kg and were highly variable; that most peanuts from western Kenya are generally safe for human consumption but that a small proportion of the samples contained very high levels of aflatoxins. The data also show that peanuts from local landraces and those harvested in the more humid agro-

ecological zones within the region were more likely to be contaminated with aflatoxins than those from improved cultivars and/or from less humid agro-ecological zones.

The data for describing the incidence of aflatoxin levels were well fitted by gamma, negative binomial and lognormal probability distributions. This observation has two key implications. First, in comparing levels of aflatoxin for any grouping variable (e.g. AEZs, cultivars, agronomic practices, etc.), the median is a more appropriate statistic than the arithmetic mean, because of the highly skewed distribution of the levels as shown by frequency plots. The second implication is that in designing sampling protocols for regulatory purposes, the skewed nature of the distributions in incidence of aflatoxin levels will need to be taken into account. By identifying the gamma distribution as the most suitable function for analysing data on the incidence of aflatoxin, the results accord well with those of Berry and Day (1973), who recommended the gamma distribution for modelling levels of aflatoxin when most samples contain undetectable levels. Their study was on dietary aflatoxin samples from the Murang'a District in central Kenya.

Over 92 per cent of the samples were within Kenya's regulatory limit (20 µg/kg), while over 87 per cent of the samples were also within the stricter EU regulatory limit of 4 µg/kg, indicating that at a household level, most peanuts have acceptably low levels of aflatoxin. These results are consistent with studies conducted elsewhere in Africa. For example, a survey carried out in Egypt reported low levels of aflatoxin in unshelled and shelled raw peanuts (El-Khadem, 1990), while in post-harvest surveys on rain-fed and irrigated peanuts in Sudan, none of the samples obtained from the households visited contained aflatoxin levels of more than 15µg/kg (Singh et al., 1989).

This study also showed lower levels of aflatoxin contamination of peanuts at household level compared to maize, as has been reported in a survey of 350 maize products conducted in Kenya in 2004, including in the Busia district, where >55 per cent of the samples exceeded the 20µg/kg limit while 35 per cent had aflatoxin levels >100 µg/kg (Lewis et al., 2005). This observation implied that the risk of human exposure to aflatoxin from consumption of peanuts is much lower than that associated with contaminated maize. The significance of this observation is clearer when one considers that in Kenya peanuts are eaten as a side dish,

sauce or snack and are consumed in relatively small amounts compared to maize, which is consumed in larger amounts of 0.4 kg/person/day (Shepherd, 2003).

Nevertheless, a market survey of peanut aflatoxin contamination would be insightful in understanding the contribution of market outlets to the risk of aflatoxin exposure since additional contamination and aflatoxin accumulation can occur at various stages in the informal peanut marketing cycle. The likelihood of higher contamination in market outlets increases when one looks at previous studies, that have documented high fungal and aflatoxin prevalence and incidence in marketed peanut kernels and their by-products (Verma and Agarwal, 2000; Ila et al., 2001; Le Anh, 2002). This infers that processing introduces greater contamination than present at harvesting and during the sale of the dried product, possibly due to the several handling stages introduced before the product gets to the end consumer.

Of all the factors studied, only the source of sample origin (district or agro-ecological zone), damage by moles, cultivar improvement status and cultivar type were significantly associated with the levels of aflatoxin in peanut samples. Previously, it was documented that significant correlations existed between AEZ and aflatoxin levels, whereby a wet and humid climate tends to aggravate aflatoxin levels. In neighboring Uganda, for example, aflatoxin levels in maize samples were higher in more humid areas compared to the drier areas (Kaaya et al., 2006). Similar results were obtained in a recent survey of maize samples from Nigeria (Atehnkeng et al., 2008). The significantly higher odds of peanuts from the Busia District being contaminated compared with those from the Homa bay District could be partly attributed to the distribution of AEZ within the districts; the wetter and humid LM1 is mainly found in Busia District while the drier LM3 is mainly found in Homa bay District. It is difficult to pinpoint the specific causes of higher levels of aflatoxin in the wet humid zone, but it is probable that high moisture does not allow for sufficient drying of nuts, that are in most cases dried on bare ground or polythene sheets in homesteads or in fewer instances dried in the field, in the study regions. This is feasible due to frequent rainfall during the peanut harvesting months of July and December. However, it is not possible to resolve the issue conclusively in the present study because the peanut samples analyzed were taken from on-farm storages, probably well after aflatoxin production had occurred. Regardless of the actual causes, strategies aimed at mitigating the aflatoxin contamination and human exposure will likely be more effective if they are targeted to the wetter and more humid areas of the Busia District.

Unimproved local varieties were associated with higher levels of aflatoxin compared to improved cultivars. These results concur with the work of Hell et al. (2003), who discovered a positive correlation between the growing of local varieties and increased aflatoxin levels of maize in Benin. The resistance status of the cultivars assessed in this study to colonization by aflatoxin-producing *Aspergillus* species is not known. However, improved varieties generally tend to be selected for increased yield and resistance to diseases that may reduce their susceptibility to infection by *Aspergillus* spp. Moreover, local landraces such as Homa bay Red, Uganda Red and Red Valencia, have been reported to be susceptible to rosette virus, stem rot and mould (Ogwang, 2006), and positive correlations between diseases and aflatoxin contamination of peanuts have been documented by many researchers (Lynch and Wilson, 1991; Udoh et al., 2000; Kasno, 2004; Robertson-Hoyt et al., 2007).

Attack by moles was also found to be significantly associated with aflatoxin levels. Damage by moles predisposes pods to colonization by aflatoxin producing fungi. Similar damage by terrestrial arthropods has been reported (Dicko et al., 1999). At the same time, the damage increases moisture levels of pods and grains, as documented by Hell et al., (2000). Pod damage also exposes the kernels to colonization by aflatoxin-producing and other saprophytic fungi (Chapin et al., 2004).

The observation that membership in a PMG was not significantly associated with levels of aflatoxin was surprising because PMG members are trained on pre- and post-harvest peanut handling practices that should result in a reduction in the level of contamination (Mutegi et al., 2007). The reasons for this observation were not investigated but it is possible that the awareness-raising program has not been undertaken long enough to have an impact. The specific message being delivered through the PMGs may also need to be reviewed to ensure that more information about aflatoxins, especially practices that reduce the level of peanut contamination, are covered. Identifying the reasons why PMGs were apparently not effective at reducing the aflatoxin contamination is essential, because long-term strategies for aflatoxin control will depend on the use of such groups as avenues for disseminating appropriate control strategies. In the short term, the risk of aflatoxin exposure in western Kenya can be minimized by focusing control strategies on the more humid agro-ecological zones such as

LM1, emphasizing planting of improved cultivars and protecting the crop from damage by rodents.[4]

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CHAPTER 5: ASSESSMENT OF FUNGI CONTAMINATING PEANUTS IN THE BUSIA AND HOMA BAY DISTRICTS OF WESTERN KENYA[5]

5.1   Introduction

Infection or infestation of major food crops such as peanuts by mycotoxins producing fungi pose a major safety concern. Contamination of produce by mycotoxins can occur at production, acquisition and handling of raw materials, processing, storage and distribution (Bastianelli and le Bas, 2002). Common fungal species capable of infecting or infesting crops and their produce include *Aspergillus*, *Penicillium*, and *Fusarium* species (Bastianelli and le Bas, 2002; Pacin et al., 2002; Gachomo et al., 2004; Khosravi et al., 2007). Most of these fungi produce mycotoxins, such as aflatoxins, ochratoxins, zearalenone and cyclopiazonic acid. Contamination of food systems by these mycotoxins pose a major health and food safety concern in many eastern and southern African countries (Siame and Nawa, 2008).

Among the mycotoxins produced by these fungi, aflatoxin has received considerable attention due to its highly potent nature (van Egmond, 1995; Wood and Trucksess, 1998). Aflatoxin producing fungi are found in section *Flavi* of the genus *Aspergillus* (Cardwell and Cotty, 2002; Horn, 2007). In particular, aflatoxins are produced mainly by *Aspergillus flavus* Link ex Fries, *Aspergillus parasiticus* Speare and to a lesser extent, *Aspergillus nomius* (Dorner, 2002; Vaamonde et al., 2003). In addition to producing aflatoxins, *A. flavus,* which is ubiquitous, also produces cyclopiazonic acid (Vaamonde et al., 2003; Dorner, 2008). *A. flavus* can infect and multiply in peanuts at both pre- and post-harvest stages (Cotty et al., 1994; Mutungi et al., 2008). Aflatoxin is also a major contaminant of several other crops including maize and tree nuts (Hill et al., 1985; Abbas et al., 2002; Abbas et al., 2004; Fandohan et al., 2004; Sobolev, 2007). Other effects of fungal contamination in peanuts include pre-emergence and seedling rot caused by *A. niger*, *A. flavus*, *Rhizopus* species*, Penicillium,* and *Sclerotium rolfsii*, among others (Subrahmanyam et al., 1992).

While the likelihood of contamination of many food commodities with aflatoxin remains high, research efforts addressing the aflatoxin problems in Kenya have focused on maize (the staple food) following outbreaks in the eastern parts of the country (Muriuki and Siboe, 1995;

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Nyikal et al., 2004; Lewis et al., 2005; Probst et al., 2007; Okioma, 2008). However, considering the diversity of Kenyan foods, under-reporting in other commodities is possible as toxicity in these foods — including peanuts—is not monitored. Ombui et al. (2001) noted that many food borne disease outbreaks are often under-reported due to inadequate investigation, monitoring and reporting systems and lack of diagnostic facilities. In addition, climatic factors and prevalence of predisposing factors such as, mechanical damage, drought stress during the late stages of pod development, and attack by pests and diseases that provide ideal environments for fungal development (Bilgrami and Choudhary, 1990; Waliyar et al., 2005a; Kaaya and Kyamuhangire, 2006) suggest the likelihood of contamination. Peanut production in Kenya is dominated by small holders whose handling practices often favour fungal contamination. For example, peanuts are either left in the field to dry, dried on polythene sheets, or directly on the ground (Mutegi et al., 2007). Nuts are stored in rooms that are not well ventilated, resulting in moisture build up. Furthermore, peanut vendors are often situated near busy and dusty roads or temporary structures where conditions increase the risk of contamination.

Previous studies have isolated fungi from peanuts in eastern Africa (Ismail, 2001; Gachomo et al., 2004). Ismail (2001) found a high prevalence of *A. flavus*, *A. niger* and *Penicillium spp.* in samples of peanut and desiccated coconut from Nairobi and Kampala. Similarly, a study by Gachomo et al. (2004) on peanut samples collected from markets in Nairobi, Kenya, found *R. stolonifer*, *Penicillium, A. parasiticus* and *A. flavus* among other fungi. However, these studies did not quantify the relationship between the incidence of specific fungal species and levels of aflatoxin. Thus the relative importance of aflatoxin-producing species in the genus *Aspergillus* with regard to peanut contamination with aflatoxin in eastern Africa is not known.

Species within the *A. flavus* group (referring to both *A. flavus* and *A. parasiticus*) are responsible for producing various types of aflatoxins (Cotty, 1997). For example, S-strain isolates of *A. flavus* produce Aflatoxin B_1 and B_2 (Kurtzman et al., 1987; Egel et al., 1994). In a recent study, a household survey of peanut production and processing in western Kenya found some very high levels of aflatoxin in samples (Mutegi et al., 2009). Further investigation is necessary to establish correlations between the levels of aflatoxin and the type and prevalence of fungi in peanut samples. This information is required to understand the potential risk of peanut contamination in order to implement and recommend measures to

reduce the associated health risks and identify atoxigenic strains that could act as biological control agents. The objectives of the study were i) to assess the prevalence of fungi in the genus *Aspergillus* in peanuts from western Kenya; ii) to determine whether the prevalence of fungi in the genus *Aspergillus* is associated with levels of aflatoxin in peanuts; iii) to identify factors correlated with the incidence of fungi in the genus *Aspergillus; iv)* to establish the prevalence of Aflatoxin B_1, B_2, G_1 and G_2, in peanuts from western Kenya, and v) to identify factors associated with the incidence of these aflatoxin types.

5.2   Methods

5.2.1   Sampling

Samples and information relating to each sample was gathered through a household survey conducted in western Kenya in 2006 and details of the sampling methodology are published elsewhere (Mutegi et al., 2009). Information was collected through personal interviews using a pre-tested questionnaire that was developed after conducting focus group discussions involving 40 and 44 participants from the Busia and Homa bay districts, respectively. A one-kilogram sample was obtained from each surveyed household and assigned to batches based on the division within the district from which they were obtained, and stored in a cold room until processed. Of the 769 samples obtained, 436 samples, consisting of 252 from Busia and 184 from Homa bay, were randomly selected and assayed for the presence of *Aspergillus* section *Flavi, Rhizopus, Penicillium* and the 4 main aflatoxin types, namely B_1, B_2, G_1 and G_2. Ten replicate plates of each sample were used during isolation. For each district, samples were selected to represent administrative divisions.

5.2.2   Isolation and identification of *Aspergillus* species

Isolation of *Aspergillus* section *Flavi* was carried out using Modified Dichloran Rose Bengal Agar (Horn and Dorner, 1998). The medium was prepared by mixing 10 g glucose, 2.5 g peptone, 0.5 g yeast extract, 1g KH_2PO_4, 0.5g $MgSO_4.7H_2O$, 20 g agar and 25 mg Rose Bengal in 1 ℓ of distilled water. The pH of this medium was then adjusted to 5.6 using 0.01 M HCl. The medium was autoclaved for 20 minutes at 121 ^{0}C and a pressure of 15 psi, and cooled in a water bath at 60 ^{0}C.   To inhibit bacterial growth and ensure the medium was

semi-selective for *Aspergillus* section *Flavi* fungi, 5 ml of 4 mg/ℓ dichloran (in acetone), 40 mg/ℓ streptomycin (in 5 mℓ distilled water) and 1 mg/ℓ chlortetracycline (in 10 mℓ distilled water) was added to the medium through a sterile 0.25 µm syringe filter after cooling to 50 ^{0}C. The medium was then poured on to 90 mm plates and allowed to settle for 2 to 3 days before use.

Preparation of samples for plating was performed by thoroughly mixing the one kilogram sample. Two sub-samples (100 g each) were weighed and blended in a kitchen grinder (Kanchan Multipurpose Kitchen Machine, Kanchan International Limited, Mumbai, India). From each of the 100 g ground samples, 10 replicates of 2.5 g each were placed in calibrated centrifuge tubes, into which 10 mℓ of 2 per cent water agar solution (prepared by adding 2 g agar in 100 mℓ sterile water) was then added and mixed thoroughly. A volume of 0.2 mℓ of the solution was then pipetted onto Modified Dichloran Rose Bengal medium in the 90 mm Petri-plates under aseptic conditions. The plates were incubated for three days at 37 ^{0}C, after which the colonies were identified and classified. Total colony counts and colony counts for *A. flavus L-strain*, *A. flavus* S-strain, *A. parasiticus*, *A. alliaceus*, *A. tamarii*, *A. niger* and *Penicillium* species per plate were recorded. The presence or absence of *Rhizopus* species in each plate was also recorded.

The medium used for identification of fungal species based on cultural and morphological characteristics was Czapek Yeast Extract Agar (CYA), which was prepared by mixing one gram K_2HPO_4, 10 mℓ Czapek concentrate, 5 g powdered yeast extract, 30 g sucrose and 15 g agar in 1ℓ of distilled water. The pH of the medium was then adjusted to 7.2 before autoclaving for 20 minutes at 121 ^{0}C and a pressure of 15 psi. Thereafter, the medium was allowed to cool in a water bath to 60 ^{0}C and approximately 20 mℓ was poured into 90 mm sterile Petri dishes and left to cool overnight under a laminar flow hood. Pure colonies on MDRB agar medium were then streaked onto the plates, and placed into an incubator at 30 ^{0}C for seven days. Different species of *Aspergillus* section *Flavi* were distinguished, based on colony colour, texture, and conidial morphology characteristics (Klich, 2002), and by comparison with reference strains obtained from Dr. Bruce Horn (USDA National Peanut Research Lab, Dawson, Georgia, United States of America).

5.2.3   Screening isolates of *A. flavus* and *A. parasiticus* for aflatoxin production

Screening of isolates for aflatoxin production was done in high sucrose yeast extract (YES) liquid medium (Horn and Dorner, 1998), from 252 and 184 isolates from Busia and Homa bay districts respectively. The YES medium was prepared by dissolving 150 g sucrose, 20 g yeast extract (Difco), 10 g soystone and 40 g glucose in 1ℓ distilled water, and an adjusted pH of 5.9 effected with 0.25 M HCl. Aliquots of 2 mℓ of the broth were dispensed into 6 mℓ vials that were then lightly screwed and autoclaved for 30 minutes at 121 ^{0}C and a pressure of 15 psi. Conidia from uncontaminated colonies of *A. flavus* and *A. parasiticus* were picked up with a sterile inoculating needle and used to inoculate the vials containing 2 mℓ of YES medium. The vials were then incubated in the dark at 30 ^{0}C for seven days, during which there was intermittent shaking using a vortex shaker. Subsequently, the vials were removed from the incubator and 2 mℓ of chloroform was pipetted into each vial. The mixture was vortexed for about 60 seconds per sample and left to stand overnight under a fume hood. Using a micro-pipette, 5 µℓ of the chloroform extract was spotted on silica gel 60 TLC plates (EMD Chemicals Inc., Darmstadt), along with analytical grade standards of aflatoxins B_1, B_2, G_1 and G_2. Toxigenic strains were used as positive controls. The plates were then allowed to develop in a solvent consisting of chloroform, acetone and distilled water in a ratio of 88:12:1.5, respectively, until the solvent covered about 90 per cent of the plate length. The plates were transferred to a dark room and scored for the presence of the four aflatoxins under UV light. The scoring was based on the presence or absence of specific aflatoxin types.

5.2.4   Analysis of peanut samples for aflatoxin content

A 200 g sub-sample was drawn from each one-kilogram sample after thoroughly mixing and grounding it into a fine powder using a dry mill kitchen grinder (Kanchan Multipurpose Kitchen Machine, Kanchan International Limited, Mumbai, India). The sample powder was then sub-divided into two equal portions. The powder was triturated in 70 per cent methanol (v/v 70 mℓ absolute methanol in 30 mℓ distilled water) containing 0.5 per cent w/v potassium chloride in a blender, until thoroughly mixed. The extract was transferred to a conical flask and shaken for 30 min at 300 rpm. The extract was then filtered through Whatman No.41 filter paper and diluted 1:10 in phosphate buffered saline containing 500 µℓ/ℓ Tween-20 (PBS-Tween) and analyzed for aflatoxin with an indirect competitive ELISA (Waliyar et al.,

2005*b*) by preparing an aflatoxin-bovine serum albumin conjugate in carbonate coating buffer at 100 ng/mℓ concentration and dispensing 150 µℓ in each well of the Nunc-Maxisorp® ELISA plates[6].

The plates were incubated at 37 °C for one hour before the toxin solution was collected and stored in a large glass bottle for disposal. The plates were washed in three changes of PBS-Tween, allowing a holding time of three minutes per wash. The plates were blocked with a 200 µℓ per well solution of 0.2 per cent bovine serum albumin (BSA) in PBS-Tween and incubated at 37 °C for one hour. The blocked plates were then washed in three changes of PBS-Tween allowing three minutes for each wash. To the washed plates, 100 µℓ of peanut extract was added followed by 50 µℓ of antiserum. Instead of the peanut extract, 100 µℓ aliquots of different concentrations of Aflatoxin B_1 (25 ng to 100 pg) were added into the first 20 wells (two rows of 10 wells each) to serve as a standard. The plates were then incubated for one hour at 37 °C to facilitate reaction between the toxins and the antibody.

The plates were subsequently washed in three changes of PBS-Tween, allowing three minutes for each wash. A dilution of 1:1000 goat anti-rabbit IgG labelled with alkaline phosphatase was prepared in PBS-Tween containing 0.2 per cent BSA. A 150 µℓ aliquot was added to each well, and incubated for one hour at 37 °C. The plates were washed in three changes of PBS-Tween, added a 150 µℓ per well of substrate solution (p-nitro phenyl phosphate prepared in 10 per cent diethanolamine buffer, pH 9.8) and incubated for about one hour at room temperature. Absorbance was measured at 405 nm in an ELISA plate reader (Multiskan Plus, Labsystems Company, Helsinki, Finland).

5.2.5   Statistical analyses

Fungal incidence was determined using frequencies tables and the number of samples from which a particular species was isolated recorded as a proportion of total number of samples assayed. Associations between the incidence of a particular fungal species with various categorical variables were investigated based on analysis of contingency tables with appropriate chi-squared tests. The categorical variables evaluated in these tests included:

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[6] Nunc A/S, Kamstrupvej 90, P.O.Box 280, DK-4000, Roskilde, Denmark

- district of sample origin;

- agro-ecological zone (AEZ) from which sample was collected;

- specific cultivar;

- cultivar type (i.e. whether a local landrace or improved);

- whether or not crop rotation was practiced during the growing period of the sample;

- whether or not the farmer used commercial fertilizer in peanut production;

- number of times a sample was weeded (0, 1-to 2 or 3 times);

- harvesting method (whether a farmer harvested by hand pulling, digging, a combination of hand pulling and digging or use of oxen);

- whether or not grading of the nuts was carried out;

- whether or not the nuts were sorted; and

- a categorical variable created by grouping samples based on their levels of aflatoxin.

Samples were grouped into three categories based on their aflatoxin content: samples with: ≤ 4 μg/kg, >4 μg/kg to ≤ 20 μg/kg, or >20 μg/kg. The ≤ 4 μg/kg category represents the European Union regulatory limit for total aflatoxins (Felicia, 2004); peanuts in the second group would be rejected in the European Union but would be accepted under the Kenya Bureau of Standards (KEBS) limits (Felicia, 2004), while nuts in the third category would be rejected both under the KEBS and EU standards. Associations between incidence of Aflatoxin B_1, B_2, G_1, and G_2 and these categorical variables were also studied by categorical data analysis.

The relationship between total colony count and aflatoxin levels per sample was analyzed with linear regression, with total colony count as the explanatory variable, and aflatoxin level as the response variable. Linear regression was also used to study the relationship between aflatoxin levels recovered in each sample (response variable) and colony counts per sample of *A. flavus* L-strain, *A. flavus* S-strain and *A. parasiticus* (as explanatory variables). Logistic regression analysis was used to investigate the relationships between the incidence of *A. flavus* L-strain, *A. flavus* S-strain, *A. parasiticus*, *A. niger*, *Penicillium* and *Rhizopus* (counts as response variables) and aflatoxin categories as well as other variables (listed for correlation studies above). All analysis was conducted using GenStat Ver 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station).

5.3   Results

5.3.1   Prevalence of fungal species in peanuts from the Busia and Homa bay Districts

The prevalence of *A. flavus* L-strain, *A. flavus* S-strain, and *A. niger* was generally high, with over 60 per cent of samples in both districts and in all agro-ecological zones showing levels of contamination (Figure 5.1 and 5.3). Conversely, there was a low prevalence of *A. tamarii, A. alliaceus* and *A. caeletus,* with incidences being less than 12 per cent, 10 per cent and 2 per cent, respectively, in both districts. The prevalence of *A. flavus* S-strain, *A. niger*, *A. tamarii*, *Rhizopus* spp. and *Penicillium* spp. was higher in Busia District compared to Homa bay District.   In contrast, the incidence of *A. flavus* L-strain, *A. alliaceus*, *A. caeletus* and *A. parasiticus* was higher in Homa bay than in Busia. Only the incidence of *Penicillium* spp. (χ^2 = 10.86; *p* < 0.001) and *Rhizopus* spp. species (χ^2 = 12.78; *p* < 0.001) was significantly correlated with districts of sample origin, with a higher prevalence for both species being noted for samples from Busia district. For example, the incidence of *Penicillium* spp. in Busia district was 50 per cent of all samples compared with 34 per cent for samples from Homa bay district (Table 5.1).
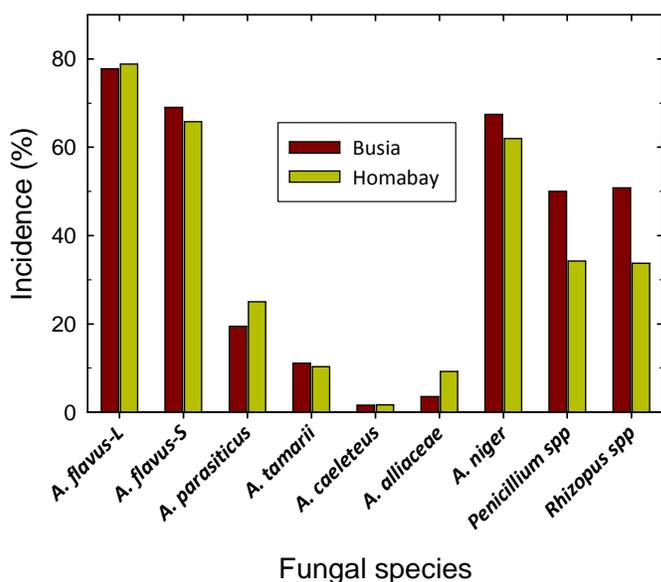
Figure 5.1: Incidence of fungal species isolated from peanuts in the Busia and Homa bay Districts of Kenya.

Table 5.1: Associations between the incidence of fungal species and cultivar type, membership to a producer marketing group, district of sample origin and grading as a post-harvest practice, August 2006

Fungal species	Descriptive factor		χ^2 value	*P* value
	Per cent of samples within each cultivar type			
		Improved Landrace		
	Local Landrace (255)	(179)		
A. flavus L-strain	82	73	4.28	0.039
A. niger	73	54	17.15	<0.001
Rhizopus spp.	48	37	5.02	0.025
	Per cent of samples belonging to a PMG group or not			
	Non PMG member (268)	PMG member (168)		
A. flavus L-strain	84	70	11.52	<0.001
A. flavus S-strain	72	61	5.97	0.015
A. niger	72	54	15.95	<0.001
Rhizopus spp.	48	37	4.98	0.026
	Per cent of samples graded/not graded			
	Do not grade (152)	Grade (284)		
A. flavus L-strain	86	74	7.70	0.006
A. flavus S-strain	74	64	4.86	0.027
	District of sample origin			
	Busia (252)	Homabay (184)		
Penicillium spp.	50	34	10.86	<0.001
Rhizopus spp.	51	34	12.78	<0.001

numbers in parenthesis indicate actual sample size evaluated.

The prevalence of *A. flavus* L-strain in LM1, LM2 and LM3 was 74 per cent, 81 per cent and 79 per cent of samples respectively, while the prevalence of *A. flavus* S-strain in LM1, LM2 and LM3 was 68 per cent, 67 per cent and 68 per cent, respectively. Despite its lower

incidence (14 per cent, 24 per cent and 28 per cent in LM1, LM2 and LM3, respectively), *A. parasiticus* was statistically significantly ($\chi^2 = 7.36$; $p < 0.025$) associated with AEZ, with incidence being highest in samples from LM3. There was also a significant ($\chi^2 = 10.36$; $p < 0.006$) association between incidence of *Penicillium* spp. and AEZ, with an incidence of 52 per cent in LM1, 35 per cent in LM2 and 48 per cent in LM3 (Table 5.2).

5.3.2   Identifying factors associated with peanut contamination by specific fungal species

Investigations on the relationship between total colony counts, colony counts of aflatoxin producing species (*A. flavus* L-strain, *A. flavus* S-strain and *A. parasiticus)* and levels of aflatoxin recovered per sample indicated that only *A. flavus* S-strain was positively correlated with aflatoxin levels ($p < 0.001$; $r = 0.545$). Figure 5.2 shows the relationship between incidence of fungal species and the three categories of aflatoxins (categories described in methodology). Samples with less than 4 µg/kg of aflatoxin levels had a 77 per cent incidence of *A. flavus* L-strain, while those in categories of 4->20 and >20 µg/kg aflatoxin levels had over 85 per cent incidence of *A. flavus* L-strain. There was a significant association ($\chi^2 = 42.19$; $p < 0.001$) between samples in specific aflatoxin level categories and the presence of *A. flavus* S-strain, whereby the percentage of *A. flavus* S-strain was significantly higher in samples that had higher aflatoxin levels. *A. flavus* S-strain was isolated in 62 per cent of samples with less than 4 µg/kg aflatoxin levels, 93 per cent of samples with between 4 µg/kg to $\leq$20 µg/kg and 100 per cent of those with >20 µg/kg (Figure 5.2).   In spite of the relatively predominant presence of *A. niger, Rhizopus* spp.*, Penicillium* spp. *and A. parasiticus*, there were no significant associations between these fungal types and aflatoxin levels.

All fungal species assayed were present in all the three agro-ecological zones. *A. flavus* L-strain*, A. flavus* S-strain and *A. niger* were predominant in all AEZs but no statistically significant difference was noted in the incidence among the zones (Figure 5.3).
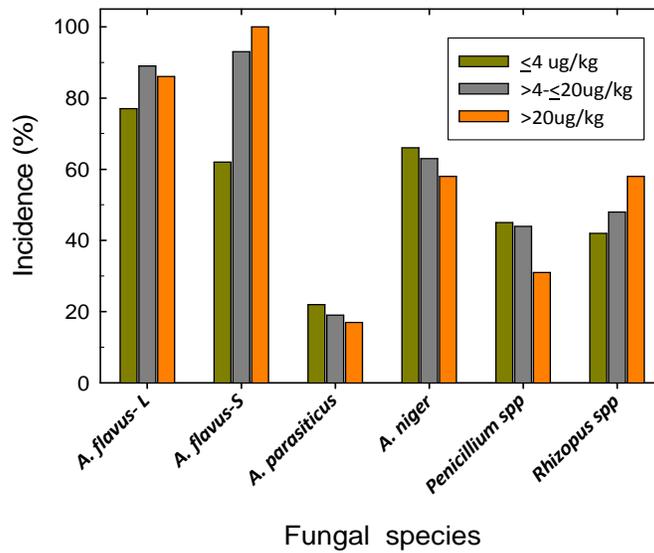
Figure 5.2: Incidence of fungal species isolated from peanuts with different levels of aflatoxin in samples obtained from the Busia and Homa bay districts of western Kenya
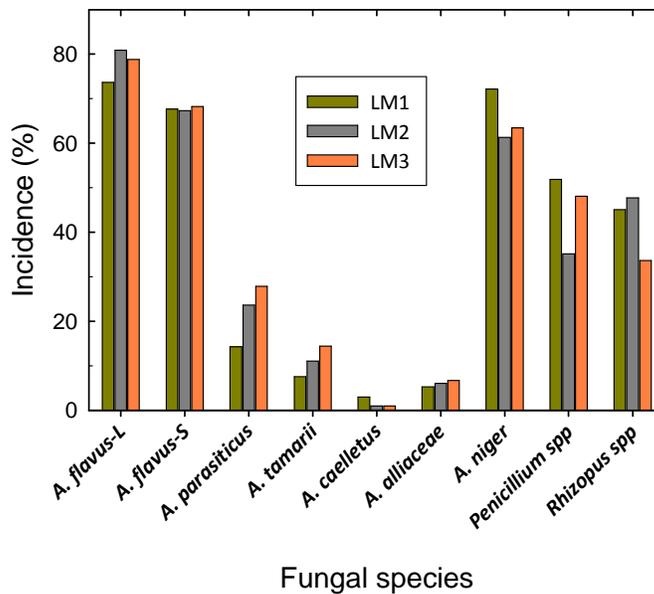
Figure 5.3: Incidence of fungal species isolated from agro-ecological zones LM1, LM2 and LM3 of the Busia and Homa bay districts in western Kenya

Table 5.2: Associations between the incidence of fungal species and AEZ, number of times that the peanut crop is weeded, and harvesting method, August 2006

Fungal species	Descriptive factor			χ^2 value	*P* value
	AEZ from which sample was collected				
	LM1 (133)	LM2 (199)	LM3 (104)		
A. parasiticus	14	24	28	7.36	0.025
Penicillium spp.	52	35	48	10.86	<0.001
	Number of times a sample was weeded				
	Once (72)	Twice (330)	Thrice (33)		
A. flavus L-strain	85	79	58	9.11	0.011
	Harvesting method				
	Hand pulling (147)	Hand digging (106)	Hand pulling and digging (181)		
Rhizopus	48	32	47	8.03	0.018

numbers in parenthesis represent actual sample size evaluated.

An assessment of the relationship between *A. niger* and individual cultivars showed a significant ($\chi^2 = 21.96$; $p < 0.003$) association, whereby the incidence of *A. niger* was higher in some local cultivars compared to improved varieties (Table 5.3). Similarly, peanuts of the Local Red variety had the highest incidence of *Rhizopus* spp. (64 per cent), while the improved cultivars Valencia Red and CG7 had the lowest percentage (26 per cent and 27 per cent, $\chi^2 = 26.14$; $p < 0.001$) of *Rhizopus* species, respectively.

There was also a significant association between cultivar improvement status (whether a sample was an improved variety or a local landrace) and *A. flavus* L-strain ($\chi^2 = 4.28$; $p = 0.039$), *A. niger* ($\chi^2 = 17.15$; $p < 0.001$) and *Rhizopus* spp. ($\chi^2 = 5.02$; $p = 0.025$), with improved cultivars showing lower contamination compared to local landraces (Table 5.1). For example, 82 per cent of the samples belonging to the local landrace category were contaminated with *A. flavus* L-strain, compared to 73 per cent belonging to the improved

cultivars, while 73 per cent of the local landraces were positive for *A. niger* compared to only 54 per cent of improved cultivars (Table 5.1; Figure 5.4).

Table 5.3: Incidence and test statistics for association between fungal species other than members of *Aspergillus* section *Flavi* and specific peanut cultivars grown in western Kenya, August 2006.

Fungal species	Cultivar							χ^2	P value
	Homabay Local	CG7	ICGV 12988	ICGV 12991	Local Red	Uganda Red	Valencia Red		
Sample size (n)^x	63	48	39	76	87	83	19		
A. niger^y	76	56	49	53	68	78	68	21.96	0.003
Rhizopus spp.^{y}	37	27	49	38	64	45	26	26.14	<0.001

^{x}number of samples analyzed for each cultivar.

^{y}per cent of samples contaminated with the species.

Grading of peanuts as a post-harvest practice significantly reduced contamination by *A. flavus* L-strain (χ^2 =7.7; *p* = 0.006) and *A. flavus-S* strain (χ^2 = 4.86; *p* = 0.027), as shown in Table 5.1. The incidence of *A. flavus* L-strain and *Rhizopus* spp. was significantly associated with method of harvesting. Farmers who first dug around the peanut crop to loosen the soil and then pulled the plant out had a significantly (χ^2 = 7.12; *p* = 0.029) lower incidence (72 per cent) of *A. flavus* L-strain, compared to samples that were harvested either by hand pulling (81 per cent) or hand digging (85 per cent) alone (Table 5.2). The incidence of *Rhizopus* spp. was significantly (χ^2 = 8.03; *p* = 0.018) higher in samples that were harvested by hand puling (48 per cent) compared to samples that were harvested by hand digging (32 per cent).
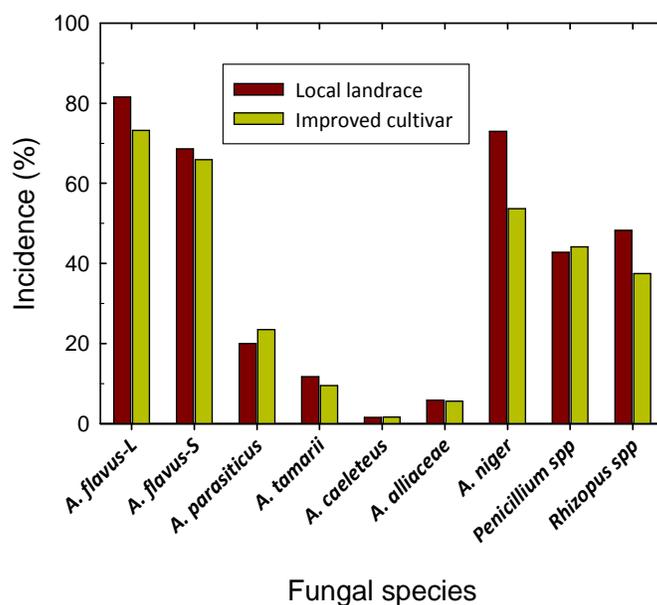
Figure 5.4: Incidence of fungal species in local and improved cultivars sampled from the Busia and Homa bay districts of western Kenya

Membership to a Producer Marketing Group (PMG) had a significant effect on the presence of four of six of the fungal species screened namely: *A. flavus* L-strain, *A. flavus* S-strain, *A. niger* and *Rhizopus* species. Higher incidences of the four species were recorded among farmers who did not belong to a PMG (Table 5.1). For example, while *A. flavus* L-strain was isolated from 84 per cent of the samples from non-PMG farmers, the incidence was reduced to 70 per cent among farmers who belonged to PMG's (χ^2 = 11.52; *p* < 0.001). Likewise, while the incidence of *A. flavus S-* strain in samples from non-PMG farmers was 72 per cent, this strain was isolated from only 61 per cent of samples from farmers belonging to a PMG (χ^2 = 5.97; *p* = 0.015). The incidence of *A. niger* was higher (72 per cent) in samples from non-PMG members compared with 54 per cent in samples from PMG farmers (χ^2 = 15.95; *p* < 0.001).

Most farmers (>85 per cent) did not use fertilizer. There was no significant association between fertilizer application and the incidence of any of the fungal species assessed. However, there was a significant (χ^2 = 4.98; *p* = 0.026) association between the number of times the crop was weeded and the incidence of *A. flavus* L-strain. As the number of weeding events increased, the incidence of *A. flavus* L-strain reduced (Table 5.2). No significant association was noted between the fungal types found and crop rotation practices.

5.3.3   Factors related to the incidence of peanut contamination

Logistic regression analysis indicated that PMG membership was the only variable significantly related with the incidence of *A. flavus* S-strain when all explanatory variables were included in the model (Table 5.4). The model rendered grading as a post-harvest practice not significantly associated with the fungus, compared to when grading was assessed without including the membership variable in the analysis (Table 5.1). The value of the odds ratio indicated that produce belonging to farmers in PMGs was only 60 per cent as likely to have *A. flavus* S-strain contamination compared to that for non-PMG members. The model found to be best suited to describe the presence of *A. flavus* L-strain was one that included the specific cultivar, district of sample origin, harvesting mode and the number of times a crop was weeded (Table 5.4). Only ICGV 12988 recorded significantly lower levels of *A. flavus* L-strain compared to the Homa bay Local variety. Samples from the Homa bay District were only 0.4 ($p = 0.035$) times as likely to be contaminated with *A. flavus* L-strain as samples from the Busia District. A combination of hand pulling and hand digging during harvesting of the peanuts also reduced the chance of contamination with *A. flavus* L-strain by almost half (odds ratio = 0.42; $p = 0.014$), compared to pulling plants from the ground. The number of times the plots were weeded was also significantly related ($p = 0.005$) with the incidence of *A. flavus* L-strain. Crops that were weeded three times had a lower incidence of *A. flavus* L-strain compared to those weeded once (Table 5. 4).

The incidence of *A. niger* was significantly ($0.01 \leq p \leq 0.032$) related to various factors that included AEZ, the specific cultivar, and whether or not the sample was taken from a farmer belonging to a producer marketing group (Table 5.4). For example, samples that were collected from LM1 AEZ were more than twice as likely to be contaminated with *A. niger* compared to samples collected from the LM3 region (Table 5.4). The odds of samples from improved cultivars of CG7, ICGV12988, ICGV 12991 being contaminated with *A. niger* were almost a quarter those of the Homa bay Local variety (Table 5.4). Moreover, the likelihood of a sample being contaminated with *A. niger* reduced by a half (0.54; $p = 0.019$) if the farmer was a PMG member, compared to samples that came from non-PMG members.

Table 5.4: Parameter estimates from logistic regression models[x] relating the incidence of fungal species with descriptive variables of peanut samples collected in August 2006.

Fungal species	Descriptive variable	Parameter estimate[y]	s.e[z]	*P*- value	Odds ratio
A. flavus S-strain	Estimate constant	0.943	0.137	<0.001	2.567
	PMG membership	-0.522	0.210	0.013	0.593
A. flavus L-strain	Estimate constant	2.728	0.710	<0.001	15.310
	ICGV12988	-1.471	0.618	0.017	0.230
	Homa bay district	-0.947	0.449	0.035	0.388
	Hand digging and hand pulling	-0.875	0.355	0.014	0.417
	Weeding thrice	-1.543	0.552	0.005	0.214
A. niger	Estimate constant	1.219	0.348	<0.001	3.384
	LM1	0.842	0.326	0.01	2.321
	CG7	-0.923	0.432	0.032	0.397
	ICGV 12988	-1.102	0.463	0.017	0.332
	ICGV 12991	-0.930	0.390	0.017	0.394
	Local Red	-1.021	0.419	0.015	0.360
	PMG membership	-0.615	0.261	0.019	0.540
Penicillium spp.	Estimate constant	0.870	0.339	0.01	2.387
	LM2	-0.842	0.276	0.002	0.431
	Homa bay district	-1.025	0.274	<0.001	0.358
	Hand pulling and hand digging	-0.659	0.274	0.016	0.517
Rhizopus spp.	Estimate constant	-0.553	0.261	0.034	0.575
	Local Red	1.145	0.344	<0.001	3.141

[x]models for each fungal species are separated by continuous lines.

[y]negative sign indicates that the incidence of the fungal species declines in relation to the variable. E. g., Incidence of *A. flavus* L-strain was lower in the Homa bay than in the Busia district.

[z]standard error of the parameter estimate.

Only the cultivar Local Red was significantly related with incidence of *Rhizopus*, with the likelihood of sample contamination by *Rhizopus* tripling (odds ratio = 3.141) in samples of this cultivar. The presence of *Penicillium* species in the samples could be explained by a model fitted with variables for AEZ, district of sample origin and harvesting mode (Table

5.4). Harvesting produce with a combination of hand digging and pulling halved the chances of contamination with *Penicillium* spp. (odds ratio = 0.517; *p* = 0.016), in comparison with samples that were harvested by hand puling alone. Samples from the Homa bay District were 0.4 times less likely to be contaminated with *Penicillium* compared to those from Busia.

5.3.4   Incidence of specific aflatoxin types and relationship with total aflatoxin levels, AEZ, cultivar type and district of sample origin

Between two and thirty-eight isolates per sample were assayed for aflatoxin levels, depending on the number of *A. flavus* and *A. parasiticus* isolates recovered. Overall, the most common toxin type was Aflatoxin B_1, followed by Aflatoxin B_2, G_1 and G_2, with a percentage incidence of 67 per cent, 46 per cent, 39 per cent and 29 per cent, respectively, among the isolates screened. There was no significant association between toxin types and AEZ or cultivar type. However, there was a significant association between the district of sample origin and the incidence of Aflatoxin G_1 contamination (χ^2 = 5.48; *p* = 0.019), with isolates from 45 per cent of samples from the Busia District producing Aflatoxin G_1, compared to only 32 per cent of samples from the Homa bay District (Figure 5.5). This was in spite of the fact that there was no significant difference in prevalence of *A. parasiticus* between the districts.
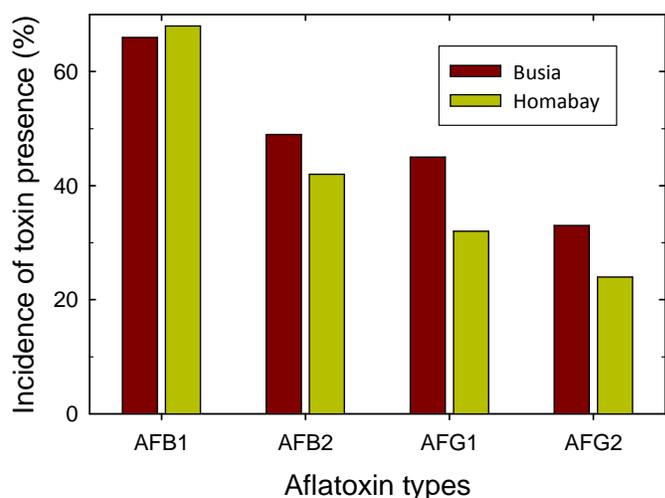
Figure 5.5: Incidence of Aflatoxin B$_1$, B$_2$, G$_1$ and G$_2$ production by *A. flavus* and *A. parasiticus* isolated from peanut samples obtained from Busia and Homa bay districts. Between two and thirty-eight isolates per sample were assayed for aflatoxin production depending on the number of *A. flavus* and *A. parasiticus* isolates recovered.

 In comparing toxin types and levels of aflatoxin, a significant association was found between toxins produced by samples and aflatoxin levels recovered in the samples (Table 5). The incidence of Aflatoxin B$_2$ in samples was 44 per cent, 31 per cent and 83 per cent (χ^2 =15.01; p < 0.001) in aflatoxin categories of <4µg/kg, 4-≥20µg/kg and >20µg/kg respectively. Similarly, the incidence of aflatoxin type G$_1$ in samples was 36 per cent, 54 per cent and 70 per cent (χ^2 =10.92; p = 0.004) in aflatoxin categories of <4µg/kg, 4-≥20µg/kg and >20µg/kg respectively (Table 5.5).

Table 5.5: Association between Aflatoxin B$_1$, B$_2$, G$_1$ and G$_2$ and categories of levels of total aflatoxin in peanut samples collected from Busia and Homa bay districts of western Kenya, August 2006.

Toxin type	Percentage of samples with different levels of total aflatoxin[x]			χ^2	P-value
	≤ 4 µg/kg	$>4 - \leq 20$ µg/kg	>20 µg/kg		
Aflatoxin B$_1$	66	77	87	5.64	0.059
Aflatoxin B$_2$	44	31	83	15.01	<0.001
Aflatoxin G$_1$	36	54	70	10.92	0.004
Aflatoxin G$_2$	27	31	23	4.05	0.132

[x]based on a total of 436 total samples assayed.

5.4   Discussion

This study assessed the prevalence of fungal species in peanuts from western Kenya, focusing on section *Flavi* of the genus *Aspergillus*, as well as fungi from other genera that may produce mycotoxins. The factors associated with the incidence of these fungi were investigated. The predominant species across the districts (i.e., species with over 60 per cent incidence) were *A. flavus* L-strain*, A. flavus* S-strain and *A. niger*, with an incidence of 78 per cent, 68 per cent and 65 per cent, respectively. These fungi have been isolated at similar levels of incidence in peanuts previously (Adebajo et al., 1994; Awuah and Kpodo, 1996; Gachomo et al., 2004), but this is the first study in the region to quantify the association between the incidences of specific species with levels of aflatoxin in peanut samples from East Africa. To the best of the authors' knowledge, this is also the first study to document the incidence of *A. caeletus, A. alliaceus* and *A. tamarii* in the region. Despite their low prevalence, *A. caeletus, A. alliaceus* and *A. tamarii* were isolated from the samples from both Busia and Homa bay districts, and their occurrence at low incidence is in line with the observations of Horn (2005), who documented these species in the United States of America.

The high incidence of *A. flavus-S* strain that produces aflatoxin (Cotty and Cardwell, 1999; Egel et al., 1994) and in particular, the more potent Aflatoxin B$_1$ and B$_2$, implies a present risk of aflatoxin contamination of peanuts from western Kenya. In as much as the incidence of *A. flavus* L-strain was high, it did not lead to a positive correlation with aflatoxin and this

could be attributed to the fact that most of the L-strains were atoxigenic. Since the factors that trigger aflatoxin production are not well understood, vigilance in pre- and post-harvest handling of peanuts is needed to avert the risk of human exposure because the toxins can be produced at both stages. The confirmation of occurrence of other species that produce toxins, such as *A. tamarii* [which produces cyclopiazonic acid (Horn et al., 1996)] and *A. alliaceus* that produces ochratoxin A (Bayman et al., 2002), underscore the need to screen peanuts not just for aflatoxin, but also for other detrimental mycotoxins. The risk of human exposure is intensified by the fact that contamination leads to rejection of nuts in lucrative markets, mostly based on aflatoxin levels. The rejected produce finds its way into local markets, increasing the risk of aflatoxin exposure to unsuspecting local consumers. For example, before a monitoring system was established to determine aflatoxin levels of peanuts destined for the European markets, peanuts that were rejected for export from Malawi due to high levels of aflatoxin were then sold on the local market (Mkoka, 2007).

There was a predominance of Aflatoxin B_1 and B_2 types across samples, and a significantly higher incidence of Aflatoxin G_1 in the Busia district, compared to the Homa bay district. Not surprisingly, the high incidence of *A. flavus* S-Strain was associated with greater aflatoxin levels. This particular strain has been found to be responsible for the production of aflatoxins, and especially the more potent B toxins. The high incidence of *A. flavus* S-strain could therefore have been responsible for the high incidence of the B toxins. of A similar trend has been found in other studies, whereby the *A. flavus* S- strain has been found to be the main source of aflatoxin (Egel et al., 1994; Cotty and Cardwell, 1999; Abbas et al., 2002) in the United States and in maize from Kenya (Probst et al., 2007) . Although the majority of the samples in this study were safe according to both the EU and KEBS regulatory limits (Mutegi et al., 2009), the high incidence of *A. flavus* L-strain and *A. flavus* S-strain, implies a likelihood of increased aflatoxin levels if safe pre- and post-harvest management practices are not adhered to. In spite of the fact that there were no significant differences in the incidences of *A. flavus* S- and L-strains or *A. parasiticus* between districts or agro-ecological zones, their incidences were high, a fact that could have contributed to high levels of aflatoxin in Busia (Mutegi et al., 2009). Logistic regression showed that samples from Homa bay were only 40 per cent as likely to be infected with *A. flavus* L-strain, as peanuts from Busia. Again the growth conditions for the fungus in Busia district that is wetter and more humid than Homa bay district could have contributed to their higher incidence in Busia, and subsequently led to

a higher incidence of the G_2 toxins that were reported to be significantly higher in Busia district as compared to Homa bay district.

The presence of other fungal species such as *Penicillium* spp., *A. niger*, and *Rhizopus* spp. indicates a likelihood of contamination from other toxins produced by these fungi (e.g., cyclopiazonic acid and patulin), because it is possible to have more than one toxin type from the coexisting fungi (Speijers and Speijers, 2004). For example, *A. niger* and *Penicillium* species can both produce ochratoxins (Sweeny and Dobson, 1987; Wilson et al., 2002). *A. flavus*, in addition to producing aflatoxins, is also capable of producing cyclopiazonic acid (Horn et al., 1996; Horn and Dorner, 1999; Vaamonde et al., 2003). Certain *Penicillium* spp. are known to produce patulin (Spadaro et al., 2009, Welke et al., 2009) and citrinin (Singh et al., 2008).The prevalence of such fungi should, therefore, be of concern since such toxins could be present in peanuts from western Kenya, even though their occurrence was not investigated in this study.

Significant associations were found between fungal species and factors such as cultivar type, specific cultivars, AEZ, aflatoxin levels and crop management practices. The higher incidence of *A. flavus*, *A. niger* and *Rhizopus* spp. in local landraces compared to the improved varieties was not surprising. Mutegi et al. (2009) have shown that local landraces have a higher likelihood of being contaminated with aflatoxin than improved cultivars. Previous studies have also shown susceptibility of local varieties to fungal contamination elsewhere (Middleton et al., 1994). Variety improvement programs are also generally tailored to reducing susceptibility to diseases, and this could explain why improved varieties were likely to show a lower incidence of fungal contamination compared to the local varieties. In addition, *A. flavus* has the ability to live as a saprophyte in parts of its life cycle.

Almost all farmers interviewed sort their peanuts (Mutegi et al., 2007) but there was no significant association between sorting and the incidence of fungal species, because the sample size of those who sorted compared to those who did not, was skewed. However, there was a significantly higher incidence of both *A. flavus* L-strain and *A. flavus* S-strain among ungraded peanuts. Grading is mainly conducted for marketed peanuts (Mutegi et al., 2007). Grading criteria includes assessing parameters such as size of nut (which, in the process, is likely to eliminate shrivelled nuts), discoloration (which, in the processes, is likely to get rid of nuts that are visibly mouldy), and broken nuts. Such criteria are therefore likely to reduce

the incidence of aflatoxin-producing fungi and have been demonstrated to be negatively correlated with levels of aflatoxins (Waliyar et al., 2008; Mutegi et al., 2009).   Moreover, logistic regression analysis showed that membership to PMGs was significantly related to the incidence of *A. flavus* S-strain. The observation that grading was not, does not contradict this conclusion. Rather, it indicates that in the model, the effects of grading in the model were confounded with those of PMG membership. This was expected, given that PMG members are trained to reduce aflatoxin contamination using approaches such as grading and sorting (Mutegi et al., 2007).

Samples that were harvested by hand-pulling or hand-digging had a significantly higher incidence of *A. flavus* L-strain compared to samples that were harvested using a combination of hand pulling and hand digging. This could be explained because of the high levels of damage to pods that occurs when a farmer uses excessive force in pulling up a peanut crop or when digging it out. Depending on which harvesting method is used, there is an increased likelihood that the pods of peanuts would be damaged, creating an entry point for fungi. For example, pulling crops from very firm ground will lead to breakages, and so does digging crops from the ground, which could wound the pods. A combination of the two methods would effectively enable for lose crop to be pulled up and for subsequent digging to be done to loosen any firm soil around the crop. Wounded plants have higher concentrations of aflatoxin and high incidences of fungal colonization (Horn, 2005).   The mode of harvesting has also been shown to affect aflatoxin levels in peanuts as evidenced by Waliyar et al. (2005a).

There was a significant association between membership to a PMG and incidence in all but two fungal species assessed. This was as expected because PMGs were established to assist farmers to strengthen their marketing abilities and to improve their profit margins, by improving both pre- and post-harvest handling practices (Mutegi et al., 2007). Therefore, it was expected that farmers who belonged to PMGs would embrace practices that improve peanut quality and safety through proper drying, grading and storage. Such practices have consistently been shown to reduce the level of contaminated peanuts (Gowda et al., 2002; Turner et al., 2005; Waliyar et al., 2008).

The most common type of toxin was Aflatoxin B_1 - the most potent of the aflatoxins (Stoloff et al., 1991). This can be explained in part by the high incidence of *A. flavus* S-strain in our

samples. Our data also corresponds well with results of other studies that have documented a similar predominance in Aflatoxin B_1 (Lisker et al., 1993; Awuah and Kpodo, 1996). It was observed that as the total aflatoxin levels increased, the incidence of Aflatoxin B_1 generally increased, which accords well with the findings of Horn and Dorner (1999), who found a positive correlation between Aflatoxin B_1 production and cyclopiazonic acid production, in both S- and L- strains of *A. flavus*.

Moreover, *A. parasiticus* was present in 22 per cent of all samples tested in this study. The presence of *A. parasiticus* would thus explain the presence of G_1 and G_2 aflatoxins in our peanut samples. Hill et al. (1985) recorded similar results, whereby peanut kernels showed 10 to 30 per cent contamination levels for *A. parasiticus*, unlike maize kernels that were almost exclusively infected by *A. flavus*. In addition to producing Aflatoxins G_1 and G_2, *A., parasiticus* is also capable of producing Aflatoxins B_1 and B_2. This could have contributed to the high proportions of the two aflatoxin types (Kurtzman et al., 1987; Egel et al., 1994).

The predominance of *A. flavus* in the samples indicates a high risk of aflatoxin contamination. The reason as to why *A. flavus* L-strain did not correlate positively with aflatoxin levels could have been due to its atoxigenic nature. The high incidence of *A. flavus* S-strain, which usually produces Aflatoxin B_1 and B_2, underscores the need for more vigilance and implementation of preventive measures that reduce the risk of aflatoxin accumulation in contaminated peanuts. The isolation of mixed cultures of fungi shows that it is likely that peanuts in western Kenya are contaminated with more than one type of mycotoxin. Further studies are required to determine if this is the case. Planting improved peanut cultivars, combined with good crop management and post-harvest handling practices is necessary to deter the proliferation of fungal species and reduce the risk of mycotoxins contamination.[7]

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CHAPTER SIX: REDUCING AFLATOXIN LEVELS IN PEANUTS: EVALUATION OF COMMON HOUSEHOLD PREPARATION TECHNIQUES USED IN WESTERN KENYA[8]

6.1   Introduction

Arachis hypogaea L. (peanut) is an important food crop of high nutritional value, widely used in food and in the production of confectionery. Peanuts are easily cultivated and require very few inputs under smallholder production systems. The nuts contain between 26 and 39 per cent protein, 47 to 59 per cent oil, and about 11 per cent carbohydrates (Nelson and Carlos, 1995; Atasie, Akinhanmi and Ojiodu, 2009). In several developing nations, peanut is used extensively in school feeding programs; therapeutic foods; weaning foods (South African National Monitoring Programme, 2004; Plahar, Okezie and Gyato, 2005); food aid supplies such as *Plumpy Nut* (Briend, 2009), a ready-to-use therapeutic food (RUTF) used by United Nations agencies in areas such as the Darfur region of Sudan and in Niger; and animal feed manufacture (Akano and Atanda, 1990; Offiah and Adesiyun, 2007).

 In Kenya, peanut is important as both a food and a cash crop (Agong, 2006). Common ways of consuming peanuts at the household level include roasting, making a peanut sauce (that is then mixed with vegetables or consumed as a side dish with starches) and boiling. Lower grade peanut is used as feed for poultry. At a commercial level, nuts are mainly sold raw, roasted or fried, and sold through middle men to cottage industries or large scale industries for making peanut-based snacks and confectioneries, or through informal markets to consumers.

Peanut is predisposed to aflatoxin contamination (Lisker, Michaeli and Frank, 1993; Dorner and Cole, 2002; Rachaputi, Wright and Kroschi, 2002; Sobolev, 2007) at both pre- and post-harvest stages (Asis et al., 2005; Waliyar et al., 2005). Developed countries manage contamination levels through strict monitoring and improved storage (Ito et al., 2001; McAlpin et al., 2002; Wilson et al., 2002). On the contrary, in many developing countries where production and utilization largely take place in small unregulated systems, contamination remains high, due to a number of factors: i) cultivation of unimproved landraces, which are associated with a higher incidence of contamination, ii) delayed

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[8] Paper in preparation for submission to Food Control Journal. Different format for referencing used due to journal requirements.

harvesting, iii) inadequate drying, iv) weak transportation systems, and v) poor storage conditions (Mutegi et al., 2007; Mutegi et al., 2009).

The impact of aflatoxin contamination is felt more in the developing countries, mainly affecting the health of the nation's population and reducing trade of contaminated products. The standards set by most developed nations for aflatoxin level limits are also difficult for producers in developing nations to attain, resulting in the rejection of agricultural products from developing nations in international markets. The rejected products are subsequently diverted to the local markets, exposing the local consumers to increased levels of aflatoxin exposure. Moreover, nuts considered as spoilt after sorting are usually not discarded, but find their way into the food chain at different stages, for example, for feeding poultry, or are sold in markets for consumption at lower prices. Therefore, an integrated approach to addressing aflatoxin contamination is required. In order to be effective and to be easily adopted, such approaches need to be affordable and to meet cultural preferences. Effective approaches can also be used as a platform to raise awareness on safe practices.

Making use of compounds that are already used by the local people during food preparation, to reduce levels of contamination in peanuts is one way of addressing the aflatoxin problem, keeping in mind people's cultures. For example, *magadi* (also referred to as *igata)*, an alkaline mineral salt used as a cooking aid mainly to soften foods, has been shown to reduce levels of aflatoxins (Mutungi et al. 2008). These authors found that the salt has a high ionic strength (5.6 x 10^2dS m^{-1}), as well as a composite nature. Alkaline media, typical of both *magadi* and local ash, enhance the opening of the lactone ring of aflatoxins, resulting in water-soluble β-keto acid derivatives (Parker and Melnick, 1966). Mutungi et al. (2008) showed that soaking *muthokoi* (dehulled maize) in *igata*, sodium hypochlorite or ammonium persulphate decreased aflatoxin levels by 28 to 72 per cent, while boiling *muthokoi* in *igata* decreased aflatoxin levels by over 80 per cent. Similarly, the alkaline nature of local ash made of peels from Irish potatoes, banana and beans, which is used to soften legumes, vegetables and grains during cooking could reduce levels of aflatoxins in peanuts during their preparation.

Research has also shown that some traditional food preparation processes such as sorting (Rheeder et al., 1992; Desjardins et al., 1998; Galvez et a., 2003) and washing (Rheeder et al., 1992; Desjardins et al., 1998) are effective in reducing aflatoxin levels in foodstuffs. The

80

three methods that are commonly used in western Kenya all involve sorting before actual cooking, while boiling involves a washing stage. Dehulling of maize during the study by Mutungi et al. (2008) was shown to reduce aflatoxins in maize by an average of 46.6 per cent. Soaking and discarding the soak water is also common during boiling. The purpose of this study was, therefore, to establish the effect of roasting, boiling and peanut sauce processing techniques on aflatoxin levels and to assess the effect of adding commonly available substances to boiled peanuts on aflatoxin levels and consumer acceptability.

6.2   Methodology

6.2.1   Sample collection

Extension staff collected samples from traders who also belonged to a women's group that marketed peanut products in the Nambale division of Busia district. The women's group was selectively sampled, based on the ease of accessing the group, even though individual traders within the group were randomly selected using their register, by assigning each member a number and randomizing these in Excel$^®$. The samples were collected a day before the experimental work was conducted, whereby a 5 kg sample of peanuts was purchased from each trader. The sample was drawn using a metallic grain sampler and portions drawn from different parts of the sack. Samples were also collected from a processing company (hereafter referred to as Company A for confidentiality reasons) and from the Gikomba market in Nairobi, which is a formal market with defined stalls. Samples from Company A were randomly selected at the factory premises, while samples from the market were bought from randomly selected vendors, with the vendors allowing us to sample from different parts of the sacks using a grain sampler. Samples from the women's group were used in field and lab experiments while samples from Company A and the Gikomba market were used only in the laboratory experiment.

6.2.2   Moisture determination

 Initial moisture content of samples was determined using the oven drying method. Samples were first ground using a kitchen blender (Senator mixer grinder, Amargum Overseas PVT

Ltd, India) for one minute. Ten grams of ground sample were placed on aluminium foil, and placed in an oven (Memmert ULM 500 Schutzart oven, Schwabach-Frg, Germany). The samples were dried at 105 ^{0}C overnight and the net weight of the dried sample was determined after drying. The moisture content was calculated as a difference between final and original weight divided by original weight of sample, and multiplied by 100 to give the percentage of moisture. Each sample was replicated three times.

6.2.3   Effect of various practices applied during three peanut preparation methods performed under field conditions, on aflatoxin levels

A previous survey by Mutegi et al. (2007) found that roasting, boiling and preparation of peanut paste are the most common household preparation methods of peanuts. This experiment was to document the progression of aflatoxin levels at each stage of the three processing methods, without any control on parameters, i.e., as they would have been prepared by each individual in their homesteads. Samples were prepared using these three techniques by the ten farmers randomly selected from the women's group from the Nambale division of Busia district. Although every effort was made to reproduce typical household settings, the exercise was executed at a centralized place for logistical reasons, as well as to dispatch experimental instructions easily to farmers. As a first step in this experiment, the women were asked to sort what they considered unfit for human consumption from a lot of 5kgs sample. The discarded produce was stored separately and transported in cooler boxes to Nairobi for analysis, to determine the levels of aflatoxins. Thereafter, 500g of produce was sampled from the clean nuts and re-sorted into peanuts for home consumption and what was considered as good quality seed for planting. Peanuts identified for home consumption (used in the subsequent stages of preparing the three different types of peanut products) were then roasted for 9 to 15 minutes (the exact duration was determined by each farmer's prior experience) at 110 to 150 ^{0}C (determined by placing a thermometer in a lot of roasting nuts), and half of the roasted sample was dehusked. The nuts were salted during roasting by sprinkling them with a salt solution (salt is usually applied in water solution to ensure uniform distribution on the nuts), prepared by dissolving 3.1 to 6.04 g of salt in 10 to 35 mℓ water, depending on each farmer. Aflatoxin levels were determined as described in section 6.2.6, for each of the samples of nuts identified for seed or home consumption (roasted and dehusked nuts).

In preparing the peanut paste, the ten farmers used another sample of about 500g each, and again sorted it for seed and for home consumption. The sample for home consumption was pounded using a mortar and pestle, mixed with 200 to 500 mℓ of water and placed on a *jiko* (a locally made charcoal-burning stove commonly used by middle and low income families in Kenya), and stirred until it was cooked. The final product had the consistency of porridge. Again, depending on the individual farmer, the cooking time ranged from 8 to 16 minutes at temperatures of between 72 ^{0}C and 94 ^{0}C. The pH of the water used ranged from 6.36 to 6.9. Salt was added towards the end of cooking (between 2.5 and 6.2 g). The aflatoxin levels of the nuts classified as intended for seed, home consumption, and cooked samples were determined as described in section 6.2.6. The volume of water added by each farmer, the time taken to cook, the amount of salt, and the pH of water used for cooking the peanut paste were recorded.

Boiled peanuts were prepared by first soaking about 500 g of sample in water overnight. The water was poured out and the peanuts were rinsed with clean water. The nuts were then boiled and allowed to simmer for 35 to 54 minutes until cooked. The pH of the water used to clean the soaked peanuts ranged from 5.15 to 6.21, while the pH of water after completion of boiling was 6.45 to 7.21. Completion of the cooking process was subjectively determined by the participants by placing a nut between the index finger and thumb and pressing it, and assessing the ease with which it crumbled. Aflatoxin levels of soaked as well as boiled peanuts were determined as described in section 6.2.6. The amount of water added, the pH and the temperature of the water during boiling were recorded. Ten replicates of each treatment were made.

6.2.4   Effect of various stages in the three peanut preparation methods performed under controlled laboratory conditions on aflatoxin levels

 Samples from two different sources were used for the laboratory tests. Ten two kilogram samples of sorted peanuts were purchased from women traders in the Nambale division of Busia district. Samples of spoilt peanuts, sold at a lower price for poultry feeding, were also obtained for testing. A second sample was sourced from a peanut processor, Company A. This sample contained peanuts that were not suitable for sale as whole nuts, but which are

used for making peanut butter. These samples were stored for 5 months without proper ventilation to accelerate aflatoxin contamination. Using the results obtained from the field experiment as a guide, optimum conditions for boiling, roasting and peanut sauce preparation were determined by conducting preliminaries in the laboratory. These conditions were used to prepare samples using the three methods (roasting, boiling and making peanut paste). For roasting 250 g of nuts were heated to 110 ^{0}C for eight minutes on a frying pan, on an electric stove. Thirty millilitres of salt solution (prepared by dissolving 15 g of common salt in 250 mℓ of distilled water) was sprinkled on the nuts during the roasting process. For the boiling treatment, 250 g of nuts were boiled in a sauce pan containing one litre of tap water. The pH of water used to wash nuts before boiling was 5.53 to 6.51. The pH of water after boiling the samples ranged from 6.45 to 7.21. The peanuts were boiled at a temperature of 92 ^{0}C for 50 minutes each. The temperature was maintained by regulating the heat from the stove. The peanut paste was prepared by cooking 500 g of ground peanuts in 500 mℓ of water (with a pH of 8.93) at 90 ^{0}C for 8 minutes. Three grams of salt were added towards the end of cooking. Ten replicates of each preparation method were made and levels of aflatoxins were determined for each replicate sample before and after the preparation.

6. 2.5   Effect of boiling of peanuts in locally available softening salts on levels of aflatoxins

 To assess the effects of locally available salts on levels of aflatoxins, an experiment was conducted, which involved a modification of the soaking and boiling stages as described above. The treatments involved soaking produce overnight in ammonium persulphate (2 per cent), or sodium hypochlorite (1 per cent) or 10 g/ℓ *magadi.* Plain water was used as a control.

The soaked nuts were subsequently boiled for 50 minutes at 92 ^{0}C in 2.5 g/ℓ of *magadi*, 50 mℓ/ℓ of locally prepared ash or 10 g/ℓ of baking powder (sodium bicarbonate), with plain water being used as a control. Thirteen samples were used, and the experiment was replicated three times, with samples from three different sources, namely, the Nambale division of Busia district, the Gikomba market and Company A. The ash was prepared by first drying 2 kgs peels from beans, 3 kgs of banana peels and 0.5 kg of Irish potato peels, the three crop residues that are locally used to prepare ash for softening food. The proportions taken were based on what was used in various households during their preparation as a

cooking aid. The dry peels were then burnt using a *jiko*. The ash was then sprinkled with 200 mℓ water and put out in the sun again to dry. The dry ash was poured into a half kg plastic tin that had minute perforations at the bottom, and the ash pressed firmly in the tin until it was half full. Water was added and allowed to percolate slowly through the ash overnight. The filtrate was collected in a plastic bottle, sealed and stored in a dark place. It was used at a concentration of 50 mℓ/ℓ of water, which is approximately the concentration used in softening the food by the local people. Moisture levels of initial samples were also recorded. Aflatoxin levels were determined prior to soaking, after soaking and after boiling.

6.2.6 Aflatoxin analysis

A 200 g sub-sample was drawn from each sample and ground into a fine powder or blended into a fine paste depending on the initial state of the sample, using a kitchen grinder (Kanchan Multipurpose Kitchen Machine, Kanchan International Limited, Mumbai, India). The ground sample was then sub-divided into two equal portions of 100 g each. The powder was triturated in 70 per cent v/v methanol (70 ml absolute methanol in 30 ml distilled water) containing 0.5 per cent w/v potassium chloride in a blender, until thoroughly mixed. The extract was transferred to a conical flask and shaken for 30 min at 300 rpm. The extract was then filtered through Whatman No.41 filter paper and diluted 1:10 in phosphate buffered saline containing 500 µl/L Tween-20 (PBS-Tween) and analyzed for aflatoxin with an indirect competitive ELISA (Waliyar et al. 2005*b*) by preparing an aflatoxin-bovine serum albumin conjugate in carbonate coating buffer at 100 ng/mℓ concentration and dispensing 150 µl in each well of the Nunc-Maxisorp® ELISA plates[9]. Absorbance was measured at 405 nm in an ELISA plate reader (Multiskan Plus, Labsystems Company, Helsinki, Finland).

6.2.7 Sensory evaluation

Peanuts of the same variety, harvested at the same time and from the same vendor were purchased from the Kawangware market. All nuts considered to be spoilt were first removed and the clean nuts sub-divided into four lots. The peanut were soaked overnight in clean, lukewarm water after which the water was drained off. Each lot was then boiled either in

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[9] Nunc A/S, Kamstrupvej 90, P.O.Box 280, DK-4000, Roskilde, Denmark

magadi, baking powder, local ash, salt or plain water at the concentrations stated above, for a period of about 50 minutes. The nuts were allowed to cool to a warm temperature, before being served to 30 trained panellists from different backgrounds that included lab technicians, scientists, security guards, cleaners, and office colleagues. Treated peanuts were presented simultaneously to each panellist, labelled as KMN (boiled in *magadi*), KNM (boiled in baking powder), NKM (boiled in ash), MNK (boiled in plain water) and MKN (boiled in water and salt). The samples were presented in identical disposable plastic plates and coded in 3-digit numbers to eliminate bias. Each panellist was asked to rank the four samples using an ordinal scale (Watts, Ylimaki, Jeffery and Elias, 1989), for flavour, appearance, texture, and overall acceptability. The most preferred sample was ranked "1" while the least preferred was ranked as "5". The panellists were also asked to give any additional comments to support their scores.

6.2.8   Statistical analyses

To study the effects of different processing techniques on changes in levels of aflatoxin at various stages of processing, values denoting actual aflatoxin levels were subjected to a natural logarithm transformation to stabilize the variance. The preparation stages analyzed were:

1. initial stage (i.e., levels of aflatoxin in the samples prior to the nuts being subjected to any sorting or preparation method), sorting for home consumption, roasting and dehusking stages for roasting technique

2. initial stage, sorting for home consumption, washing and boiling for the boiling technique; and

3. initial stage, sorting for home consumption and cooking of peanut paste stages for peanut paste processing technique.

The differences in aflatoxin levels between one stage and another were presented as percentages. To determine if there was any significant difference between two different stages, sources, or methods, a repeated measures ANOVA was performed, and significance determined at a 5 per cent confidence level. Effects of soaking and boiling produce in different compounds on the aflatoxin levels was determined by first converting aflatoxin levels at initial, soaking and boiling stages into their natural logs. The

differences in aflatoxin level at each stage were recorded as percentage differences of the log. Data on each attribute assessed in the sensory evaluation experiment was ranked for each sample and a total rank obtained. Least significant differences were used to compare summative rankings for each sample at the 5 per cent confidence level. Comparisons were made in relation to the sample boiled in water and salt. All analyses were performed, using Genstat (Discovery Edition, copyright 2007, Lawes Agricultural Trust Rothamstead Experimental Station).

6.3   Results and discussions

6.3.1   Results for the sorted peanuts

Of all the ten samples of spoilt peanuts, 80 per cent had moisture levels below 8 per cent, while only two samples had moisture content of slightly over 8 per cent (8.92 and 8.37 per cent). The levels of aflatoxin in the spoilt samples were high ranging from 15.93 to 6762.81µg/kg (Table 6.1). Fifty percent of the nuts discarded by the traders had levels of aflatoxin greater than 4000 µg/kg, while 30 per cent had over 200µg/kg, with one sample having 76.7 µg/kg.

**Table 6.1: Aflatoxin content of discarded nuts from ten traders
in Busia district**

Sample no	Moisture content (%)	Aflatoxin level (µg/kg)
Farmer 1	7.08	6762.81
Farmer 2	6.90	76.69
Farmer 3	7.13	4613.35
Farmer 4	8.37	8502.24
Farmer 5	6.66	4126.50
Farmer 6	8.92	4968.74
Farmer 7	7.01	15.93
Farmer 8	7.24	277.45
Farmer 9	7.10	508.25
Farmer 10	6.93	926.70
Range	2.26	8486.31

Only one farmer's produce had aflatoxin levels less than 20 µg/kg. The high levels of aflatoxin observed in the discarded component of the samples were within the ranges that were reported previously from different samples obtained in the region (Mutegi et al. 2009). In that study, samples were analyzed prior to sorting and it is likely those with high levels of aflatoxin would have been discarded after sorting.

Based on a pair-wise T-test of 17 samples, there were no differences (P=0.135) in levels of contamination between samples identified as suitable for home consumption and seed, with some of the seed sample having higher aflatoxin levels compared to home consumption samples, even though participants classified nuts sorted for seed as having the best quality. For example, after sorting, Sample 17 had aflatoxin levels of 3.7 µg/kg in the nuts intended for home consumption, compared to 9.3 µg/kg in the nuts destined for seed use (Table 6.2). The moisture content of all but one sample of nuts that excluded spoilt produce was below 6.5 per cent (Table 6.2). These results accord with those of Galvez et al. (2003) who also showed that sorting can substantially reduce levels of aflatoxin in contaminated nuts, even when initial levels are very high. The results also indicated that whereas sorting can substantially reduce the risk of exposure to aflatoxin, its effectiveness cannot be solely relied upon as peanuts that look physically clean could still be contaminated. Moreover, the subjectivity of the person sorting could lead to disparities in sorting, necessitating the need to combine sorting with other safety practices.

Despite participants' awareness of the poor quality of discarded nuts, such nuts eventually find their way into products in the food chain. For example, discarded nuts are usually sold at a lower price as poultry feed or given to poultry at home, or sold to cottage industries for making peanut butter. This can lead to aflatoxin poisoning since aflatoxin has been shown to accumulate in animal products (El-Gohary, 1996; El-Sayed et al., 2000) as well as to contaminate peanut butter (Omer et al., 1998; Shephard, 2003). The seed category is usually considered the cleanest and hence all peanuts of a lower standard than seed quality is used to prepare food at home rather than for planting. However, it is clear that sorting does not guarantee the consumer of a safe product.

Table 6.2: Aflatoxin and moisture content of sorted peanut samples[x] collected from members of a farmer group in Busia district, Kenya

Sample No	Moisture content (%)	Aflatoxin content of samples destined for home consumption (µg/kg)	Aflatoxin content of samples intended for planting (µg/kg)
1	6.2	4050	3.4
2	6	26.3	3.7
3	6.2	4050	4.6
4	6.1	1.7	4.7
5	6.1	1.5	5.8
6	6.1	10.3	5.2
7	6.1	4050	4.3
8	6.1	6.7	4.3
9	6.2	5.7	6
10	6.2	6.2	4.8
11	9.4	6.6	3.7
12	6.4	4.1	3.7
13	6.2	3.7	4.3
14	6.2	6.2	3.7
15	6.3	3.7	11.1
16	6.3	8.9	4.7
17	6	3.7	9.3
18	6	8	3.7
19	6	8.5	3.7
20	6	5	3.7

[x]Samples analyzed were those used for roasting and preparation of peanut paste. Sample 1, 2 and 7 were restricted during analysis to avoid their leverage on the output. SE of the mean=1.580.

6.3.2   Aflatoxin levels at different stages of processing

The changes in aflatoxin content were more notable in samples with initially high levels of aflatoxin. For example, for the boiling experiment, the peanut sample from Company A

which had initially averaged 675.4 µg/kg (SE=1.23) aflatoxin declined by 38.7 per cent after sorting compared with 17.8 per cent decline ($P<0.001$) from peanuts from the field source that had lower initial levels of aflatoxin that averaged 12.0 (SE=1.23). Previous studies have shown a similar trend, whereby samples with initially higher aflatoxin levels had resulted in larger declines of aflatoxin compared to those with less. Tabata et al. (1994) obtained a 66 per cent reduction in aflatoxin concentration in maize samples treated with 1.0 per cent w/v ammonium persulphate compared with over 90 per cent degradation of pure aflatoxins treated with 1 per cent w/v ammonium persulphate at 20 ^{0}C. Lopez-Garcia and Park (1998) also suggested that aflatoxin distribution in maize fractions during processing may be influenced by contamination levels. Fondahan et al. (2005) also found no aflatoxin in discarded hulls and embryo in the preparation of *mawe* (a solid-state fermented dough used in Benin, Togo and Nigeria for cooking several dishes-Hounhouigan et al., 1993) from maize, as the aflatoxin levels were already so low in the cleaned initial sample.

In the field experiment where peanut preparation procedures were not controlled, there was a significant decline in levels of aflatoxin at various stages of processing, within the same method ($P = 0.043$; LSD 1.331; Table 6.3). For example, washing of nuts reduced the aflatoxin levels by an average of 36.2 percent, while sorting of peanuts for peanut paste processing reduced aflatoxin levels by 86.9 percent from the initial peanuts. Reduction of aflatoxin levels resulting from sorting has been observed in other studies (Rheeder et al., 1992; Desjardins et al., 1998). As the sorting is done manually by hand, its success in reducing aflatoxin levels is subjective. Fondahan et al. (2005) noted that hand sorting of visibly mouldy grains with the aim of reducing mycotoxin levels was likely to depend on the ability of the people responsible for this activity.

There was no difference ($P = 0.213$) in levels of aflatoxin due to source of nuts over the different stages within each method (boiling, preparation of peanut sauce and roasting) in the lab experiment. This implied that the change in aflatoxin levels for peanuts from the farmers in Nambale and those from Company A were similar. This observation enabled us to combine the results from the two sources of peanuts within the laboratory experiment and look at the means at each processing stage for each method. There was a general decline in the levels of aflatoxin during processing, but this was not always significant.

Table 6.3:. Log means[x] of aflatoxin levels at different stages of peanut processing in the field

Peanut preparation stages	Boiling	Peanut paste making	Roasting
Initial	3.28a,b	2.06a	1.91a
Sorting for home consumption	4.09b	3.85b	1.75a
Washing	2.61a	-----	-----
Boiling	5.24c	-----	-----
Peanut paste making	-----	2.80ab	-----
Roasting	-----	-----	1.73a
Dehusking	-----	-----	2.05a

[x]LSD between means within the same method = 1.331

In the boiling experiment, aflatoxin levels decreased after sorting by 32.1 per cent, while after washing, the aflatoxin content reduced by 37.2 per cent. Percentage decline of levels of aflatoxin because of boiling was 20.9 per cent. There was, however, no significant decline in aflatoxin levels between the washing and boiling stages (Table 6.4).

Table 6.4:. Log means[x] of aflatoxin levels at different stages of peanut processing in the laboratory

Peanut preparation stages	Boiling	Peanut paste making	Roasting
Initial	4.70c	4.06b	3.65b
Sorting for home consumption	3.19b	3.31b	3.44b
Washing	2.00a	-----	-----
Boiling	2.25a	-----	-----
Peanut paste making	-----	2.096a	-----
Roasting	-----	-----	3.84b
Dehusking	-----	-----	2.46a

[x]LSD between means within the same method and with combined sources =0.8686

During peanut paste processing, there was a decline of 57.8 per cent in aflatoxin levels between the nuts sorted for home consumption and the cooked paste. There was an 11.9 per cent decline in levels of aflatoxin because of roasting, while dehusking peanuts after roasting reduced levels of aflatoxin by 35.9 per cent over the roasted nuts. The study by Njapau et al. (1997) also showed a substantial decline in roasted peanuts. Overall, the percentage decline between sorted nuts for home consumption and boiled nuts, peanut paste or dehulled nuts was 46.7, 62.5 and 39.7 per cent respectively. This suggests that these three methods are viable processes for reducing aflatoxin levels in peanuts. In particular, this study showed that the sorting performed in the three processes was very effective in reducing aflatoxin levels. Such effectiveness has been illustrated in past studies, including that of Fondahan et al. (2005), who found that practices such as sorting, winnowing and washing reduced mycotoxins by up to 91 per cent, during preparation of maize products using traditional methods. The effectiveness of washing produce before cooking has been illustrated by Shetty and Bhat (1999), who found that 74 per cent of fumonisins were removed by washing maize grains, immersing them in water and removing the upper fraction. The mechanism behind reducing aflatoxin levels during washing and sorting is the physical removal of spoilt nuts during sorting, while the wash water during washing process is bound to carry along aflatoxins that are not bound.

Dehusking peanuts is practiced by some people locally before eating roasted peanuts, even though it is not common practice. In this study, dehusking reduced levels of aflatoxins. Its effectiveness in reducing aflatoxin levels implies that it could be encouraged as a practice during consumption of roasted peanuts. The reductions in levels of aflatoxins recorded in our study because of dehusking match those of Mutungi et al. (2008) who recorded an average decline of 46.6 per cent in aflatoxin levels of dehulled maize, during the preparation of *muthokoi*. However, Siwela et al. (2005) noted a 92 per cent decrease in aflatoxin levels of dehulled maize meal. It is important to note that dehulling does not eliminate all aflatoxin. This can be explained by the fact that although surface colonization of seeds by aflatoxin producing fungi is predominant, the fungi are also capable of penetrating the seed (Lopez and Christensen, 1967). Aflatoxins have been found to be relatively heat stable (Alberts et al., 1990; Sinha, 1998), and this could explain why the greatest reductions in aflatoxin levels occurred during the stages prior to actual cooking. Inconsistency in sorting and preparation processes between traders during the field experiment could explain inconsistency in some of the results, as evidenced in Table 6.2.

6.3.3   Effect of soaking and boiling peanuts using various salts

Soaking produce in different solutions significantly reduced ($P = 0.027$; LSD=17.88) levels of aflatoxin, regardless of the solution used. Peanuts soaked in water, *magadi*, NaOCl, and ammonium persulphate recorded percentage declines of 27.7, 18.4, 18.3, and 1.6 per cent, respectively (Figure 6.1).
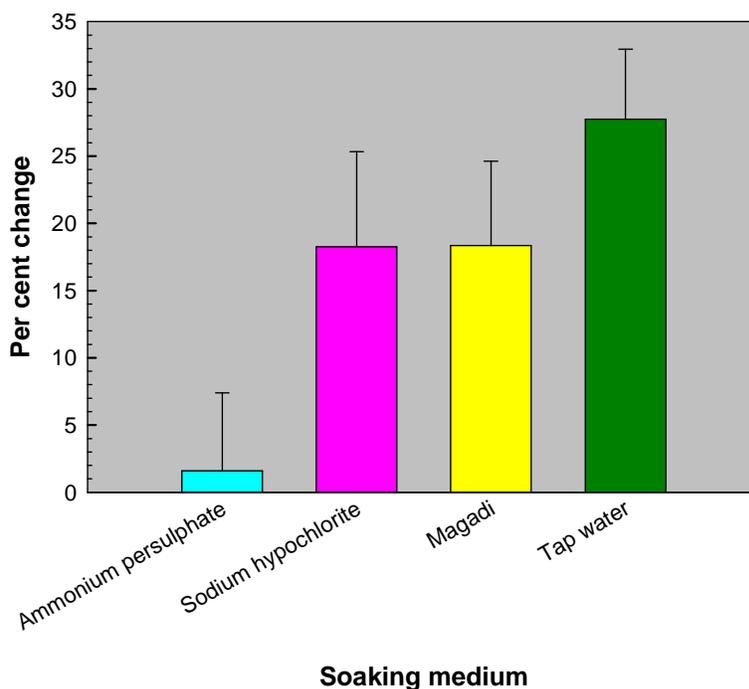
Figure 6.1:. Percentage overall means for reduction levels of aflatoxin in peanuts soaked in different salt solutions (LSD=17.88; n=39).

The action of soaking peanuts in water and throwing away the wash water before boiling is therefore an important stage in preparation of boiled peanuts. Alternatively soaking the peanuts in *magadi* is also effective as shown above. A significant difference ($P < 0.001$) was also observed with regard to where the peanuts came from (Table 6.5).   For example, percentage decline of aflatoxin levels in peanuts soaked in ammonium persulphate was 15.5 per cent, 7.8 per cent and -18.2 per cent for samples obtained from Company A, Busia and

Gikomba markets, respectively. Samples from Gikomba had the lowest initial levels of aflatoxin, with only two samples having more than 20 µg/kg. Soaking of produce in water reduced levels of aflatoxin as shown in the study by Mutungi et al. (2008). This could be explained by the fact that aflatoxins are relatively soluble in water (Cole and Cox, 1981), and could have been washed out with the soaking water. However, the decline in aflatoxin levels in peanuts soaked in ammonium persulphate were not as high as those noted by Mutungi et al. (2008), who assessed the effect of ammonium persulphate on the decontamination process of naturally contaminated maize, by soaking it in 0.5 per cent ammonium persulphate for 6 or 14 hours, at 25 ^{0}C. Soaking peanuts in sodium hypochlorite and *magadi* significantly reduced aflatoxin levels, even though the percentage decline in maize was higher than what we recorded (Mutungi et al. 2008). Significant losses of aflatoxin after soaking in *magadi* solution has been shown to occur over prolonged soaking periods (over 6 hours) and has been attributed to a slow hydrolysis of hemicelluloses in the grain pericarp by sodium bicarbonate, a major component of *magadi* (Mutungi et al. 2008).

Boiling the peanuts in *magadi,* local ash, baking powder and water reduced levels of aflatoxin in the peanuts by 43.8 per cent, 41.8 per cent, 28.9 per cent and 11.7 per cent, respectively, relative to the initial sample (Figure 6.2).
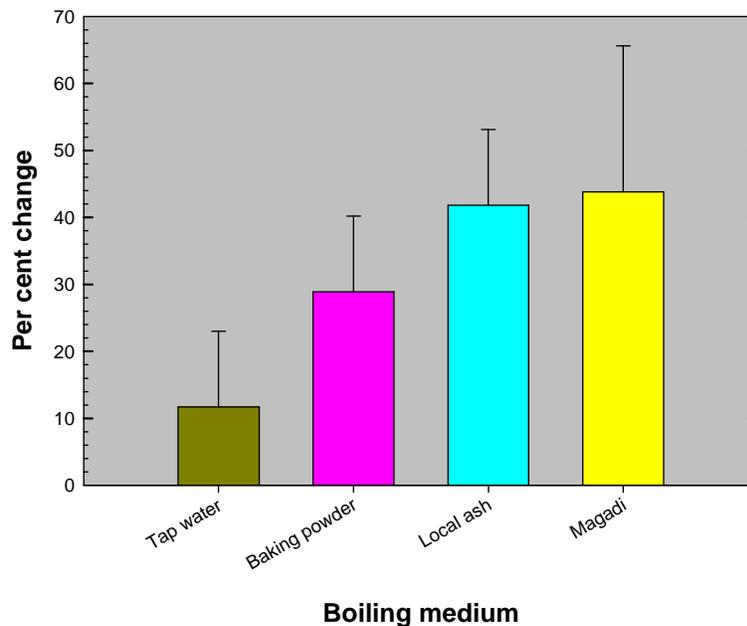

Figure 6.2:   Means for percentage changes in aflatoxin levels of boiled peanuts (LSD=41.80; n=39)

However, there was however, no significant difference in per cent reduction of aflatoxin because of boiling the peanuts in any of the three solutions tested implying that all the boiling media were effective. Again, the largest percentage decline was noted for samples that originated from Company A, which also had the highest initial aflatoxin levels (Table 6.5). For example, samples from Company A resulted in a decline in aflatoxin levels of 52.4, 48.3, 29.6 and 13.4 per cent when the nuts were boiled in locally prepared ash, baking powder, *magadi* and plain water, respectively (Table 6.5). Use of *magadi* also led to the highest percentage decline in levels of aflatoxin from various sources (76.1 per cent, 33.2 per cent and 29.6 per cent drop in levels in samples from Busia, Gikomba and Company A, respectively). The results above therefore show that boiling peanuts in *magadi*, local ash or baking powder are all effective in reducing aflatoxin levels in boiled peanuts. Their effectiveness can be attributed to their alkaline nature (Parker and Melnick, 1966; Mutungi et al. 2008).

Table 6.5: Means of changes in levels of aflatoxin in soaked[x] and boiled[y] peanuts from three sources

Source	Busia	Gikomba	Company A
Soaking medium			
Ammonium persulphate	7.79 ± 9.98	-18.2 ± 9.98	15.15 ± 9.98
Igata	3.46 ± 17.28	13.49 ± 9.98	34.28 ± 9.98
Sodium hypochlorite	40.81 ± 9.98	5.91 ± 12.22	12.32 ± 9.98
Water	89.98 ± 9.98	-20.25 ± 8.64	24.15 ± 8.64
Boiling medium			
Local ash	33.7 ± 21.6	37.4 ± 18.7	52.4 ± 18.7
Baking powder	29.9 ± 21.6	8.8 ± 18.7	48.3 ± 18.7
Igata	76.1 ± 21.6	33.2 ± 18.7	29.6 ± 18.7
Tap water	0 ± 37.5	37.5 ± 13.4	13.4 ± 37.5

[x]LSD=31.16; n=39, for comparing means within the same column due to the soaking medium

[y]LSD=72; n=39, for comparing means within the same column of boiling medium

6.3.4   Sensory evaluation of peanut samples cooked in different media

The tabulated critical value for 32 panellists and 5 samples at $P = 0.05$ (Watts et al. 1989) was 35. With regard to taste, only boiling in plain water gave a significantly different taste as compared to boiling in *magadi*, baking powder, local ash or table salt, and was the least preferred sample (Table 6.6). The most preferred peanuts were those boiled in *magadi* and salted water. Peanuts that were boiled in *magadi* were the most preferred with regard to colour, with the least preferred being those boiled in plain water. Samples boiled in *magadi*, salted water and baking powder were ranked as having significantly better colour compared to those boiled in water (Table 6.6).

Table 6.6: Tabulated sums of ranking[x] for acceptance test for peanuts[y] boiled in various treatments shown to reduce levels of aflatoxin

Sample boiling treatment	Taste	Colour	Texture	Overall acceptability
Peanuts boiled in plain water	119	129	106	110
Peanuts boiled in igata	85	67	79	79
Peanuts boiled in baking powder	93	86	108	98
Peanuts boiled in local ash	102	104	95	114
Peanuts boiled in salt water	81	92	93	79

[x]Tabulated critical value at $P=0.05$ for 32 panellists and 5 samples is 35

[y]Only safe peanuts purchased from the local store were used in this test

With regard to texture, no significant difference was noted by the panellists between samples. With regard to overall acceptability, panellists ranked peanuts boiled in *magadi* as highly as peanuts boiled in salted water (Table 6.6). The least preferred peanuts were those boiled in plain water or in local ash. The results show that peanuts boiled in *magadi* are as acceptable as peanuts boiled in salt and water. The fact that peanuts soaked in various salts scored equally or better with regard to various attributes compared to the conventional boiling means adoption would not be hindered. In addition to adding flavour to the peanuts, the compounds also double up as softening agents, saving on cooking time. They have an economic

advantage of being readily available and affordable, with no cost for ash and only about $0.8 dollars/kg of *magadi.*

6.4 Conclusions

The study investigated the effectiveness of boiling, peanut paste preparation and roasting of peanuts in reducing levels of aflatoxin. The results showed that sorting, washing, dehusking, that are preparation stages are effective in reducing aflatoxin in the peanuts. Roasting peanuts before grinding in the preparation of peanut paste can significantly reduce aflatoxin levels.

Boiling peanuts in locally available salts was found to be effective in reducing aflatoxin. In addition to reducing aflatoxin levels in the peanuts, boiling in *magadi* also enhanced the sensory attributes of the boiled peanuts. The multipurpose nature of *magadi,* local ash and even baking powder as both softening agents as well as compounds that reduce aflatoxin in peanuts makes their adoption easy. During this study, specific concentrations of ammonium persulphate, sodium hypochlorite and *magadi* were used to soak peanuts for a specific time. As further investigations, various concentrations of these compounds should be tested over various times to see if their effect on reducing aflatoxins could be optimized.

Further investigation on the ionic composition of locally prepared ash to determine its ionic strength is recommended. Overall, the study recommends further investigations on traditional processing of peanuts in reducing their aflatoxin levels. The study also recommends further exploration of both *magadi* and local ash in boiling of peanuts, as they are easily available, affordable, easily prepared and have a multipurpose use.

Peanuts given to poultry at household level were highly contaminated, and in spite of the success of sorting in reducing aflatoxin in peanuts for home consumption, it does not guarantee a safe product. The continuous need to raise awareness through extension staff on quality and safety aspects of peanuts, and explore decontamination options for already contaminated nuts cannot be over emphasized. This ought to be done bearing in mind that the

safety standards in the country are now stricter, with safe limits in aflatoxin levels being reduced to 10 µg/kg compared to the previous limit of 20 µg/kg[10].

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CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

Food safety is key to achieving food security. The importance of peanuts in food security is underscored by its high nutritive value and importance as a cash crop. However, its value is threatened by its susceptibility to aflatoxin contamination. Despite the crop's significance in the diets of people in western Kenya, the extent of aflatoxin contamination in peanuts in the region is not documented.

Therefore, this study set out to determine the quality of peanuts with regard to aflatoxin levels and *Aspergillus* section *Flavi* contamination from households in western Kenya. Aflatoxin levels in samples were quantified and factors associated with the levels were investigated. Fungi in the *Aspergillus* section *Flavi* group were also assessed. Other fungi isolated from the samples included *Penicillium* spp. and *Rhizopus* spp. Aflatoxin B_1, B_2, G_1 and G_2 were identified as these types have direct impact on the potency of aflatoxin contamination. Lastly, household peanut preparation techniques were assessed for their effectiveness in reducing levels of aflatoxin in the peanuts.

To achieve these objectives, aflatoxin analysis was performed by indirect competitive ELISA method. This method had the advantage of allowing analysis of numerous samples. Information on pre- and post-harvest peanut practices was collected by using personal interviews. The protocols for identifying various fungi are well documented and recognised. Protocols were drawn from existing methods. These included using Modified Dichloran Rose Bengal (MDRB) agar for isolation of *Aspergillus* section *Flavi*, *Penicillium* and *Rhizopus* spp. and identification of specific fungal species with Czapek yeast extract Agar (CYA). Screening isolates for aflatoxin production by *A. flavus* and *A. parasiticus* was done in high sucrose yeast extract (YES) liquid medium, and the aflatoxin types identified on TLC plates, using analytical grade standards of aflatoxin B_1, B_2, G_1 and G_2.

Typical household preparation methods for peanuts were selected and conducted under controlled conditions in the laboratory to explore if common practices controlled contamination.

The findings showed that 7.54 per cent of samples surpassed the KEBS tolerance limits. However, the range of aflatoxin levels varied considerably among samples, and was between zero and 2,687.6 µg/kg in Busia and zero and 7,525 µg/kg in Homa bay. Busia District had a larger area that was covered by the wetter Lower Midland 1 and Lower Midland 2 zones, compared to Homa bay District that had a larger area covered by the drier Lower Midland 3 agro-ecological zone. There was a significant (χ^2=11.983; P=0.0005) association between AEZ and aflatoxin levels, with the percentage of contaminated samples decreasing with lower precipitation. Thus, the drier Lower Midland 3 had more samples with lower aflatoxin levels compared to the wetter Lower Midland 1 and Lower Midland 2 agro-ecological zones. It was not surprising therefore, that samples from Busia had significantly higher levels of aflatoxin compared to those from Homa bay. While about 10.70 per cent of samples from the Busia District had aflatoxin levels greater than 20 µg/kg, only 4.09 per cent of samples from Homa bay fell into this category.

There was a significant (χ^2=9.748; P=0.0018) association between levels of aflatoxin and cultivar, with improved cultivars showing lower contamination in samples compared to local cultivars. Samples from farmers who experienced problems with moles in their peanut fields had significantly higher levels of aflatoxin because of pod damage.

The predominant fungi in the peanuts from either district were *A. flavus* L-strain, *A. flavus* S-strain and *A. niger*. There was a significantly higher incidence of *Rhizopus* spp. and *Penicillium* spp. in samples from Busia compared to samples from the Homa bay District. Total aflatoxin levels were also found to be significantly correlated with the colony counts of *A. flavus* S-strain, with aflatoxin levels increasing with increase in colony counts of *A. flavus* S-strain. A higher per cent of *A. flavus* S-strain was isolated from samples that had higher aflatoxin levels. Therefore, samples with less than 4 µg/kg aflatoxin had lower isolates of *A. flavus* S-strain compared to samples with more than 20 µg/kg aflatoxin content.

Improved cultivars showed a significantly lower incidence of *A. flavus* L-strain, *A. niger* and *Rhizopus* spp. compared to local cultivars, while grading as a post-harvest practice significantly reduced the levels of *A. flavus* S- and L-strains. Higher incidences of *A. flavus* S- and L-strains, *A. niger* and *Rhizopus* spp. were recorded in samples from farmers who did

not belong to a PMG. Aflatoxin B_1, B_2, G_1 and G_2 were found in isolates of *A. flavus* and *A. parasiticus*, with the most common being Aflatoxin B_1.

Rejected peanuts that are fed to poultry had high levels of aflatoxins (ranged from 15.93 to over 6000 µg/kg). There were no significant differences in aflatoxin levels between what the farmers considered peanut for seed and peanut for household consumption, even though the farmers considered peanut for seed to be less contaminated with aflatoxin than that for household consumption.

Changes in aflatoxin levels at different stages of cooking were more notable in samples that had initially high levels of aflatoxin. Sorting and washing peanuts significantly reduced levels of aflatoxin. De-husking nuts after roasting also reduced aflatoxin levels. Overall, peanut paste, cooking, roasting and boiling of peanuts all led to declines in aflatoxin levels, albeit at different rates. Soaking peanuts before boiling in either water, *magadi*, NaOCl or ammonium persulphate all lead to declines in aflatoxin levels in the peanuts, with soaking in *magadi* being as effective as the conventional soaking in water. Boiling peanuts in *magadi*, local ash and baking powder (sodium bicarbonate) were all equally effective in reducing aflatoxin levels. Peanuts boiled in *magadi* had the best taste and compared well with the conventional peanuts boiled in water and salt. There was no difference in appearance between peanuts boiled in *magadi*, salt or baking powder, and respondents reported that these peanuts tasted better than peanuts boiled in plain water. Overall, the most preferred peanuts were those boiled in *magadi* and conventional salt.

7.1   Conclusions

In conclusion, the level of aflatoxin varied considerably among the samples but the majority of samples had aflatoxin levels within the Kenya Bureau of Standards and European Union tolerance levels for total aflatoxins. However, the incidence of aflatoxin producing fungi was high including contamination by *A. flavus* S- and L-strain, and *A. niger. Rhizopus* spp. and *Penicillium* spp. were also prevalent, but other groups of *Aspergillus* section *Flavi* such as *A. caeletus*, *A. tamarii* and *A. alliaceus* are not prevalent. Aflatoxin B_1, B_2, G_1 and G_2 were found in the samples, Aflatoxin B_1 and B_2 being the most predominant. The high levels of aflatoxin producing fungi may exceed tolerance levels if safe pre- and post-harvest practices are not adhered to. Control strategies during peanut production should be directed to the

wetter Lower Midland 1 and Lower Midland 2 agro-ecological zones. Improved cultivars and reduced pest damage reduced aflatoxin levels in the peanut samples. Producer Marketing Groups promoted peanut grading and provided training on this. Grading produce significantly reduced the levels of fungal contamination in peanuts. Commonly used household preparation techniques (roasting, boiling and making of peanut paste) were effective in reducing levels of aflatoxin in already contaminated produce, as did sorting and dehusking contaminated peanuts in food preparation. Locally available salts effectively reduced aflatoxin levels, without negatively affecting sensory attributes.

7.2   Recommendations

The study illustrates the presence of contamination in peanuts and the possibility of contamination levels increasing in the absence of safe pre- and post-harvest practices among sampled households in western Kenya. Various actions are urgently required to prevent, control and mitigate contamination and its effect on human and animal health and its negative impact on export market opportunities.

Prevention can be addressed by raising awareness through campaigns and promoting sound practices at all stages of the value chain. Varieties, especially those introduced by ICRISAT, have been found to be promising in reducing levels of aflatoxin. Farmers ought to be encouraged to grow these varieties. The improved varieties are also bred for disease resistance, and are therefore less prone to disease compared to the local varieties.

Control strategies should include encouraging peanut production in regions less conducive to aflatoxin contamination - such as in the Lower Midland 3 agro-ecological zone. Preventive measures should be adopted in regions that are likely to predispose peanuts to aflatoxin contamination such as the wetter Lower Midland 1 and Lower Midland 2 areas where peanuts are an important crop.

Farmers need to be encouraged to join producer marketing groups that train farmers in grading, sorting and other helpful control techniques and practices and encourage the use of these and provide cost-effective collective opportunities for quality control through the Kenyan Bureau of Standards. The success of PMGs in the marketing of peanuts should be

used by government institutions to scale up the concept to other regions. Government extension staff can use the PMGs as avenues to train farmers on various aspects of peanut production, processing and marketing.

There is a need to encourage and facilitate Hazard Analysis and Critical Control Points (HACCP) training programs for various peanut products, owing to the continued increase in peanut production and its potential as a cash income crop. This could be done effectively through the Kenyan Bureau of Standards. Already, such efforts are being realised with the help of collaborative efforts between Peanut Collaborative Research Support Programme (CRSP), ICRISAT, KARI and KEBS, where extension workers and peanut processors are being trained on HACCP management systems for peanuts and peanut products.

Mitigating approaches for aflatoxins in peanuts need to include conventional peanut preparation processes that should be encouraged as an adoptable way of managing aflatoxins. Due to their affordability and familiarity, adoption of these practices is simplified. Emphasis needs to be laid on specific processing such as sorting of peanuts and, de-husking, and even washing nuts before cooking. Home Economics Departments need to include modifications to peanut boiling in their training programs as a means of encouraging safe eating habits among farmers. *Magadi*, which is locally available and cheap needs to be encouraged during boiling as it has superior sensory attributes and is an effective medium for reducing levels of aflatoxin in boiled peanuts.

7.3   Recommendations for further research

Recently, the tolerance level for aflatoxins in peanuts and other food commodities by the Kenya Bureaus of standards was lowered from 20 µg/kg to 10 µg/kg. An assessment on the implications of such a move on the food availability *vis a vis* health implications needs to be carried out. Further, as a possible control measure, research on the use of atoxigenic strains of *A. flavus* as a possible bio-control agent needs to be pursued in addition to breeding for resistance, as this would be a cost effective means of managing aflatoxins in peanuts. The research should be built on the high presence of *A. flavus* L-strains found in the region.

Post harvest mitigating strategies can be advanced by studying the ionic properties of locally available ash. In addition, the success of other clays or ash concoctions that are used to either soften food or as mineral supplements especially by pregnant women need to be investigated for their effectiveness in reducing aflatoxin levels. The soils eaten by women should be investigated for their ability to bind aflatoxins in human and animal food/feeds.