FUNGAL SPECIES AND AFLATOXIN CONTAMINATION IN PEANUTS FROM FORMAL AND INFORMAL MARKETS IN ELDORET AND KERICHO TOWNS IN RIFT VALLEY, KENYA

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DECLARATION

Declaration by the Candidate

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DEDICATION

To my beloved husband (Joseph), children (Jean Victor, Vincent de Paul, Jonathan, Anicet, Hellenia, Peter and Angela) and Dr Charity Mutegi.

ABSTRACT

Aflatoxins are a group of mycotoxins mainly produced by *Aspergillus flavus* and *A. parasiticus* mostly on cereals and peanuts. Aflatoxins adversely affect food and feed safety thereby affecting human and animal health as well as trade. Conditions for growth of fungal contaminants and production of mycotoxins vary from region to region and among different products. This study investigated levels of fungal and aflatoxin contamination in marketed peanuts in Kericho and Eldoret towns. A total of 228 samples were collected using stratified systematic sampling from both towns: 140 from formal (raw: 30; roasted coated: 66; roasted de-coated: 44) and 88 (raw: 48; roasted coated: 35; roasted de-coated: 5) from informal markets. Diversity and populations of fungal species contaminating peanuts were determined by culturing ground samples on Modified Dichloran Rose Bengal Agar. Aflatoxin levels were quantified using indirect competitive enzyme-linked immunosorbent assay. Correlation between the incidence of major aflatoxin-producing fungal species and aflatoxin contamination levels was also established. Seven major fungal species and strains isolated from peanut samples were *Aspergillus flavus* L strain, *A. flavus* S strain, *A. parasiticus, A. tamarii, A. caelatus, A. alliaceus* and *A. nige*r. Other isolated fungal genera were *Fusarium*, *Penicillium, Mucor* and *Rhizopus.* Aflatoxin levels averaged 146.8 µg/kg (range: 0 to 2345 µg/kg) for raw, 56.5 µg/kg (range: 0 to 382 µg/kg) for roasted coated and 19.9 µg/kg (range: 0 to 201 µg/kg) for roasted de-coated peanut samples. The level of aflatoxin contamination differed significantly ($p \leq 0.05$) between raw peanut samples from formal and informal markets, both in Eldoret and Kericho towns. There was no significant difference ($p \geq 0.05$) in aflatoxin contamination levels of roasted de-coated peanut samples from formal and informal market outlets. Overall, the total aflatoxin levels in 43% of peanuts and peanut products exceeded the 10 µg/kg regulatory limit set by the Kenya Bureau of Standards. Raw peanuts were the most contaminated with more than 50% of the samples having aflatoxin levels above10 µg/kg. There was a positive and significant correlation ($R^2 = 0.63$; $p \leq 0.05$) between aflatoxin levels and the population of major aflatoxin producing fungal species (*Aspergillus flavus* L and S strain and *A. parasiticus* combined) in raw peanuts sampled from formal market outlets in Eldoret. Total aflatoxin in raw peanut samples from Kericho informal market outlets was positively and significantly correlated ($R^2 = 0.81$; $p \leq 0.05$) with the population of *Aspergillus flavus* (L and S strains). In roasted coated peanut samples from formal market outlets in Eldoret, aflatoxin levels correlated positively and significantly with *Aspergillus flavus* S strain (R^2=0.37; $p \leq 0.05$). Roasted de-coated peanuts were the least contaminated with only 40% and 22% of samples exceeding Kenya Bureau of Standards threshold in informal and formal markets, respectively. Roasting, de-coating and packaging of peanuts reduced the incidence of aflatoxin-producing fungi and aflatoxin production. There is need to create awareness among peanut traders and the public on proper handling of peanuts and health risks associated with consuming unsafe peanut products.

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ACRONYMS AND ABBREVIATIONS

AFB_1:	Aflatoxin B_1
AFG_1:	Aflatoxin G_1
ANOVA:	Analysis of variance
CFU:	Colony forming unit
FB_1:	Fumonisin B_1
FDA:	US Food and Drug Administration
ICRISAT:	International Crops Research Institute for the Semi-Arid Tropics
KEBS:	Kenya Bureau of Standards
SDI:	Simpson Diversity Index
UNWFP:	United Nations World Food Programme

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background Information

Peanut (*Arachis hypogaea* L) also known as groundnut or earthnut is an annual crop grown for its edible oil and protein rich kernel seeds borne in pods that develop and mature below the soil surface (Atasie *et al.*, 2009). It is native to Eastern South America (Atasie *et al.*, 2009) but is mainly grown in China, India, USA and many Sub-Saharan African countries including Kenya (Campos- Mandragon *et al.,*2009; Mutegi, 2010). Within Kenya, peanuts are mainly grown in parts of Nyanza, Western, Rift Valley, Coast and Eastern Provinces. In these regions, the crop is grown in at least two seasons per year (Mutegi, 2010). By 2010, World Food Programme in Kenya was reported to buy about 50,000 metric tones of cereals and groundnuts from Kenya (Oywa, 2010a).

According to Wu *et al.*, (2011) the trend of peanut production by developing countries including Kenya has been increasing over the last decade and is predicted to continue for the next three decades. From 1990-1992, Kenya produced 0.05% (10 million kg) of the World's peanuts production when developing countries produced 91% of the global statistics (23.94 million tons). From 2001-2003, Kenya's production increased to 0.09% (30 million kg) of the world's production (34.46 million tons), with 2030 projection of 0.05% of the World's production when developing countries will be producing 93% of the global statistics (44.33 million tons of peanuts). In 2010, FAO statistics indicated a production of 99,072 metric tones of groundnuts with shell in Kenya, harvested from 19,291 hectares (FAOSTAT, 2012) .Peanut is high in protein (26 to 39%), fat (47 to

59%) and carbohydrates (11%) (Nelson and Carlos, 1995; Atasie *et al.*, 2009). It also contains several minerals, including sodium (42.0 mg/100g), potassium (705.11 mg/100g), magnesium (3.98 mg/100g), calcium (2.28 mg/100g), iron (6.97 mg/100g), zinc (3.2 mg/100g) and phosphorus (10.55 mg/100g) (Atasie *et al.*, 2009), as well as vitamins E, K and B. Due to its high nutritional value, it has several uses such as in weaning, therapeutic food, confectionery, and as an animal feed.

A major challenge in peanut production is fungal contamination. Fungi are among various plant pathogens and major spoilage agents of food and feed. According to Makun *et al.* (2010), infection of plants by fungi not only results in reduction in crop yield and quality with significant economic losses but also contamination of grains with mycotoxins. Mycotoxins are toxic secondary metabolites that are produced by some fungal species and contaminate food and feed (Jay, 1991; MacLauchlin and Little, 2007; Richard, 2007; Russell *et al.*, 2010). *Aspergillus flavus* and *A. parasiticus* are fungal species that have a particular affinity for nuts and oilseeds, and are the main producers of aflatoxins which are the most toxic and carcinogenic compounds among the known mycotoxins (Yu *et al,* 2004). Peanuts and maize are the main sources of human exposure to aflatoxin because they are highly consumed worldwide (13.3 million tons of peanuts were consumed in 2001-2003 with a projected consumption of 16.32 million tons in 2030) and unfortunately are the most susceptible crops to aflatoxin contamination (Waliyar *et al.,* 2009; Mutegi *et al.*, 2009; Mutegi, 2010; Wu and Khlanwiset, 2010).

1.2. Problem Statement and Justification

Aflatoxins are potent mycotoxins that cause impaired growth and immune system suppression. They are carcinogenic, mutagenic and teratogenic. Aflatoxins affect quality of the produce and food safety. Recent reports indicate that aflatoxins are common in peanuts and grains in different parts of Kenya (Mutegi, 2010; Mutegi *et al.*, 2010; Oywa, 2010a, b) and are a serious health problem. In order to minimize consequences of aflatoxin on food security, trade, health; and meet international and national mycotoxin regulatory standards, there is need to monitor fungal species and mycotoxin contamination periodically. The purpose of this study was therefore to investigate the level of fungal and aflatoxin contamination in marketed peanuts in Eldoret and Kericho towns in Kenya with a view to assessing health risks of consuming peanuts and identifying strategies of minimizing aflatoxin production.

1.3. Objectives

Broad objective

To assess the fungal and aflatoxin contamination levels of different peanut products from various market outlets in Eldoret and Kericho towns of Rift Valley region in Kenya.

Specific objectives

1. To determine the incidence of aflatoxin producing fungi in peanut products marketed in different outlets in Eldoret and Kericho towns.
2. To establish the prevalence of aflatoxin in different peanut products from Eldoret and Kericho towns.

3. To compare aflatoxin contamination levels in peanut products from various market outlets in Eldoret and Kericho towns.

1.4. Hypotheses

1. The incidence of aflatoxin producing fungi does not vary in different peanut products in different outlets from Eldoret and Kericho towns.

2. There is no variation in the prevalence of aflatoxin in peanut products marketed in Eldoret and Kericho towns.

3. There is no difference in aflatoxin contamination levels in peanut products from various market outlets in Eldoret and Kericho towns.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Mycotoxins

Mycotoxins are fungal toxic secondary metabolites that cause health problems in humans and animals (Park *et al.*, 2007; Hedayati *et al.*, 2007). Mycotoxins are by-products of metabolism that are not essential for fungal growth and development. They serve as a defensive force for molds, because they can actually limit the role of competitive microorganisms in the vicinity. They are produced by a few fungal species when they colonize crops in the field or during storage (MacLauchlin and Little, 2007; Wagacha and Muthomi, 2008). Mycotoxins are a problem in both tropical and temperate regions in indigenous and imported crops (MacLauchlin and Little, 2007). They attract worldwide attention because of the significant economic losses associated with their impact on human and animal health, productivity, economics of their management and trade (Wagacha and Muthomi, 2008). Some mycotoxins are poisonous if eaten in sufficient quantity. Numerous mycotoxins have been studied and identified. Of particular interest are mycotoxins that affect man.

2.2. Mycotoxins in Crops

Many grains, fruits and vegetables can support fungal growth under certain conditions (Garbutt, 1993). Agricultural attention to mycotoxins has focused on maize, nuts, and fruit crops because of their susceptibility to mold growth and their importance in the human diet. Maize and peanuts are the most frequently affected crops by aflatoxin (Jacobsen *et al.*, 2007; Battilani, 2010). Other susceptible crops include wheat, sorghum,

millet and cassava. Mycotoxins contaminate crops into two phases (Bhat and Vasanthi, 2003; Cotty *et al.* 2008; Battilani, 2010; Hell and Mutegi, 2011): The first phase is during crop development (pre-harvest stage): fungal species contaminate physically damaged and physiologically stressed crops during the growing season. Contamination builds up after maturation when the crop is exposed to warm humid conditions. The second phase may occur to the crop prior to harvest in field or after harvest, which is, during transportation, storage or at any point until the crop is consumed. In addition to climatic conditions, socio-economic and agronomic factors also contribute to mycotoxin contamination of food and feed in Africa (Wagacha and Muthomi, 2008; Hell and Mutegi, 2011). Farm and food industries make every effort to eliminate mycotoxins because they can not only cause illness but also devalue crops.

2.3. Major Groups of Mycotoxins

Major groups of economically damaging mycotoxins include aflatoxins, ochratoxins, trichothecenes and zearalenone. Aflatoxins, a group of polyketide-derived furanocoumarins, are the most toxic and carcinogenic compounds among the known mycotoxins (Yu *et al.,* 2004). Among at least 16 to 20 structurally related aflatoxins, there are four major groups: B_1 (AFB_1), B_2 (AFB2), G_1 (AFG1) and G_2 (AFG_2) so designated based on their blue and yellow-green fluorescence (Olweny, 1977; Wu *et al.*, 2011). They are produced by many species of *Aspergillus* including *A. flavus*, *A. parasiticus* and *A. nomius*. Others are *A. pseudotamarii*, *A. bombycis*, and *A. ochraceoroseus* (Varga *et al.*, 2003; Cary *et al.*, 2005). These toxins contaminate

agricultural commodities and pose a potential risk to livestock and human health (Yu *et al.*, 2004; Hedayati *et al.*, 2007).

Ochratoxins consist of a group of at least seven structurally related secondary metabolites including ochratoxin A, B and C (Jay, 1991; MacLauchlin and Little, 2007). Ochratoxin A, discovered in the mid- 1960s (MacLauchlin and Little, 2007), is the most toxic and prevalent as a natural contaminant of stored grains (Benford *et al.*, 2001; MacLauchlin and Little, 2007). It is primarily produced by *Aspergillus* especially *A. ochraceus* in tropical regions. Gachomo *et al.*, (2004) isolated this species in fresh peanuts marketed within Nairobi, Kenya. Ochratoxin A is also produced by *Penicillium* notably *P. verrucosum* in cereals and cereal products in temperate regions (Benford *et al.*, 2001; USDA, 2006). Ochratoxin A is also found in many other raw and processed foods (for example peanuts, beans, soya beans, barley, wheat, pulses, dried fruits, dried fish, citrus fruits) and beverages such as wine, beer and grape juice (Garbutt, 1993; Benford *et al.,* 2001; USDA 2006, MacLauchlin and Little, 2007). Serious health effects of ochratoxin A in humans and livestock (for example poultry and pigs) have been documented (MacLauchlin and Little 2007) and include impairing immune system function, causing neural abnormalities and development of urinary tract tumours. Contamination of humans by Ochratoxin A can be through eating contaminated foods such as pork and bread (USDA, 2006).

Trichothecenes are a group of mycotoxins produced by *Stachybotrys* spp. and *Fusarium* spp. (Samson *et al.,* 1995). They are deadly mycotoxins if ingested in large amounts and can severely damage the entire digestive tract and cause rapid death due to internal

hemorrhaging (Garbutt, 1993). Jacobs *et al.*, (2010) reported that exposure to toxigenic mold *(Stachybotrys chartarum)* led to infantile pulmonary hemosiderosis (a condition in which victims cough up blood from lungs) in Cleveland in 1990s. Chronic exposure to T-2 toxin and HT-2 toxin, categorized as trichothecenes A, can cause damage to the bone marrow, the immune system and eventually death (Garbutt, 1993). T-2 toxin and HT-2 toxin were reported to be responsible for alimentary toxic aleukia during the Second World War (Lutsky and Mor, 1981). Bhavanishankar and Shantha (2006) found T-2 toxin up to 38.89 mg/kg in peanuts marketed in Mysore, India. Type B trichothecenes that include deoxynivalenol, also referred to as vomitoxin, and nivalenol are majorly produced by *Fusarium cerealis*, *F. culmorum* and *F. graminearum* (Samson *et al.,* 1995) in wheat, barley, maize, rice and sorghum (Canady *et al.*, 2001; Muthomi *et al.,* 2004). Symptoms associated with deoxynivalenol include hemorrhage and necrosis of the digestive tract, neural problems, immune system suppression, lack of blood production in the bone marrow and spleen, and possible reproductive problems such as birth defects and abortion (USDA, 2006).

Zearalenones include at least five naturally occurring compounds that are produced by *Fusarium* species, mainly *F. graminearum*, *F. culmorum* and *F. tricinctum.* These fungal species are associated with maize and invade the field at the silking stage especially during heavy rainfall. Moisture levels above 20% following harvesting cause fungi to grow and produce toxin. Susceptible crops include wheat, barley, sorghum and rice (Jay, 1991). To avoid growth of *Fusarium* spp. in grains during storage the moisture level

should be less than 14%. Zearalenone causes infertility, abortion or other reproduction problems (USDA, 2006).

2.4. Historical Perspective of Aflatoxicosis Outbreaks

Aflatoxicosis refers to an infection caused by consuming commodities contaminated by aflatoxins. Aflatoxins were first isolated in 1961 after a disastrous outbreak termed turkey-X disease resulted in the death of over 100,000 turkeys in England as a result of using a contaminated poultry feed that contained peanuts imported from Brazil (Sargeant *et al.*, 1961). In 1974, four hundred people were affected and one hundred died in northwest India as a result of consumption of heavily aflatoxin contaminated maize containing up to 15,000 parts per billion (ppb) while other smaller outbreaks have been reported from Thailand and Malaysia (MacLauchlin and Little, 2007).

In 1977, significant correlation of liver cancer with aflatoxin ingestion was established from a study carried out in Murang'a District of Kenya (Olweny, 1977). In 2004, severe cases of aflatoxin ingestion were reported in Kenya when over 450 people were affected and 125 of them died after consuming aflatoxin contaminated maize (Probst *et al.*, 2007; Oywa, 2010a). The deaths were mainly associated with homegrown maize that had not been properly dried before storage. In addition, harvested maize grain had not fully matured and was more susceptible to infection. Following the outbreak, a survey of 65 markets in Kenya was undertaken and it was established that 55% of maize samples exceeded the then Kenya regulatory limit of 20 ppb (this has since been revised to 10 ppb

for total aflatoxin), 35% exceeded 100 ppb while 7% exceeded 1000 ppb (Probst *et al.*, 2007).

In 2010, maize harvests in 29 districts from Coast and Eastern Provinces were declared to be unfit for human consumption by the Government of Kenya after 2.3 million bags, equivalent to nearly two-thirds of the total grain harvest were confirmed having aflatoxin exceeding the regulatory threshold (Oywa, 2010a; Oywa and Nzia, 2010). This contamination was attributed to prolonged rainfall that did not allow the maize to dry (moisture content of the harvest was up to 16% while the recommended is 13-13.5%), poor post-harvest handling practices and unfavorable storage conditions (Oywa, 2010a,b).

2.5. Aflatoxins and Aflatoxigenic Fungi

Aflatoxins are primarily produced by *Aspergillus* species (Jay, 1991; Garbutt, 1993; MacLauchlin and Little, 2007). *Aspergillus flavus* and *A. parasiticus* are the predominant species responsible for aflatoxin contamination of crops prior to harvest or during storage (Yu *et al.*, 2004; Klich, 2007; Oywa, 2010a; Wu *et al.*, 2011). At least 16 to 20 different types of aflatoxin are produced, with aflatoxin B1 considered to be the most toxic. The presence of *A. flavus* does not always indicate harmful levels of aflatoxin (Jacobsen *et al.*, 2007). *Aspergillus flavus* produces AFB_1 and AFB_2 while *A. parasiticus* produces AFB_1, AFB_2, AFG_1 and AFG_2 (Yu *et al.*, 2004). Other aflatoxigenic species include *A. nomius* that produces B and G aflatoxins (Dorner, 2002)*, A. pseudotamarii, A. bombycis* and *A. ochraceoroseus* (Varga *et al.*, 2003; Cary *et al.*, 2005).

Aflatoxin is a problem particularly in developing countries in the tropics and subtropics (Battilani, 2010). It is estimated that more than 4.5 billion people in developing countries may be chronically exposed to aflatoxin in their diets (Oywa, 2010b). According to the World Health Organization (WHO), Kenya is among the African countries faced with serious aflatoxin problems.

Peanut is a substrate of choice for extensive aflatoxin production by *A. flavus* (Bankole and Adebanjo, 2003). *Aspergillus flavus* also grows in a wide range of dietary components including maize, wheat, beans, soyabeans, cotton seed, and tree nuts (Olweny, 1977; Jay, 1991; Gachomo *et al.*, 2004; MacLauchlin and Little, 2007). *Aspergillus flavus* is common and widespread in nature and is most often found when certain grains are grown under stressful conditions such as drought. The mold occurs in soil, decaying vegetation (Bankole and Adebanjo, 2003), hay and grains undergoing microbiological deterioration and invades all types of organic substrates whenever and wherever the conditions are favorable for its growth (high moisture content and high temperature). *Aspergillus parasiticus* is most prevalent in peanuts and uncommon in aerial crops such as maize and cottonseed (Horn *et al.*, 2009).

2.6. The genus *Aspergillus*

2.6.1. *Aspergillus* section *Flavi* group

The genetic diversity in the population of *Aspergillus* section *Flavi* has been documented by Horn (2007). *Aspergillus flavus* belongs to section *Flavi* which contains an assemblage of phylogenetically related aflatoxigenic and non-aflatoxigenic species

(Peterson, 2008). Abdel-Hadi *et al.* (2011) identified 18 strains of *Aspergillus* section *Flavi* group, 13 of which were aflatoxin-producers, from Egyptian peanuts. Giorni *et al.,* (2007) isolated 70 strains within *Aspergillus* section *Flavi* from maize destined for animal feed in Italy. The strains included toxigenic *A. flavus* (dominant at 93%) and *A .parasiticus.* Mutegi *et al.* (2012) recovered *A. flavus* S strain*, A. flavus* L strain*, A. parasiticus, A. tamarii, A. caelatus* and *A. alliaceus* members of section *Flavi* group in peanuts sampled from Western Kenya.

2.7. Factors Affecting Occurrence and Production of Aflatoxin in Peanuts

Contamination of crop seeds by *A. flavus* and *A. parasiticus* may occur in field or during storage (Klich, 2007). Biological factors (crop susceptibility and its compatible toxigenic fungi), environmental factors, harvesting, storage, handling and processing all contribute to aflatoxin contamination of crops (Mahuku and Nzioki, 2011). Mutegi *et al.*, (2007) noted that peanut production in Kenya is dominated by small holders whose handling practices favour fungal contamination. According to Hell and Mutegi (2011), aflatoxin contamination of food and feed in Africa is increasing due to environmental, agronomic and socio-economic factors. Fungal growth and aflatoxin contamination are therefore the consequence of interactions among the fungus, the peanut and the environment. The appropriate combination of these factors determines the infection and colonization of peanuts, and the type and amount of aflatoxin produced. In addition, a suitable substrate is required for fungal growth and subsequent toxin production, although the precise factors that initiate toxin formation are not well understood. Substrate and insect damage of the host plant are major determining factors in mold contamination and toxin

production. Aflatoxin formation is also affected by associated growth of other molds or microbes (Bhatnagar *et al.,* 2002).

Environmental and agronomic factors are major factors that influence pod and seed infection by the aflatoxin-producing fungi and aflatoxin production (CAC/RCP, 2004). These factors vary from one location to another and between seasons within the same location. To be effective in reducing aflatoxin production incidences, pre-harvest and post-harvest control measures of peanuts must be taken into consideration (CAC/RCP, 2004).

2.7.1. Environmental factors

Fluctuation in climatic factors influences aflatoxigenic fungi in the environment and predisposes the host to contamination by altering crop development and affecting insects that create wounds on which aflatoxin-producers proliferate (Cotty and Jaime-Garcia, 2007). According to Bankole and Adebanjo (2003), mycotoxin contamination is favoured by plant stress, high ambient humidity that prevents thorough drying, and inappropriate storage practices.

Dry hot conditions affect crop in field while warm and wet conditions favour the produce in storage (Cotty and Jaime-Garcia, 2007). According to Wottom and Strange (1987), peanut susceptibility increases due to reduction in phytoalexin production during drought stress. Stress of the plant in field and poor storage conditions such as excessive heat and moisture, pest crop damage and long period of storage of the produce are the main

predisposing factors to aflatoxin contamination (Wu *et al*., 2011). Under high humidity, dry seed absorbs water hence increasing its moisture content that favours the extent of fungal contamination (Cotty and Jaime-Garcia, 2007). Some environmental conditions are favorable to fungal infection and subsequent aflatoxin contamination of peanuts. Contamination of peanuts by aflatoxin is influenced by high temperatures, prolonged drought conditions, high moisture content before and during harvesting, heavy rains during the growing season and poor post-harvest handling by farmers (Oywa, 2010a). The recommended safe moisture content for peanuts is below 10 percent (CAC/RCP, 2004), that is, lower than that of maize which is 10 to 13.5 per cent (Oywa, 2010b; Hell *et al.*, 2003). Inadequate drying or improper storage of food commodities are also factors influencing the occurrence of aflatoxin in humid tropics. According to Bhat and Vasanthi (2003), climatic conditions such as high temperature and moisture, unseasonal rains during harvest, and flash floods lead to fungal proliferation and production of mycotoxins. The optimum temperature for aflatoxin production has been found to be between 24°C and 28°C (Jay, 1991), 26°C and 32°C (USDA, 2006).

2.7.2. Agronomic factors

Aspergillus flavus is widespread and often found when certain grains are grown and harvested under stressful conditions. The mould occurs in soil, decaying vegetation, hay, and grains undergoing microbiological deterioration, invading all types of organic substrates whenever the conditions are favorable for its growth (Oywa, 2010b). Seed quality (variety, size, damage, diseased), specific crop growth stages, poor fertility, high crop densities, and weed competition have been associated with increased mold growth

and toxin production (CAC/RCP, 2004). In addition, the practice of allowing peanuts to dry out in the field predisposes the kernels to *A. flavus* infection (CAC/RCP, 2004).

2.7.3. Biotic factors

Susceptible developing crops are predisposed to fungal contamination through mechanical damages by birds, mammals and insects (Guo *et al.*, 2003). Diverse insects (like corn borers on maize, pink bollworm on cotton, lesser corn stalk borer on peanut) vector aflatoxin-producing fungi and they cause damage that allows the fungi to gain access, increasing the chances of aflatoxin contamination (Stephenson and Russell, 1974; Diener *et al.*, 1987; Dowd, 2003; Guo *et al.*, 2003). Storage pests also play significant role in contamination of foods with toxigenic fungi (Hell *et al.*, 2003; Lamboni and Hell, 2009).

2.8 . Implications of Aflatoxins on Human and Animal Health

2.8.1. Aflatoxins and human health

Aflatoxins are heat stable and survive most cooking methods (Olweny, 1977). Aflatoxin contamination makes commodities unfit for human consumption. There is a general consensus that aflatoxin contamination of foods affects people and their livelihoods; it is particularly serious for poor people who have little choice regarding the food they consume. USAID (2012) reported that exposure to aflatoxin is a health problem rooted in the whole food chain and requires a multidisciplinary approach for analysis and management.

Aflatoxicosis is a disease produced through poisoning by exposure to aflatoxin (Wu and Khlangwiset, 2010). Exposure to aflatoxin is mostly through ingestion of contaminated food (Richard, 2007; Wagacha and Muthomi, 2008; Mutegi, 2010) but also by the dermal and inhalation routes (Wagacha and Muthomi, 2008; Mutegi, 2010). The disease can be acute or chronic. When aflatoxin is consumed, it may alter intestinal integrity or modulate the expression of cytokines and proteins that signal to each other and to immune system components resulting in stunted growth in children and immune suppression (Wu and Khlangwiset, 2010).

The ingestion of high levels of aflatoxin can be fatal, while chronic exposure may result in serious health conditions such as cancer and liver cirrhosis, weakened immune systems, and stunted growth (Wu and Khlangwiset, 2010). Aflatoxin plays an important role in human hepatocellular carcinoma (liver cancer), increase susceptibility to diseases especially in children and childhood pre-five years mortality and reduced life expectancy (Makun *et al.,* 2010). In 1987, the International Agency for Research on Cancer (IARC) placed aflatoxin B1 on the list of human carcinogens (IARC, 1987). Specific P450 enzymes in the liver metabolize aflatoxins into a reactive oxygen species (aflatoxins-8, 9-epoxide), which may bind to proteins and cause acute aflatoxicosis or to DNA and induce liver cancer (Wild and Gong, 2010; Wu and Khlangwiset, 2010). The primary disease associated with intake of aflatoxin is liver cancer. According to WHO (2008), liver cancer is the third- leading cause of cancer death with 550 thousand to 600 thousand new cases each year. Strosnider *et al.* (2006) reported that 80% of these deaths occur in East Asia and Sub-Saharan Africa. In addition to liver cancer, aflatoxins cause cancer of the

gut, lungs and human breast (Cotty and Jaime-Garcia, 2007). These toxins also impair child development (underweight, neurological impairment), suppress immune system and lead to death when in severe acute exposure (Cotty and Jaime-Garcia, 2007).

The syndrome of acute aflatoxicosis is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidneys, and heart (Williams *et al*., 2004). Humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth. Such exposure is unavoidable because fungal growth in foods is not easy to prevent. Heavily contaminated food supplies are not permitted in the market place in developed countries, but concern still remains for the possible adverse effects that result from long-term exposure to low levels of aflatoxin in the food supply. More than 5 billion people in developing countries worldwide are estimated to be at risk of chronic exposure to aflatoxins through contaminated foods (Strosnider *et al.*, 2006). Evidence of acute aflatoxicosis in humans has been reported from many parts of the world including Kenya, Uganda, Taiwan, India, and many others (Jacobsen *et al.*, 2007; Probst *et al.*, 2007).

The expression of aflatoxin-related diseases in humans may be influenced by age, sex, nutritional status, and/or concurrent exposure to other causative agents such as viral hepatitis (HBV) or parasite infestation (Bennet and Klich, 2003). The limited availability of food, environmental conditions that favor fungal development in crops and commodities, lack of regulatory systems for aflatoxin monitoring and control are among conditions that enhance the likelihood of acute aflatoxicosis in humans (Wu *et al.*, 2011).

2.8.2. Aflatoxins and animal health

Some mycotoxins are acutely toxic and can cause vomiting and feed refusal by livestock (Lubulwa and Davis, 1998). Several studies have shown that aflatoxin B1, aflatoxin M1, and aflatoxin G1 cause various types of cancer in different animal species. The susceptibility of individual animals to aflatoxin varies considerably depending on species, age, sex, and nutrition (Bennett and Klich, 2003). Ingestion of aflatoxin by animals may cause liver damage, decreased milk production, decreased egg production, immune suppression, embryo toxicity, growth impairment, increased mortality rate, anemia among others (Lubulwa and Davis, 1998; Wu *et al.*, 2011). Young animals are the most susceptible to aflatoxin effects among the other age groups. Since aflatoxins can also affect animals with devastating results, then contaminated food should not be converted into animal feeds.

2.9. Testing for Aflatoxin

The presence of aflatoxin-producers in a given food/feed does not mean that aflatoxins are present, but does increase the risk for aflatoxin production (Jacobsen *et al.*, 2007). Aflatoxins are not destroyed during food and feed manufacturing processes. Given the potential long-term effects of aflatoxins leading to significant economic and health hazards, it is essential to test the food and food products for aflatoxins before their consumption (Waliyar *et al.*, 2009) and ensure that their levels in food supply are minimized (Makun *et al.*, 2010). Several testing methods and technologies used in identifying the level of contamination in various foodstuffs have been developed. Mycotoxins are analyzed by either quick tests or confirmatory tests.

2.9.1. Quick tests

Quick tests or screening are rapid solutions that can be useful in-field analysis and as a first set of measures for rejection/acceptance of food/ feed. They are used to determine whether samples are positive for some mycotoxins (Yiannikouris and Haladi, 2012; Vincelli *et al.*, 2012). Quick tests include immunoassays, or Enzyme Linked Immunosorbent Assay (ELISA), which use antibodies to detect mycotoxins, and thin layer chromatography (TLC) testing (Eurofins, 2008-2011; Yiannikouris and Haladi, 2012; Vincelli *et al.*, 2012). These rapid tests provide fast results and may take only a few minutes to one day.

ELISA is commonly chosen due to its speed and the significant number of samples that can be analyzed, and is available as semi-quantitative tests for various concentration levels and ranges. ELISA requires a simple preparation and inexpensive equipment and is highly sensitive, good for screening but can sometimes lead to false positive results (Vincelli *et al.*, 2012). Thin-layer chromatography, (TLC) test is simple, inexpensive test for aflatoxin and can be applied in the same way as ELISA, with better repeatability and less cross-reactivity, but TLC requires further sample clean-up and a consequent increase in the amount of time needed to obtain a precise ratio (Waliyar *et al.*, 2009; Yiannikouris and Haladi, 2012; Vincelli *et al.*, 2012).

2.9.2. Use of lateral flow devices

Other strategies used as alternative to quick tests include using lateral flow devices such as immunostrips, immunodipsticks and immunofiltration with immobilized antibodies on their surface. These techniques are able to rapidly test for the main groups of regulated mycotoxins, but need to be used with caution due to cross-reactivity and false-positives (Yiannikouris and Haladi, 2012)

2.9.3. Confirmatory tests

Quantitative confirmatory tests use high-performance liquid chromatography (HPLC), or gas chromatography coupled with UV or fluorescent detection (FLD) (Yiannikouris and Haladi, 2012; Vincelli *et al.*, 2012). HPLC-UV or HPLC-FLD is used to separate, identify and quantify compounds according to their chemical properties. They are used when quick tests are strongly positive. A number of HPLC methods exist for analyzing aflatoxin, usually based on the AOAC approved method for corn and peanut butter. These methods are capable of lower quantitation limits and usually show more specificity. They can confirm results and provide more accurate information about the amount of mycotoxin present. They are highly sensitive, very specific and accurate (Waliyar *et al.*, 2009; Yiannikouris and Haladi, 2012; Vincelli *et al.*, 2012). The disadvantages of these methods include more expensive instrumentations, much more skilled technical requirements, higher cost per analysis, long run time for analysis (several days to a week), and focus on group of toxins .of similar chemical structure at one time. Thus, with these methods, testing for a range of toxins requires significant time and investment.

2.10. Regulation of Aflatoxin Contamination in Peanuts

To protect human and animal health, action and tolerance levels of different aflatoxin types and aflatoxin total in food and feed have been established by different institutions or organizations in different countries. The US Food and Drug Administration has set the maximum safe level for total aflatoxin to be 20 μg/kg for peanuts for human consumption. The European Union set the maximum limit for aflatoxin B1 at 2 μg/kg for peanuts and its processed products and 4 μg/kg for total aflatoxin μg/kg (Guo *et al.*, 2003; Kubo, 2012); while the Government of Kenya (through Kenya Bureau of Standards (KEBS) and the UN World Food Programme have set the maximum safe level of total aflatoxin in maize and peanuts at 10 μg/kg for human consumption down from 20 μg/kg that was allowed before levels were reviewed (KEBS, 2007).

2.11. Strategies for Aflatoxin Control and Prevention

Aflatoxins contaminate agricultural commodities and cause health problems in humans and animals. Measures and strategies to control and prevent aflatoxin will lead to economic gains and health improvement. Many solutions against aflatoxin production have been proposed by mycotoxin researchers (Bankole and Adebanjo, 2003).

2.11.1. Prevention of mold contamination and growth

According to Hell and Mutegi (2011), field management practices that increase yield can reduce the risk of aflatoxin production. Pre-harvest procedures for aflatoxin reduction with reference to fungi include the use of resistant varieties, crop rotation, well-timed planting, weed control, reduction of insect infestation, application of fertilizer to reduce

plant stress and irrigation to avoid drought stress (Hell and Mutegi, 2011; Okoth, 2011). Rapid and proper drying, sorting, sanitation, smoking, use of botanicals or synthetic pesticides as storage protectants, proper transportation as well as packaging are among post- harvest strategies to prevent fungal contamination and subsequent aflatoxin production (Bankole and Adebanjo, 2003; Hell and Mutegi, 2011).

2.11.2. Drying

The moisture content of the produce after harvest is usually 26 to 35% which must be reduced to 12-14% on weight basis for safe storage with minimal deterioration (Noomhorm and Cardona, 1989). According to CAC/RCP (2004), safe moisture content level in peanuts should be less than 10%. Rapid drying of newly harvested peanuts is one of the mycotoxin control and prevention measures to avoid losses in quality and quantity of the produce (Noomhorm and Cardona, 1989; Bankole and Adebanjo, 2003; Hell and Mutegi, 2011). Sun drying which is the oldest and common way of reducing the moisture content of any crop is normally slower than using mechanical driers which are equipments that artificially use heated air during the drying process (Noomhorm and Cardona (1989).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Eldoret and Kericho towns (Figure 3.1) within Rift Valley region, in Kenya. Eldoret town (0^o31’54’’N, 35^o15’58’’E) is located in Western Kenya at 2100-2700 m above sea level, 300 km North West of Nairobi on the trans-African highway and 65 km North of the equator. Eldoret is the fifth largest town in Kenya with a total urban population of 252,061 by 2009 census (Republic of Kenya, 2009). It is the administrative centre of Uasin Gishu County in the Rift Valley region. Its climate is cold and wet, with an average temperature of 27^oC and mean annual rainfall of 1,124 mm.

Kericho town (0^o22’0’’S, 35^o16’59’’E) lies within the highlands west of the Great Rift Valley in Kenya, adjacent to Kenya’s biggest water catchment area, the Mau forest. It is located in the south west of the country at 2096 m above sea level, 263 km North West of Nairobi. It is centrally located at 70 km to Kisumu, 100 km to Nakuru and 130 km to Eldoret. The climate in Kericho is characterized by cool temperatures ranging between16°C and 20°C, and high rainfall averaging between 1,400 mm and 2,000 mm per annum. Kericho’s total urban population was estimated at 42,029 by 2009 census (Republic of Kenya, 2009).

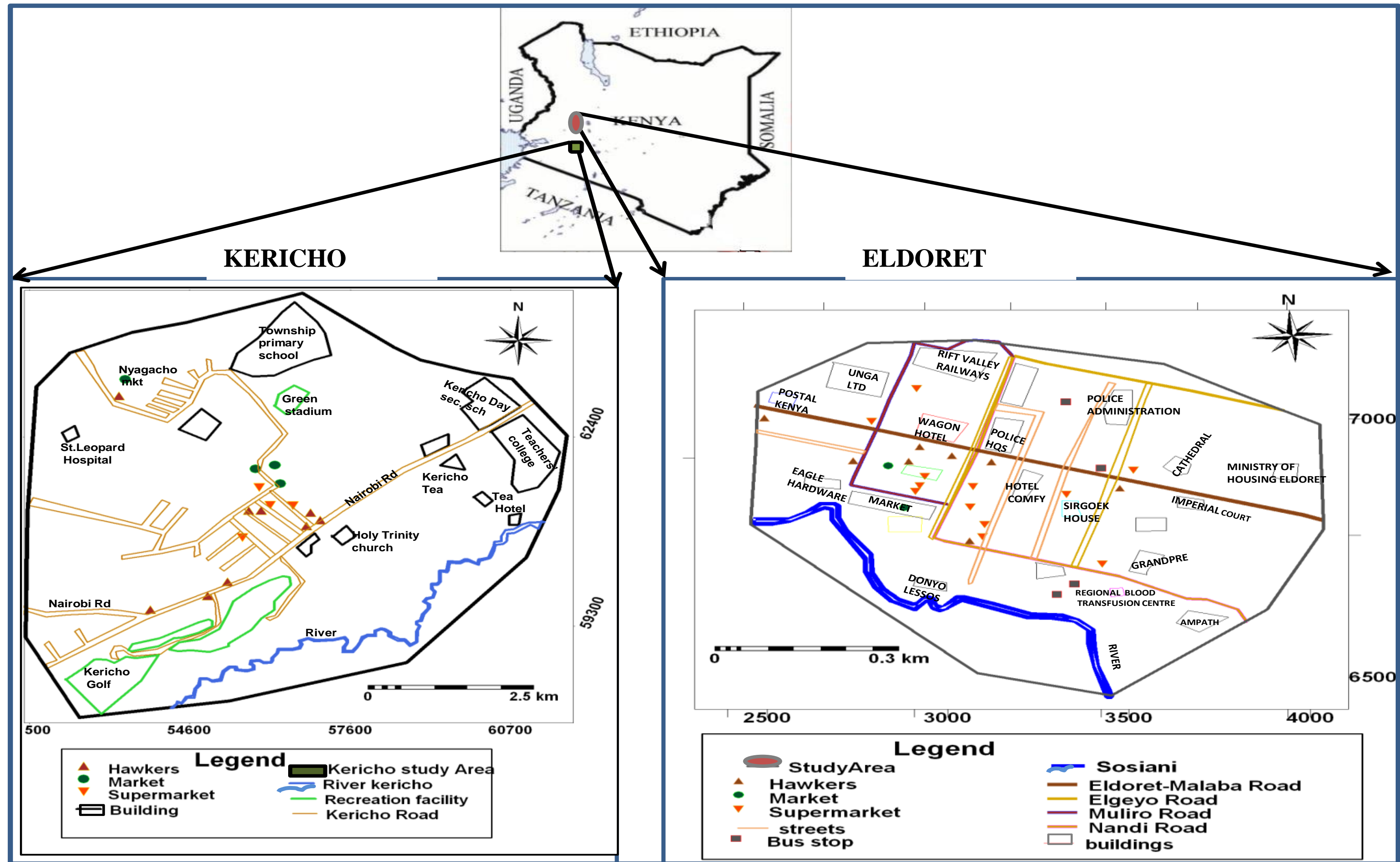

Figure 3.1: Peanuts sampling points in Kericho and Eldoret towns, Rift Valley, Kenya. (Source : Author, 2013)

3.2. Field Survey and Collection of Peanut Samples

Two hundred and twenty eight (228) peanut samples of 0.5 kg each were collected from formal and informal markets in both study towns: one hundred and eighteen (118) from Eldoret and one hundred and ten (110) from Kericho towns. One hundred and forty peanut samples were from formal (raw: 30; roasted: 66; roasted de-coated: 44) and 88 (raw: 48; roasted: 35; roasted de-coated: 5) from informal markets. . Within each town, stratified systematic sampling plan was followed in acquiring samples of peanut products on sale. The sampling points within the towns are shown in Figure 3.1 for Kericho and Eldoret. Samples were collected from hawkers, open markets, retail shops, and supermarkets/stockists. Sizes of peanut samples from each market outlet are shown in Appendix 1. Formal markets referred to stockists, shops and supermarkets while informal markets included hawkers and open markets. Half a kilogram sample of raw shelled, roasted and roasted de-coated peanuts was collected from each vendor operating formal and informal market outlets. The collected samples were packaged, well sealed in a sterile polythene bag and then transported to the laboratory where they were kept in a cool dry place at room temperature (20 – 25 ^{o}C) until laboratory analyses. Figure 3.2 shows different types of peanut products sampled from informal markets in Kericho and Eldoret towns. Background information on the source and handling of peanuts on sale was gathered through direct observations and face-to-face interviews using a semi-structured questionnaire. Information sought included the type of peanut product, nature of market outlet, packaging material, source of the peanut products, mode of transport to the market, storage conditions, whether peanuts were sorted before selling and criteria used, and the time interval between buying and selling.

Figure 3.2: Different types of peanuts and peanuts products being marketed in informal market outlets of Kericho and Eldoret towns, Kenya, during the study period.

(A) Informal roasting of peanuts, (B) De-coated roasted peanuts, (C) Packaged coated peanuts, (D) Packaged coated and de-coated peanuts, (E) Raw peanuts displayed in an open market.

(Source: Author, July 2011)

3.3. Sample Preparation

Each thoroughly mixed peanut sample weighing 250g, (drawn from 500 g peanut sample) was ground to a fine powder using a Black and Decker blender machine (BX525-B5 Type 02, Shanghai, China). Two replicates of 100 g each were weighed using an analytical weighing machine. One replicate was used for mycological analysis; the other for aflatoxin analysis and the remaining 50g for moisture content determination.

3.4. Moisture Content Determination

Low constant temperature oven method was used as prescribed by the International Seed Testing Association (1985, 2005). Two replicates of 1g each (wet weight) were drawn from 50 g peanut powder sub-sample before oven-drying. Aluminum foil papers were weighed using an analytical weighing machine and labeled. Each 1 g replicate was then wrapped in the weighed aluminum foil paper and placed in the oven which was maintained at a temperature of 103 ± 2°C. The wrapped peanut powder was dried to a constant dry weight. For each replicate, moisture content was calculated using the following formula and the two replicate values were thereafter averaged.

$$\text{Moisture content (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

3.5. Microbial Analysis

3.5.1. Preparation of media for isolation and identification of *Aspergillus* species

Isolation and identification of different fungal species, mainly *Aspergillus* spp., in the peanut samples was carried out on Modified Dichloran Rose Bengal (MDRB) Agar medium (Horn and Dorner, 1998). The medium was prepared by mixing 10 g glucose, 2.5 g peptone, 0.5 g yeast extract, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 20 g agar, and 25 mg rose Bengal in 1 L distilled water. The pH of this medium was adjusted to 5.6 using 0.01M HCl. The medium was autoclaved for 20 minutes at 121°C and a pressure of 15 psi, and cooled in a water bath at 60 °C. To inhibit the growth of bacteria and making the medium semi selective for *Aspergillus* species, 5ml of 4mg/l dichloran (in acetone), 40 mg/l streptomycin (in 5 ml distilled water) and 1 mg/l chlortetracycline (in 10 ml distilled water) were added to the medium through a sterile 0.25 µm syringe filter paper after cooling to 50°C. The medium was dispensed (approximately 20 ml per plate) in disposable Petri dishes and allowed to settle for two to three days before use.

3.5.2. Preparation of peanut samples for plating

From the 100 g ground peanut sub-sample, two sub-samples of 2.5 g each were weighed and transferred into falcon tubes into which 10 ml of 2% water agar solution (2 g of agar dissolved in 100 ml sterile distilled water) were added and mixed thoroughly. The first sub-sample was serial diluted to 10^{-1} and the second to 10^{-2}. Six replicates for each sample (three replicates for 10^{-1} and three replicates for 10^{-2}) comprising 0.2 ml each of the solution was pipetted onto MDRB medium plates under aseptic conditions. The plates

were incubated for seven days at 30 ^{o}C after which colonies were observed and identified.

3.5.3. Identification of fungal species and counting of colonies

After seven days of incubation, fungal colonies were identified and differentiated to species or strain level based on their cultural and morphological characteristics. For different *Aspergillus* species, macroscopic identification was done using reference cultures obtained from the ICRISAT Plant Pathology laboratory. These reference cultures were sub-cultured at the same time of plating of the peanut samples. *Aspergillus flavus* L-strain and *A. flavus* S-strain were identified based on the colour of spores and size of sclerotia. *Aspergillus parasiticus* colony is low, yellow and smooth. *Aspergillus alliaceus* colony is characterized by formation of big screrotia. *Aspergillus tamarii* has raised colony that is yellow-dark green in centre, cottony and granular. Colonies of other isolated fungal contaminants were also recorded and in some cases identified to genus level. Digital photographs were taken using High sensitivity Sony Camera 7.2 Mega Pixels (Model no. DSC-S650, Sony cybershort 7.2 mega pixels, Quezon City, Philippines) to show macroscopic distinctive features of identified fungal species isolated from various peanut samples. The colony forming units (CFUs) of each fungal species were counted using the Gallenkamp colony counter (Gallenkamp manufacturer, Frodsham, England). The population (CFU/g peanuts) of the fungal species was determined using the formulae:

$$\text{CFU/g peanuts} = \frac{\text{colony counts}}{(\text{volume plated} \times \text{dilution factor})}$$

Volume plated was 0.2 ml while dilution factors were 0.25 for first dilution (10^{-1}) and 0.025 for second dilution (10^{-2})

3.6. Aflatoxin Analysis

The level of total aflatoxin in each composite sample was determined by indirect competitive Enzyme Linked Immunosorbent Assay (ELISA) method approved by Association of Analytical Chemists (AOAC). A 100 g sub-sample powder was well mixed and 20 g taken, triturated in 70% vv methanol (70 ml absolute methanol in 30 ml distilled water) containing 0.5% w/v potassium chloride in blender for about 2 minutes. The extracts were transferred to a conical flask and shaken for 30 minutes at 300 rpm. The extract was filtered through Whatman number 41filter paper, and then transferred to a sterile container, stored in a freezer until analysis for total aflatoxin. The extracts were analyzed for aflatoxin level at the Plant Pathology laboratory in ICRISAT-India.

3.7. Data Analyses

Data on CFUs and aflatoxin levels were compared among market types (formal and informal), peanut product types (raw, roasted coated and roasted de-coated) and towns (Eldoret and Kericho). The diversity of fungal species contaminating peanut products in various market types and different towns was compared based on Simpson diversity index values using the formula:

$$D = \frac{1}{\sum_{i=1}^{s} p_i^2}$$

Where S = number of species; i= 1, 2, ….10; $p_i = \frac{\text{CFUs for species i}}{\text{Total CFUs}}$

Lower index value indicates that a few species dominate over the others.

For aflatoxin levels, samples were grouped into four categories based on their aflatoxin content as per standards by European Union (EU), Kenya Bureau of Standards (KEBS) and Food and Drug Administration (FDA): < 4 µg/kg, 4-10 µg/kg, >10-20 µg/kg and > 20 µg/kg. Peanut in the first category (< 4 µg/kg) could be accepted under EU. Peanuts in second category (4-10 µg/kg) could be rejected by EU but accepted by KEBS. Category three contains peanuts that could be rejected by both EU and KEBS and accepted under FDA while those in last category could be rejected by EU, KEBS, and FDA.

The response variable aflatoxin level was not normally distributed and did not have constant variance; therefore the assumptions for parametric t-test did not hold (Shapiro-Wilk test for Normality: $p < 0.001$ and Bartlett's test for homogeneity of variances: $p < 0.001$). Hence in comparing any two groups of the variables, the non-parametric Mann-Whitney U (Wilcoxon rank-sum) statistical test was used to analyze the data. In comparing more than two groups, data were analyzed using ANOVA under unbalanced design (GenStat version 14). The means were separated using Least Significant Difference (LSD) at 5% level of significance. SPSS version 16 statistical software was also used to generate frequencies and tests of significance including correlation analysis.

CHAPTER FOUR

4.0. RESULTS

4.1. Background Information on Marketed Peanuts in Kericho and Eldoret Towns

During the study period, existing formal market outlets for peanuts were found to be supermarkets in Kericho and both supermarkets and retail shops in Eldoret. The informal outlets were municipal open markets (for both towns), stationed hawkers who roasted peanuts as they sell (in both towns) and mobile/errant hawkers (in both towns) (Figure 4.1. There were no stockists of peanuts in either town.

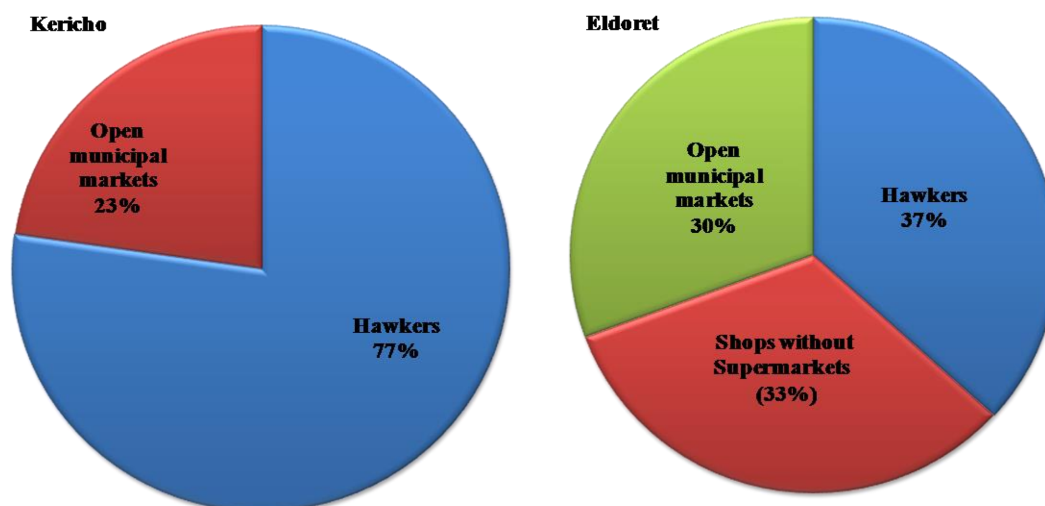

Figure 4.1: Proportion of market outlets in Kericho and Eldoret towns, Rift Valley, Kenya.

Suppliers to traders and supermarkets in both towns did not have their base in the same towns. Six means of transport for peanuts in informal markets were used but dominated by walking and use of trucks (Figure 4.2). In Eldoret town, peanuts were supplied through open (40%) and closed (35%) trucks, and minibus (25%). In addition, walking

and bicycle means were mainly used from different estates around Eldoret town. In Kericho, peanuts are mainly supplied through open trucks (60%).

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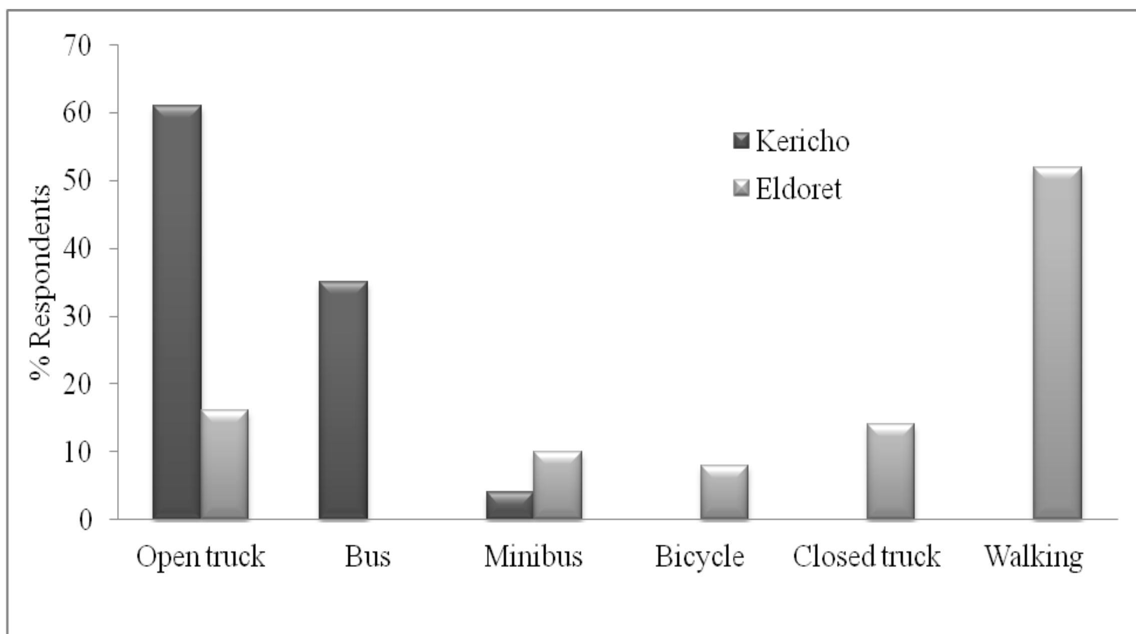


Figure 4.2: Mode of transport for peanuts in informal markets in Kericho and Eldoret towns, Rift Valley, Kenya.

Three types of peanut products - shelled raw peanuts, roasted coated peanuts and roasted de-coated peanuts – were marketed in Kericho and Eldoret towns. Raw peanuts marketed in informal market outlets in Kericho and Eldoret towns were sourced from Busia, Migori and Oyugis in Kenya, and from Malawi and Tanzania (Figure 4.3).

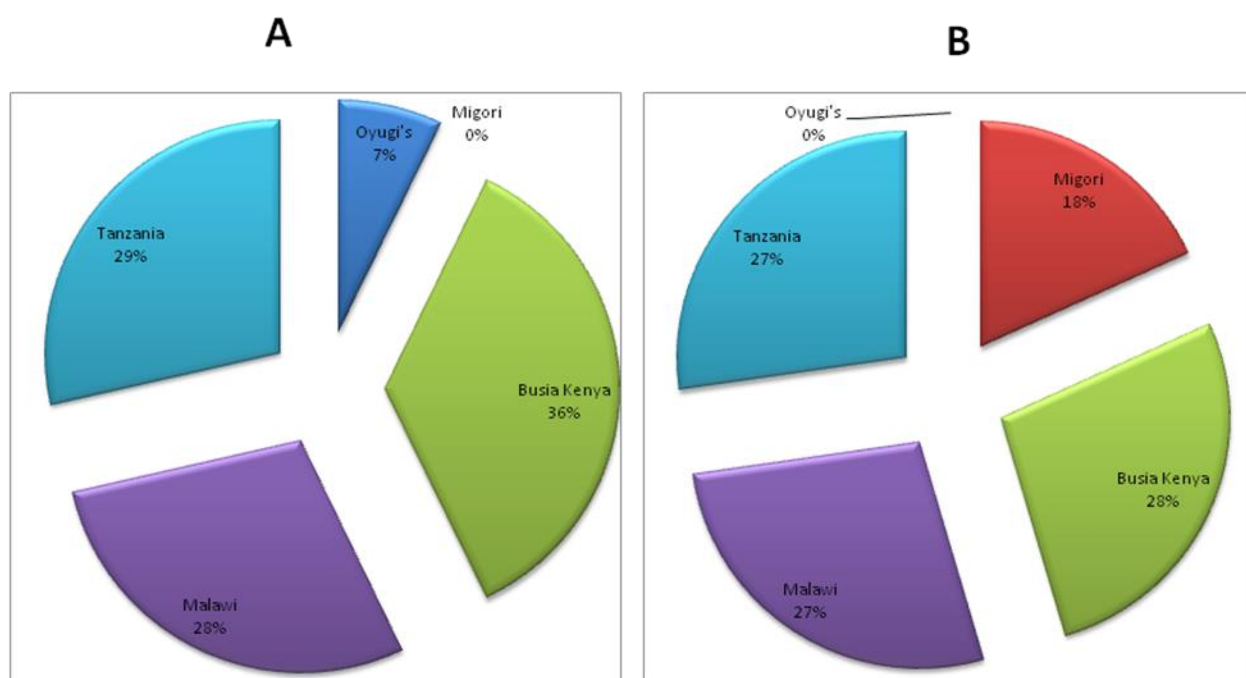


Figure 4.3: Sources of raw peanuts marketed in informal markets in Kericho (A) and Eldoret (B) towns, Rift Valley, Kenya.

Identity of raw and roasted peanuts in formal market outlets were labeled on packaging materials indicating the name of supermarket or industry/enterprise that packaged or roasted them (Appendices 1.1, 1.2 and 1.3). Seventeen different industries/enterprises of peanuts displayed in formal markets were recorded. In some cases, the source of packaged peanuts could not be ascertained from the labeling. However, roasted peanuts that were being marketed packaged in informal were not labeled and their exact geographical sources could not be identified and traders did not know the exact source of their nuts.

Different packaging materials for peanuts marketed in Kericho and Eldoret towns included used newspaper, used printing paper, and polythene paper (Figure 4.4). Two

types of packaging materials (Appendices 1.4 and 1.5) dominated in informal markets for roasted peanuts sold by hawkers: air tight plastic bags (32% in Kericho; 41% in Eldoret) and used newspapers (45% in Kericho; 22% in Eldoret). All raw peanuts from informal markets were stored in polypyrene bags and were not packaged.

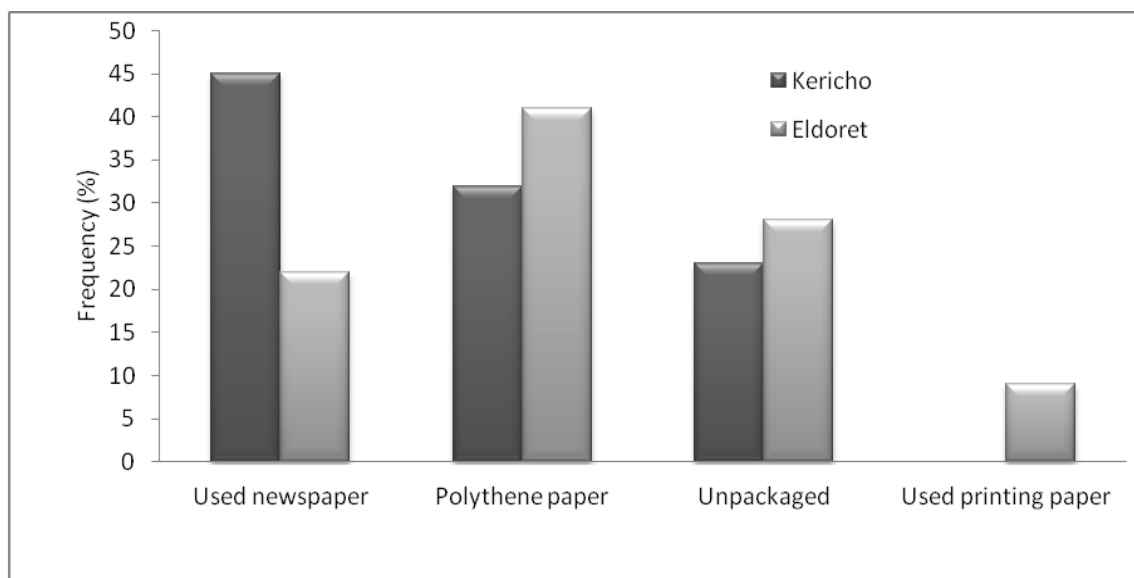


Figure 4.4: Types of packaging materials used in informal peanut markets in Kericho and Eldoret towns, Rift Valley, Kenya.

Storage of peanuts in market outlets was done under various conditions: home room temperature (20-25°C), generally practiced by hawkers, stall (top covered with iron sheet and free moving air underneath), Shop, store made of wooden structure, raised and ventilated, and store made of stones/ bricks with ventilation - wire mesh window). The proportion of vendors who stored their peanuts (raw peanuts) in stalls and home room temperature/ house (mainly roasted peanuts) dominated in both Kericho and Eldoret towns (Figure 4.5).

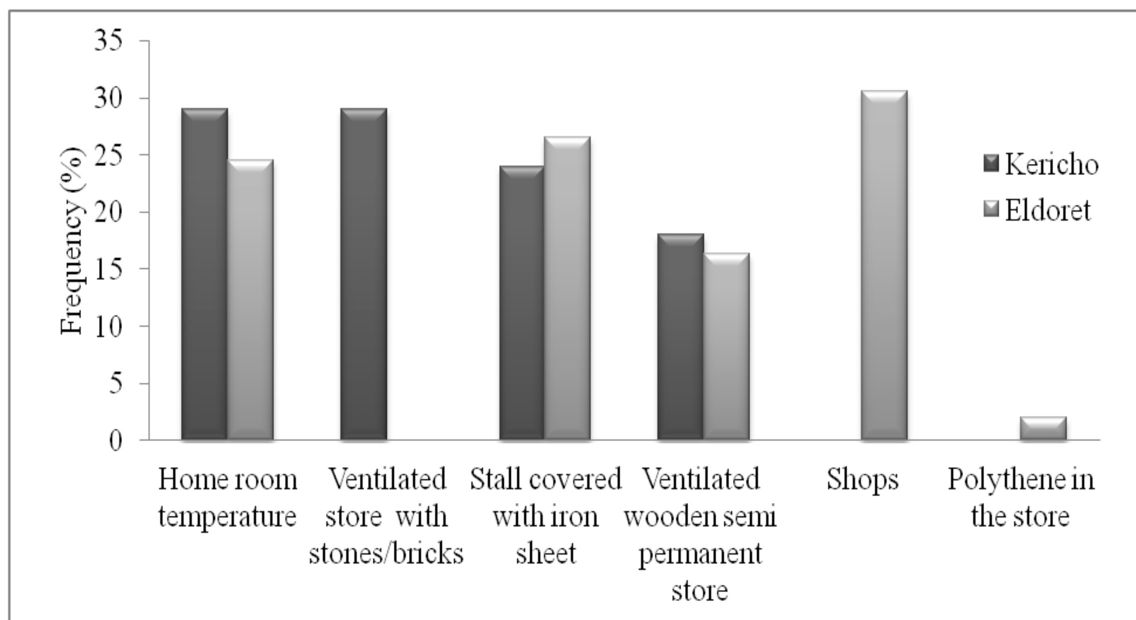


Figure 4.5: Storage structures and conditions of peanuts marketed in Kericho and Eldoret towns, Rift Valley, Kenya.

Most of peanut vendors (65%) sold the products that were not sorted. However, Eldoret town had higher proportion (33%) of vendors who practiced thorough sorting of peanuts than in Kericho town (14%). Criteria used in sorting focused on removing rotten, undersized seeds, shrunk, damaged seeds and impurities (Figure 4.6). Thoroughness of sorting was judged based on the number of sorting criteria that were combined in a given sorting activity. Combinations of criteria were therefore retained in Figure 4.6 as captured from interviewees.

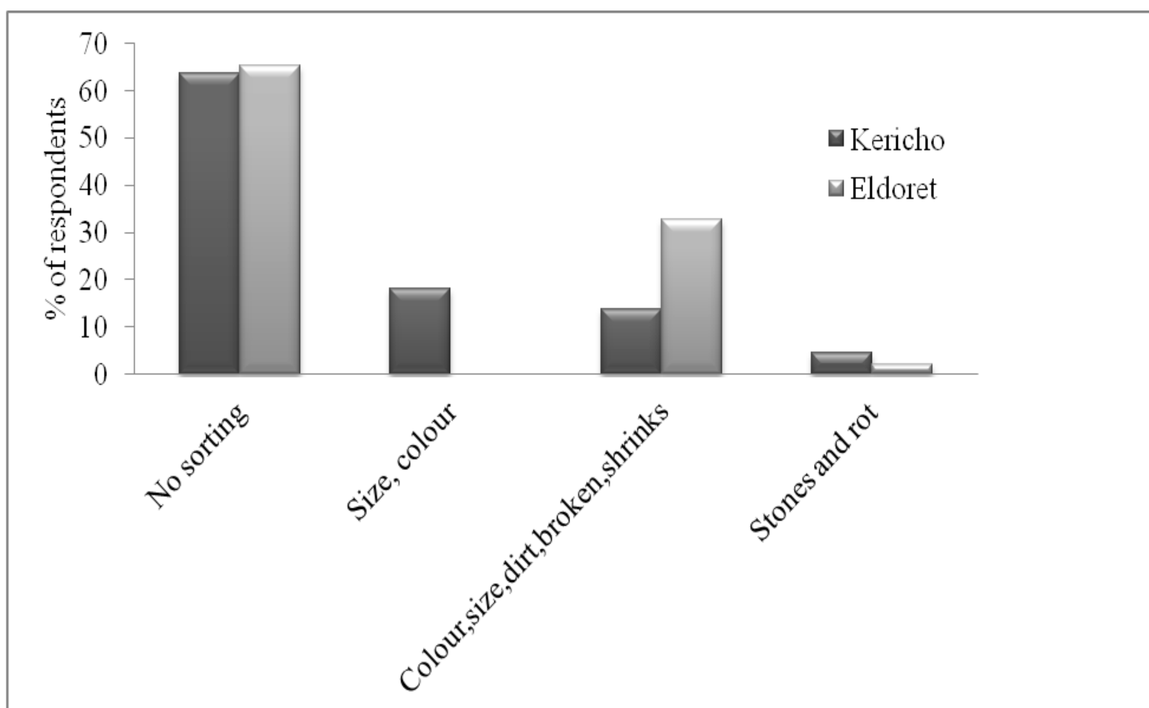


Figure 4.6: The proportion (%) of criteria used in sorting of peanuts in Kericho and Eldoret towns, Rift Valley, Kenya.

Time interval between buying and selling (length of storage period) of peanuts in informal markets ranged between 1- 90 days. The proportion of those who sold their products within a day was higher (71%), generally for roasted peanuts that are sold through hawking, while 11% of raw peanut vendors sold their products between 60 and 90 days. The length of period taken between buying and selling was mostly less than seven days (Figure 4.7). Most of peanut products in formal markets (supermarket) were sold between one and six months (Figure 4.8).

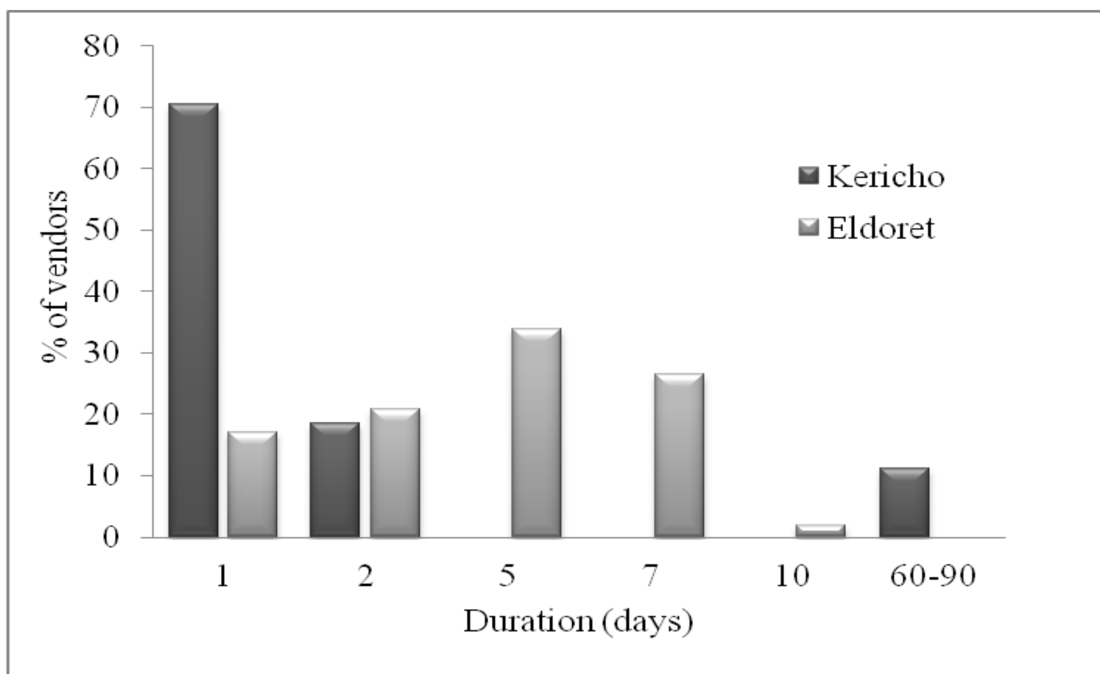


Figure 4.7: The length of storage period (days) of peanuts in Kericho and Eldoret towns, Rift Valley, Kenya.

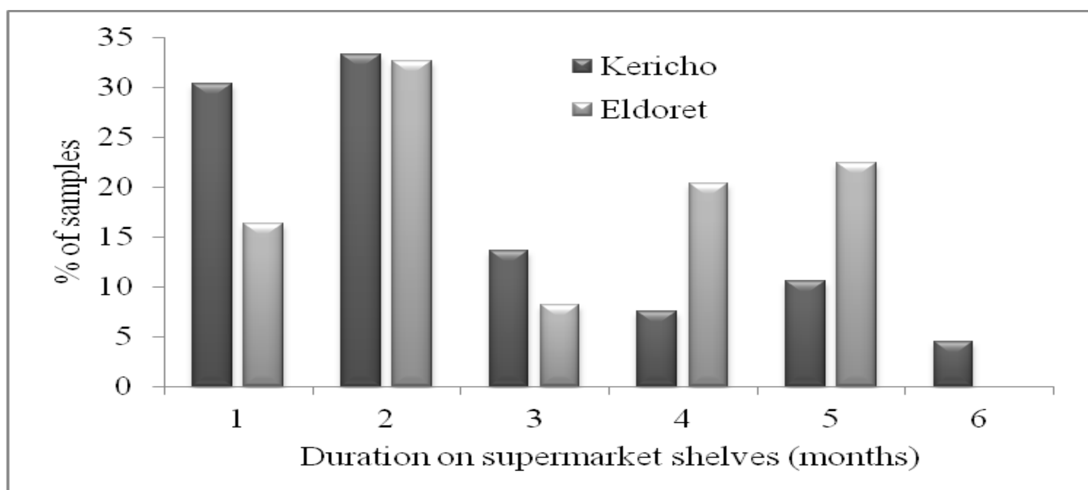


Figure 4.8: The Length of storage period (months) for peanuts sold in formal market (supermarket) in Kericho and Eldoret towns, Rift Valley, Kenya.

4.2. Moisture Content of Marketed Peanuts in Kericho and Eldoret Towns

The moisture content of peanut products sampled from Kericho and Eldoret towns ranged from 0.0 (from roasted peanuts) to 9.6% (from raw peanuts). Roasted peanuts had lower moisture content than raw peanuts (Table 4.1). However, there was no significant ($p \geq 0.05$) difference in moisture content levels among the analyzed peanut products.

Table 4.1: Moisture content (%) on wet weight basis of different peanut products sampled from Eldoret and Kericho towns, Rift Valley, Kenya

Peanut product	Eldoret		Kericho	
	Formal	Informal	Formal	Informal
Raw	4.67a	4.43a	3.32a	4.20a
Roasted coated	3.60a	1.76a	3.45a	2.78a
Roasted de-coated	1.76a	N/A*	2.66a	2.92a
Mean	3.34	3.10	3.14	3.30

"N/A" means that during the sampling period, the product was not found on sale in informal market of Eldoret. Means followed by same letter in the table are not significantly different (Multivariate analysis, Fisher's protected LSD test, $p \geq 0.05$).

4.3. Fungal Species Identified from Various Peanut Products

Seven *Aspergillus* species - *A. flavus* L strain, *A.flavus* S strain, *A.parasiticus, A. tamarii, A. caelatus, A. alliaceus* and *A. nige*r - were isolated from various peanut samples. Some peanut samples were infected with one type of *Aspergillus* species or strain (Figure 4.9); while others were contaminated with more than one fungal species (Figure 4.10). Other

isolated fungal genera included *Mucor, Rhizopus, Fusarium* as well as unidentified genera.

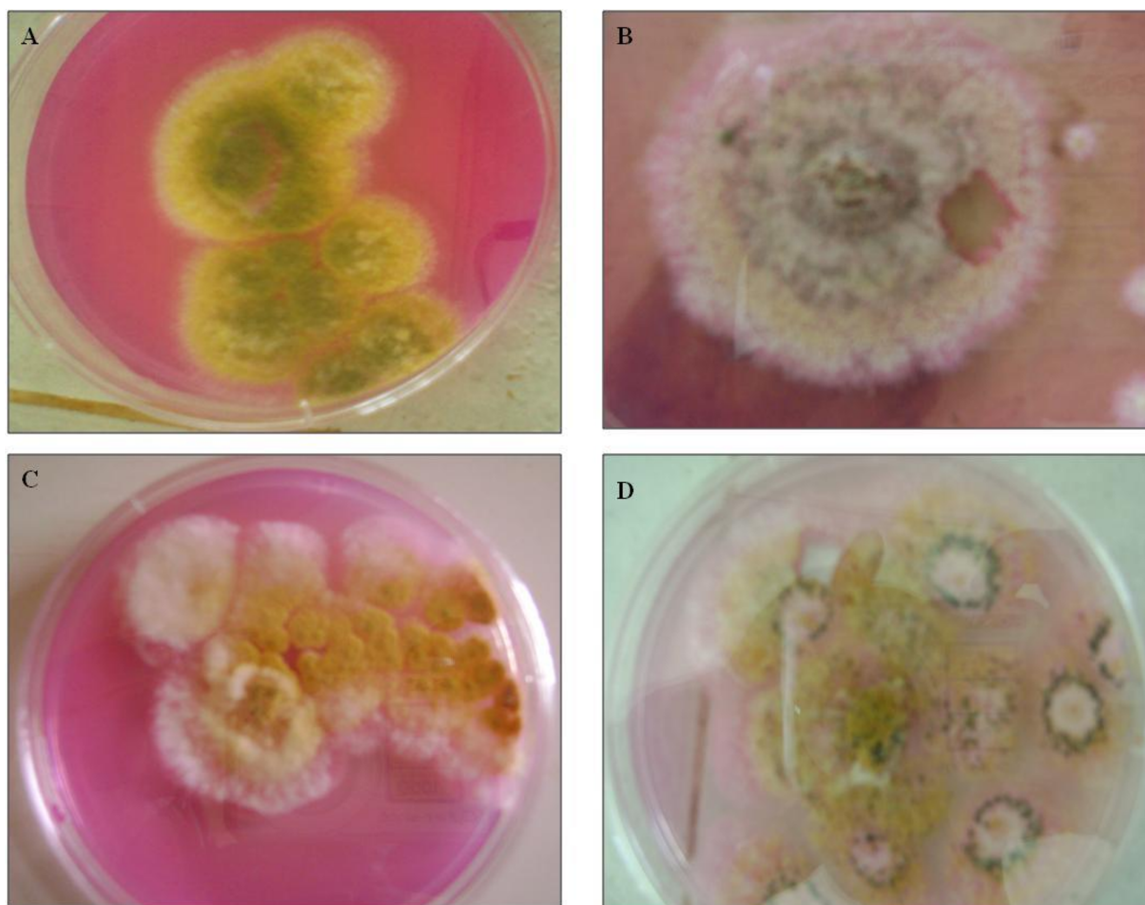


Figure 4.9: Macroscopic distinctive features of identified fungal species isolated from peanut products sampled from Eldoret and Kericho towns, Rift Valley, Kenya.

Aspergillus flavus L strain with yellow-green smooth spores (plate A); *Aspergillus flavus* S strain with formation of numerous small screlotia (plate B); *Aspergillus parasiticus* with low colony, golden and smooth spores (plate C); *Aspergillus alliaceus* colony characterized by formation of big screlotia (plate D). **(Source: Author, March 2012)**

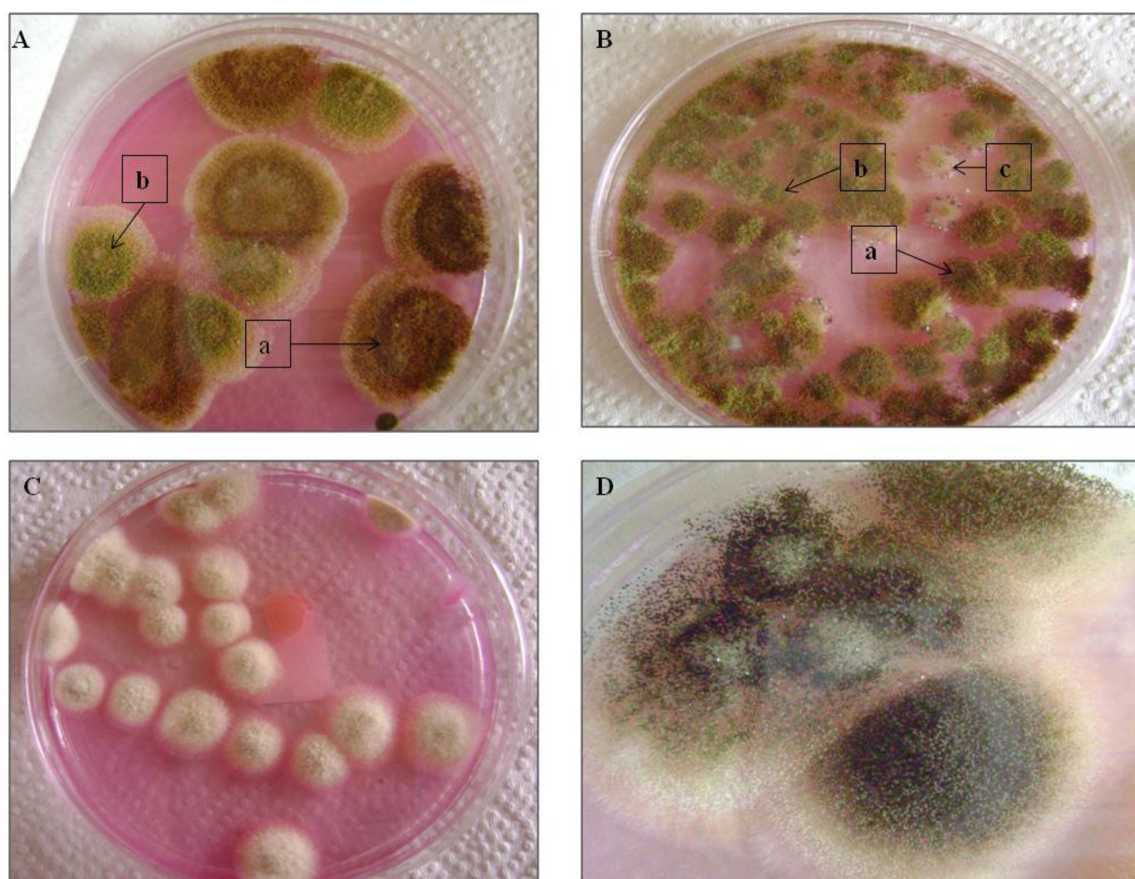


Figure 4.10: Multiple fungal species (plates A & B) and unidentified fungal species (Plates C & D) isolated from peanut samples from Eldoret and Kericho towns, Rift Valley, Kenya. [A] *Aspergillus tamarii* (a) and *A. flavus* L strain (b); [B] *A. tamarii* (a), *A. flavus* L strain (b) and *A. alliaceus* (c). (**Source: Author, March 2012)**

Figure 4.11 shows the population of each of the isolated fungal species (all product types and market types combined). *Aspergillus flavus* L strain was isolated with the highest frequency (mean = 574 CFUs/g peanuts), followed by *A. tamarii* (mean = 109) and *A. flavus* S strain (mean = 97). *Aspergillus niger*, *A. parasiticus*, *A. alliaceus* and *A. caelatus* were isolated in low frequency with averages of 39, 18, 4 and 3 CFUs/g substrate,

respectively. Other isolated fungal genera included *Penicillium* (18), *Mucor* and *Rhizopus* spp.

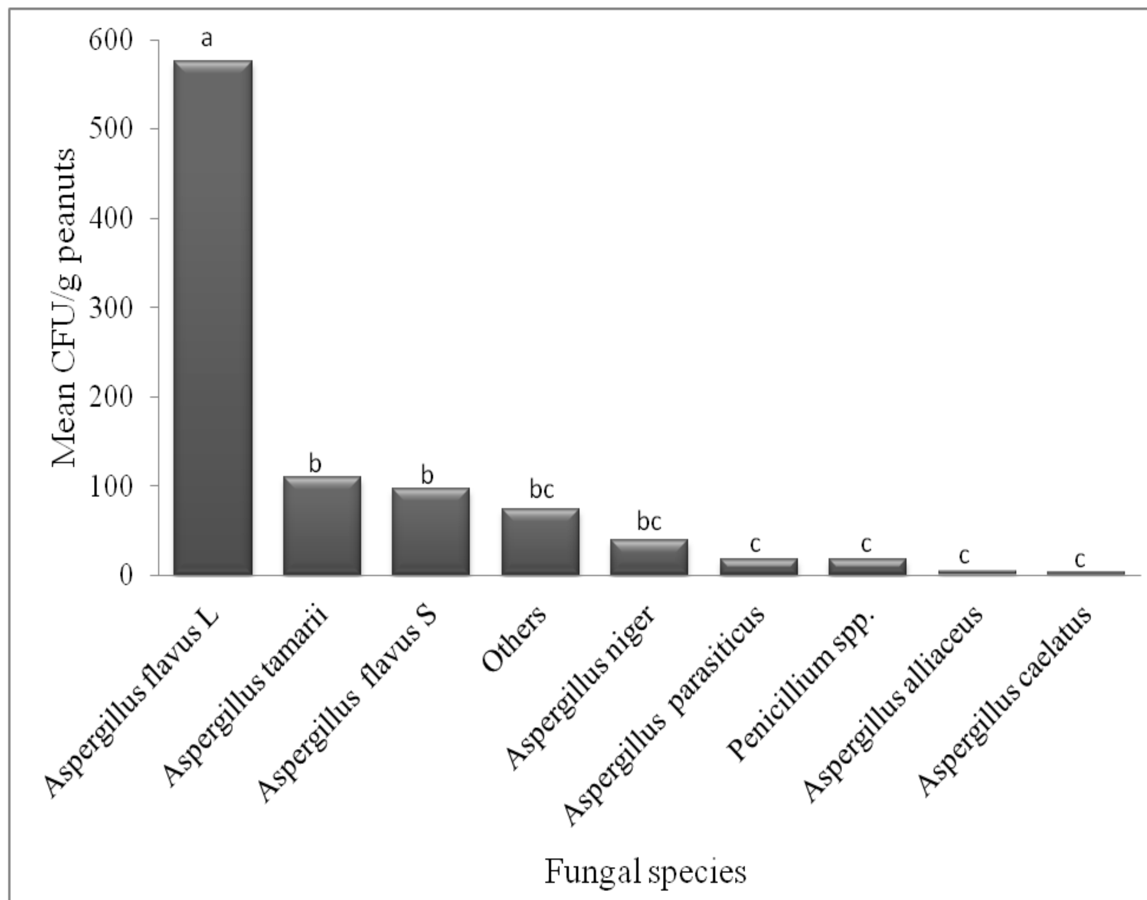


Figure 4.11: Incidence (CFUs/g peanuts) of fungal species isolated from peanut samples from Eldoret and Kericho towns, Rift Valley, Kenya. Bar values followed by same letter in the graph are not significantly different at 95% confidence level.

4.4. Fungal Population in Different Peanut Products and Market Types

Generally, the incidence of fungal contaminants in peanut samples from informal markets was significantly higher ($p \leq 0.05$) than that from formal markets (Table 4.2). The

incidence of fungal contaminants in raw peanuts from informal market outlets was significantly higher ($p \leq 0.05$) than from formal market outlets. However, roasted coated peanuts from formal markets were more contaminated than samples from informal markets. Fungal infection levels of raw and roasted de-coated peanuts from Kericho town were higher than from Eldoret town. However, there was no significant difference ($p \geq 0.05$) in the incidence of fungal contaminants for roasted de-coated peanuts in Kericho between formal and informal markets. The incidence of fungal species in peanut samples was significantly different ($p \leq 0.05$) between the formal and informal market outlets for raw peanuts, both in Eldoret and Kericho towns. However, the incidence of fungal contaminants was not significantly different ($p \geq 0.05$) between roasted coated peanuts and roasted de-coated peanuts from formal and informal markets, in Eldoret and Kericho towns. Level of fungal contamination was not significantly different in all peanut products between Kericho and Eldoret towns ($p \geq 0.05$) for formal as well as informal markets. Overall, the incidence of fungal species was significantly ($p \leq 0.05$) lower in formal than in informal markets regardless of towns and products.

4.5. Diversity of Fungal Species in Different Peanut Products and Market Types

The fungal species diversity was higher in some peanut products from Eldoret than Kericho towns (Figure 4.12). However, there was no significant difference ($p \geq 0.05$) between fungal species diversity in different peanut products.

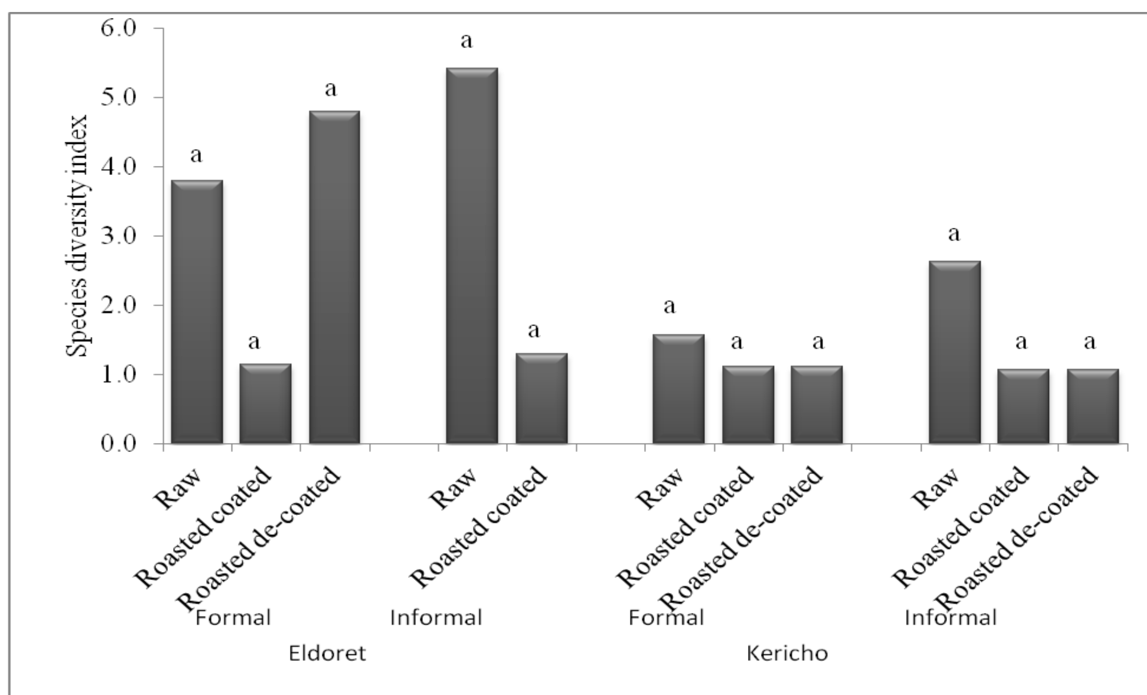


Figure 4.12: Levels of fungal species diversity in peanut products marketed in formal and informal markets in Kericho and Eldoret towns, Rift Valley, Kenya. Bar values followed by same letter in the graph are not significantly different at 95% confidence level. .

4.6. Population of Aflatoxin-producing Fungi in Peanut Samples

The incidence of aflatoxin-producing species was significantly higher ($p \leq 0.05$) in peanuts from Kericho than in Eldoret towns. The incidence of these fungal species was not significantly different in roasted peanuts from formal and roasted de-coated from informal market outlets of Kericho town (Figure 4.13). In Eldoret town, the incidence of aflatoxigenic fungal population was significantly lower in raw peanuts from formal than in informal markets. *Aspergillus flavus* L strain was pre-dominant in roasted coated and de-coated peanuts from formal markets with an incidence of 98.9 and 94.9%, respectively. The corresponding incidence in raw, roasted coated and roasted de-coated

peanuts from informal markets was 68.5, 99.9 and 100% (Table 4.2). The incidence of *A. flavus* S strain was significantly higher (82%) in peanuts from formal markets than from informal markets (28%). *Aspergillus parasiticus* was recovered in low incidence of 5 and 6% in peanuts from formal and informal markets, respectively. The incidence of the major aflatoxin producing fungi (*A. flavus* L strain, *A. flavus* S strain and *A. parasiticus*) was significantly higher ($p \leq 0.05$) in raw peanuts from informal markets than formal markets.

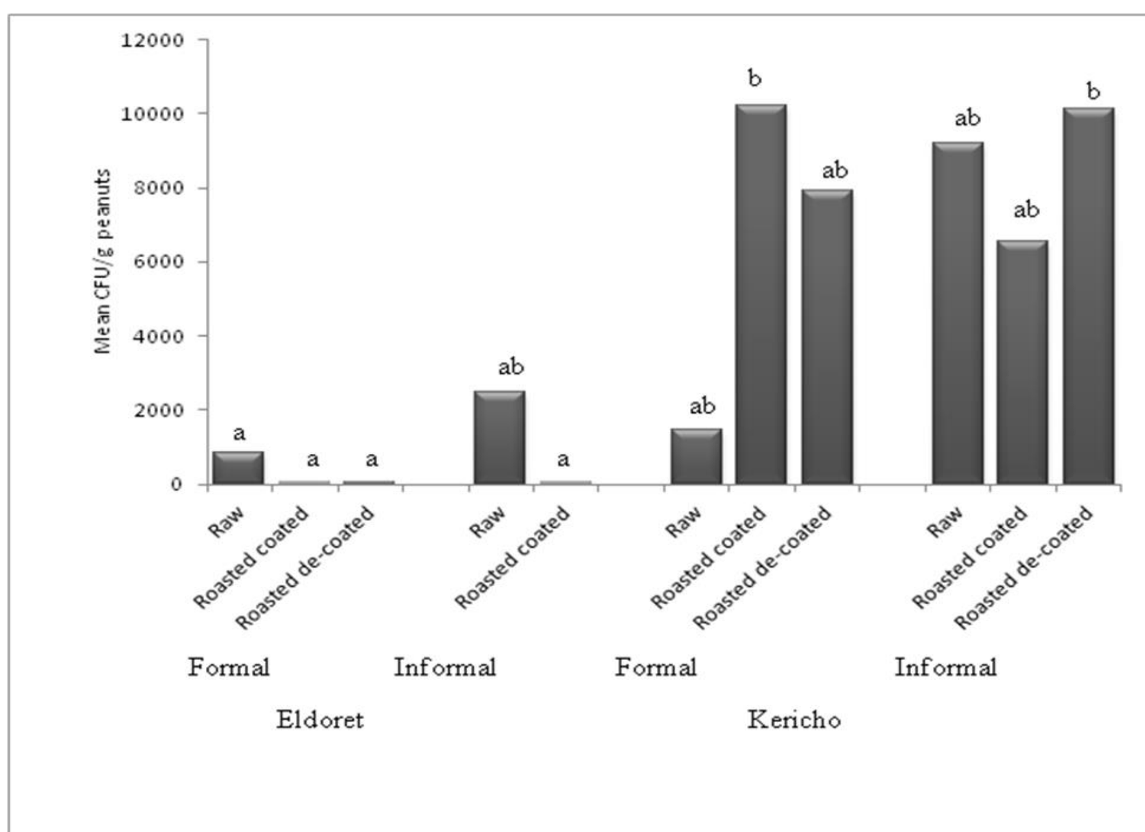


Figure 4.13: Colony forming units (CFU/g substrate) of *Aspergillus flavus* (L and S strains) and *A. parasiticus* in different peanut products sampled from formal and informal markets in Eldoret and Kericho towns, Rift Valley, Kenya. Bar values followed by same letter in the graph are not significantly different at 95% confidence level.

Table 4.2: Colony forming units (CFU) of fungal species per gram of different peanut products sampled from formal and informal market outlets in Eldoret and Kericho towns, Rift Valley, Kenya

	Town	Peanut product	Population of fungal species (CFUs/g substrate)									Total	Mean ±SE
			AFL	AFS	AP	AT	AC	AA	AN	PEN	Others		
Formal market	Eldoret	Raw	43.8	114.0	1.6	8.9	11.3	1.3	250.7	43.6	236.0	711	79.0±33.2
		Roasted coated	0.4	0.1	0.3	1.4	1.8	0.0	0.4	0.9	78.1	83	9.3±8.6
		Roasted de-coated	5.8	1.9	4.4	9.1	1.7	0.0	1.6	0.4	11.5	36	4.0±1.3
		Mean ± SE	16.7±13.7	38.7±37.7	2.1±1.2	6.5±2.5	4.9±3.2	0.4±0.4	84.2±83.2	15.0±14.3	108.5±66.6		30.8±12.9
	Kericho	Raw	9.3	217.3	19.8	0.0	3.1	0.0	25.8	0.0	0.0	275	30.6±23.6
		Roasted coated	1685.9	17.9	0.0	71.9	0.0	0.0	1.1	0.1	0.2	1777	197.5±186.2
		Roasted de-coated	1254.9	56.4	4.9	437.9	0.0	0.0	0.0	0.0	0.0	1754	194.9±140.8
		Mean ± SE	983.4±502.7	97.2±61.1	8.2±6.0	169.9±135.6	1.0±1.0	0±0	9.0±8.4	0.0±0.0	0.1±0.1		141.0±76.7
		Grand Mean ±SE	500.0 ±311.9	67.9 ±34.7	5.2 ±3.0	88.2 ±70.8	3.0 ±1.7	0.2 ±0.2	46.6 ±41.0	7.5 ±7.2	54.3 ±38.4	772.7^{a} ±328.7	
Informal market	Eldoret	Raw	188.5	191.1	33.5	83.8	12.6	36.0	156.8	136.5	442.8	1282	142.4±43.8
		Roasted coated	0.2	0.0	1.7	0.0	0.0	0.0	0.0	0.2	14.0	16	1.8±1.5
		Roasted de-coated	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	NA	NA
		Mean ± SE	94.4±94.2	95.6±95.6	17.6±15.9	41.9±41.9	6.3±6.3	18±18.0	78.4±78.4	68.4±68.2	228.4± 214.4		72.1±27.3
	Kericho	Raw	1326.9	448.5	110.0	441.5	1.7	0.0	47.9	0.0	6.5	2383	264.8±146.3
		Roasted coated	1094.4	0.0	0.0	25.8	0.0	0.0	0.0	0.0	4.9	1125	125.0±121.2
		Roasted de-coated	1685.3	0.0	0.0	13.3	0.0	0.0	0.0	0.0	0.0	1699	188.7±187.1
		Mean ± SE	1368.9±171.	149.5±149.	36.7±36.7	160.2±140.7	0.6±0.6	0±0	16±16.0	0±0	3.8±2.0		192.8±86.1
		Grand Mean ±SE	859.1 ±327.4	127.9 ±88.3	29.0 ±21.2	112.9 ±83.4	2.9 ±2.5	7.2 ±7.2	40.9 ±30.4	27.3 ±27.3	93.6 ±87.3	1301.0^{b} ±388.1	

Different letters accompanying total CFUs grand means indicate that the means are significantly different at 5% level of significance. SE: standard error; AFL: *Aspergillus flavus* L strain; AFS: *A. flavus* S strain; AP: *A. parasiticus;* AT: *A. tamarii;* AC: *A. caelatus;* AA: *A. alliaceus;* AN: *A. niger;* PEN: *Penicillium* spp.

4.7. Aflatoxin Levels in Different Peanut Products

Total aflatoxin contamination levels varied between peanut samples from formal and informal market outlets, Eldoret and Kericho towns as well as among peanut products (Table 4.3). Eighty one percent (185 out of 228) of the peanut samples analyzed had detectable levels of total aflatoxin. Levels of aflatoxin in the peanut products ranged from 0 to 2344.8 μg/kg. Generally, raw peanuts had the highest levels of total aflatoxin (mean = 146.8 ppb) while roasted de-coated peanuts were the least contaminated (mean = 19.9 ppb) (Table 4.3). Similarly, raw peanuts from informal markets had higher levels of aflatoxin (mean = 210.2 ppb) than formal market outlets (mean = 83.4 ppb). In contrast, roasted coated peanuts from formal markets were more contaminated (mean = 74.3 ppb) than samples from informal markets (mean = 38.8 ppb). However, their levels of contamination were higher in Eldoret town. Raw peanut samples from informal markets in Kericho town had high levels of total aflatoxin (Mean = 340.2 μg/kg with 83% contaminated samples while roasted de-coated samples from formal markets in Eldoret had low levels of total aflatoxin (mean = 7.9 μg/kg with 74% contaminated samples). Finally, in both Eldoret and Kericho town, levels of aflatoxin contamination were higher in informal (mean = 97.1 μg/kg) than formal market outlets (mean = 55.5 μg/kg). Observed differences were nonetheless statistically not significant ($p \geq 0.05$).

Table 4.3: Aflatoxin levels (µg/kg) in different peanut products sampled from formal and informal markets in Eldoret and Kericho towns, Rift Valley, Kenya.

Peanut product	Formal market			Informal market			Grand mean
	Eldoret	Kericho	Mean	Eldoret	Kericho	Mean	
Raw	37.8	129.0	83.4	80.1	340.2	210.2	146.8±67.1[a]
Roasted coated	93.1	55.5	74.3	48.1	29.4	38.8	56.5±13.4[a]
Roasted de-coated	7.9	9.6	8.8	-	42.3	42.3	19.9±11.2[a]
Mean	46.3	64.7	55.5	64.1	137.3	97.1	
± SE	±25.0	±34.8	±19.6	±13.1	±101.5	±58.6	

Roasted de-coated peanuts were not on sale in informal markets Eldoret town.
Grand mean values followed by same letter in the column are not significantly different at 95% confidence level.

4.8. Aflatoxin Level Categories in Peanuts

Overall, out of the 228 peanut samples analyzed, 45% contained <4 µg/kg of total aflatoxin, 12% contained total aflatoxin in the range of 4 - 10 µg/kg, 11% in the range of >10-20 µg/kg, and 32% contained levels that exceeded 20µg/kg (Table 4.4). Forty three percent of the peanut samples had total aflatoxin levels that exceeded the KEBS limit of 10 µg/kg. Out of 228 samples analyzed, the proportion of roasted de-coated peanut samples that were within the acceptable limits of KEBS was as follow: 78.2% and 90.4% from formal markets in Eldoret and Kericho, respectively, and 60% from informal market outlets of Kericho town (Table 4.4). The proportion of raw peanut samples from formal and informal markets which had levels of total aflatoxin above 10 µg/kg was 50 and 52%, respectively. Similarly, 44 and 60% of raw peanut samples from Eldoret and Kericho towns respectively did not meet the KEBS regulatory standards.

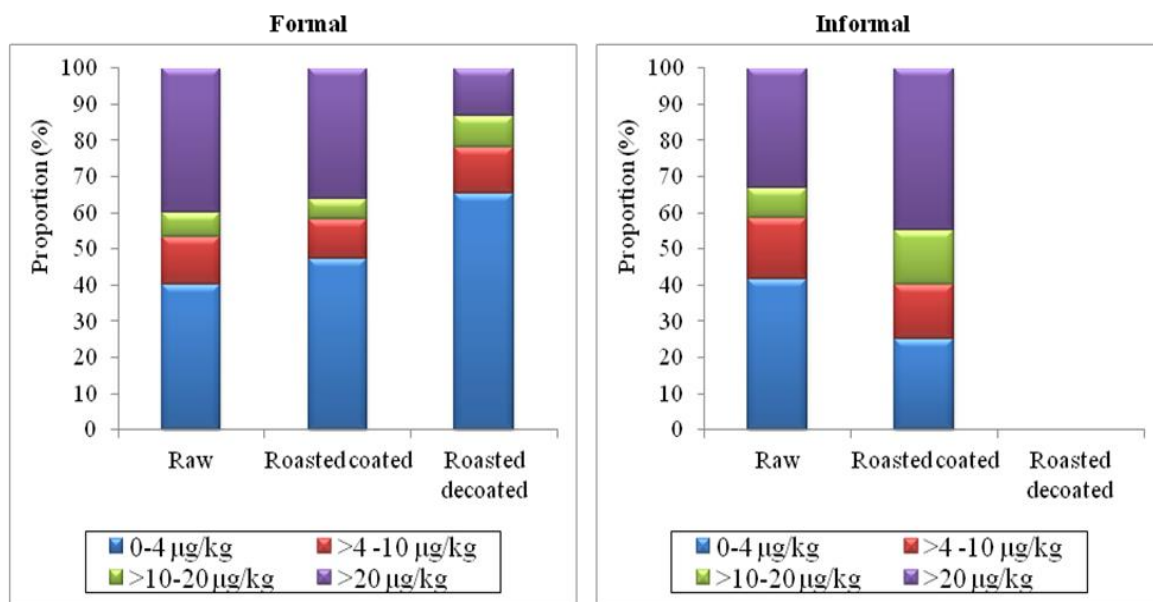
Table 4.4: Proportion (%) of aflatoxin contamination level categories (µg/kg) for peanut products sampled from formal and informal market outlets in Eldoret and Kericho towns, Rift Valley, Kenya.

Peanut product	Formal market outlets					Informal market outlets				
	0-4	>4 -10	>10-20	>20	n	0-4	>4 -10	>10-20	>20	n
a. Eldoret Town										
Raw	40	13.3	6.7	40.0	15	41.7	16.7	8.3	33.3	24
Roasted	47	11.1	5.6	36.1	36	25.0	15.0	15.0	45.0	20
Roasted de-	65	13.0	8.7	13.0	23	-	-	-	-	
Mean	50	12.5	7.0	29.7		33.4	15.9	11.7	39.2	
b. Kericho Town										
Raw		0.0	20.0	33.3	15	33.3	4.2	8.3	54.2	24
Roasted coated	26	16.7	20.0	36.7	30	53.3	13.3	13.3	20.0	15
Roasted de-coated	71	19.0	0.0	9.5	21	60.0	0.0	20.0	20.0	5
Mean	48	11.9	13.3	26.5		48.9	5.8	13.9	31.4	

- Represents missing samples for the types of product that were not on the market during the sampling period.

The proportion of peanut product samples from formal market outlets (supermarkets and shops) in the different aflatoxin level categories was as follows: <4 µg/kg (48.6%), 4 - 10 µg/kg (12.9%), >10-20 µg/kg (10.0%) and > 20 µg/kg (28.6%) (Figure 18). More than a third (38.5%) of the 140 peanut samples had total aflatoxin contamination level above the 10 µg/kg limit set by KEBS. The proportion of peanut product samples from informal market outlets (open markets and hawkers) in the different aflatoxin level categories was as follows: <4 µg/kg (38.6%), 4 - 10 µg/kg (11.4%), >10-20 µg/kg (11.4%) and > 20 µg/kg (38.6%). Fifty percent of the 88 peanut samples from informal market outlets had total aflatoxin contamination level above the 10 µg/kg limit set by KEBS.

Eldoret market outlets



Kericho market outlets

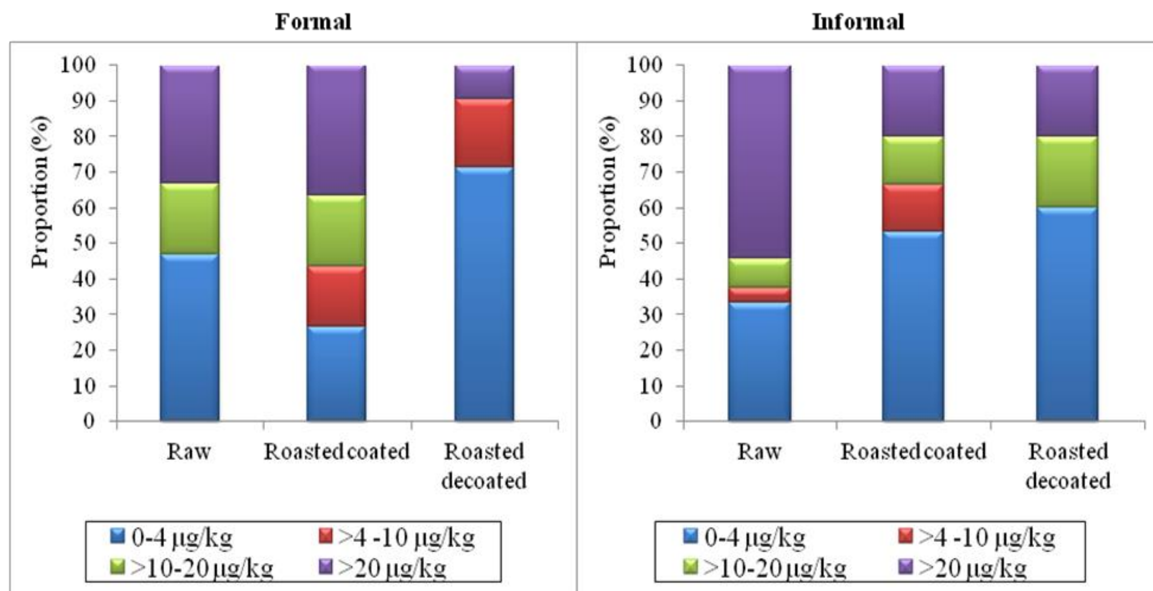


Figure 4.14: Proportion (%) of aflatoxin contamination level categories (µg/kg) for peanut products sampled from formal and informal market outlets in Eldoret and Kericho towns, Rift Valley, Kenya.

4.9. Correlation Between Sample Type, Market Type, Population of Aflatoxigenic Species and Aflatoxin Contamination

The population of *A. flavus* and *A. parasiticus* in raw peanuts had significant positive correlation with aflatoxin level (R^2= 0.69; $p \leq 0.05$) (Figure 4.15 and Appendix 2). However, there was no significant ($p \geq 0.05$) correlation for the same in roasted peanuts (Figure 4.16, Figure 4.17 and Appendix 2). In Eldoret town, aflatoxin levels in raw peanuts from informal markets were not significantly correlated ($R^2 = 0.06$; $p \geq 0.05$) with the population of aflatoxin-producing species (*A. flavus* L strain, *A. flavus* S strain, *A. parasiticus*) isolated from peanut samples. However, in formal markets, the combined population of aflatoxigenic species (*A. flavus* L strain, *A. flavus* S strain, *A. parasiticus*) significantly influenced the levels of aflatoxin produced (Linear regression, $R^2 = 0.63$; $p \leq 0.05$). Aflatoxin levels in roasted coated peanuts from informal markets were not significantly correlated (R^2 =0.09; $p \geq 0.05$) with the population of the major aflatoxigenic fungal species. However, in formal markets, the population of *A. flavus* S strain significantly positively correlated with the levels of aflatoxin produced ($R^2 = 0.37$; $p \leq 0.05$). The levels of aflatoxin produced was not significantly correlated ($R^2 = 0.102$; $p \geq 0.05$) with the population of aflatoxin producing fungal species isolated from roasted de-coated peanut samples in formal markets.

In Kericho, correlation was highly significant between aflatoxin concentration and the population of both *A. flavus* L strain and *A. flavus* S strain ($R^2 = 0.807$; $p \leq 0.05$) in raw peanuts from informal markets. However, in raw peanuts from formal markets, only the population of *A. flavus* S strain significantly influenced aflatoxin production ($R^2 = 0.48$; p

≤ 0.05). For roasted coated and de-coated peanuts from all types of markets (formal and informal), aflatoxin production was not significantly correlated with the population of aflatoxin producing fungal species ($p \geq 0.05$).

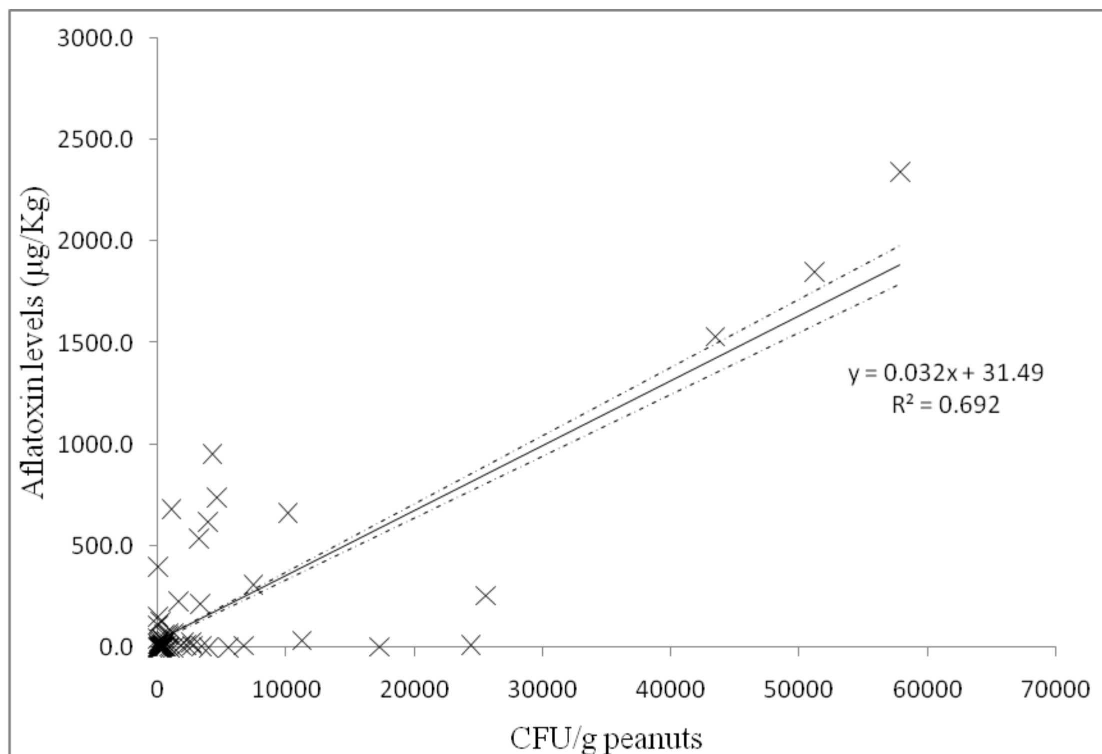


Figure 4.15: Scatter plot of the population [CFU/g peanuts] of *Aspegillus flavus* (L and S strains) and *A. parasiticus* against aflatoxin level in raw peanuts marketed in Kericho and Eldoret towns, Rift Valley, Kenya. Aflatoxin levels = 31.50 ($p \geq 0.05$) + 0.032038 CFU ($p \leq 0.05$; $R^2 = 0.69$).

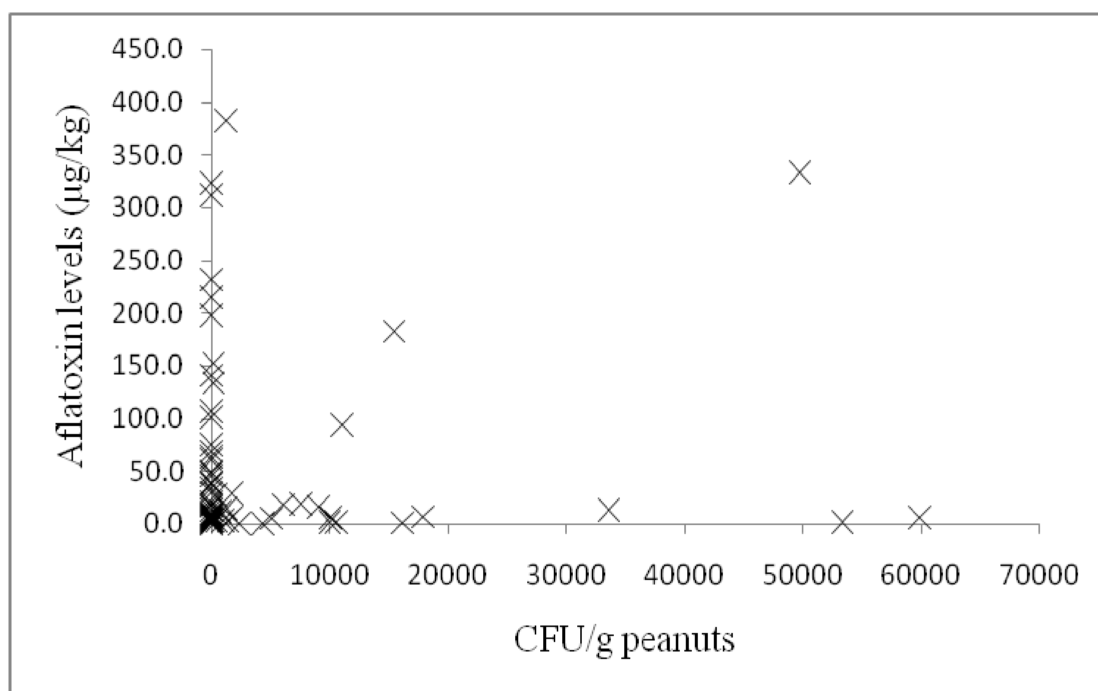


Figure 4.16: Scatter plot of the population [CFU/g peanuts] of *Aspegillus flavus* (L and S strains) and *A. parasiticus* against aflatoxin level in roasted coated peanuts, marketed in Kericho and Eldoret towns, Rift Valley, Kenya. No significant correlation ($p \geq 0.05$).

Figure 4.17: Scatter plot of the population [CFU/g peanuts] of *Aspegillus flavus* (L and S strains) and *A. parasiticus* against aflatoxin level in roasted de-coated peanuts, marketed in Kericho and Eldoret towns, Rift Valley, Kenya. No significant correlation ($p \geq 0.05$).

CHAPTER FIVE

5.0. DISCUSSION

5.1. General Overview on Quality of Peanuts in Kenya

In Kenya peanuts are produced under small holder conditions characterized by mechanical damage to pods, poor harvesting, drying and storage methods that predispose peanuts to fungal contamination and subsequent aflatoxin production (Mutegi *et al.,* 2007). In addition, marketing of most peanuts is through informal market outlets where environmental conditions favour fungal growth (Mutegi *et al.,* 2010; Mutegi, 2010). Researchers have established that peanuts at market level are more contaminated with aflatoxin than those stored by farmers (Kaaya, 2005; Mutegi *et al.*, 2010). In the market, peanuts are not properly protected from environmental influence and are not properly packaged; making them susceptible to fungal contamination. The peanuts collected from Eldoret and Kericho towns were not grown there but were from different parts of Kenya and neighbouring countries. It is therefore, probable that transportation, storage conditions and handling influenced contamination leading to the decline in quality for these products at the selling points.

5. 2. Incidence of Aflatoxin Producing Fungi in Peanut Products

The fungal species isolated from different peanut products sampled from formal and informal markets in Eldoret and Kericho towns were *A. flavus* L strain, *A. flavus* S strain, *A. parasiticus, A. tamarii, A. caelatus, A. alliaceus* , *A. nige*r, and other fungal genera that included *Mucor, Rhizopus, Fusarium* among others that were unidentified. The study established that out of 228 peanut samples, 67% were contaminated with the major

aflatoxigenic fungi (*A. flavus* L strain, *A. flavus* S strain and *A. parasiticus*). The incidence of these fungi was similar as previously reported in peanuts from western Kenya (Mutegi, 2010). Two morphotypes of *A. flavus,* the S and L strains isolated in this study have also been identified in previous studies in Kenya (Mutegi, 2010; Mutegi *et al.*, 2012).

The existence of *A. tamarii, A. alliaceus*, and *A. caelatus* in peanuts is also documented by Mutegi *et al.* (2012). The incidence of fungal species in raw peanuts was significantly different between formal and informal market types both in Eldoret and Kericho towns. Generally, in both towns, peanuts from informal markets tend to have higher fungal species diversity in raw peanuts and this observation concurs with the findings by Gachomo *et al.* (2004). Results also show that the type of market outlets influenced the incidence of pathogenic fungi in peanuts. This could be attributed to the superior packaging, sorting and storage conditions (ventilated stores) that were found in formal markets. In contrast, raw peanuts marketed in informal markets were not generally packaged or sorted and were stored in stalls exposed to weather fluctuations. In addition, some peanuts were marketed in open air systems subjecting them to weather changes and abrupt rainfall which could promote fungal proliferation. Furthermore, one container used to weigh the different products, as commonly practiced in open markets, may vector microbes from one bag to another through dust content.

The incidence of aflatoxin producing fungi was significantly higher in peanuts sampled from markets in Kericho than in Eldoret town. The reason for these findings is that in

Eldoret, some raw peanuts from informal markets were being marketed under covered places compared to most of those from Kericho that were marketed under open air. Based on information gathered through personal interviews with vendors, hawkers in Eldoret bought small quantities (1 kg on average) of raw peanuts from open markets, roasted and sold them within one day thereby reducing the time interval between roasting and selling. The practice was different in Kericho where most packaged roasted peanuts were supplied and not processed by hawkers themselves. Generally, peanut roasting and de-coating processes reduce fungal population present in and/or on kernels (Kaaya and Harris, 2006). Indeed, during roasting process, peanuts are exposed to dry heat at high temperatures (Okello *et al*., 2010) that kill or reduce the present fungal population. According to Harris (1999) burning charcoal can produce fire temperature well over 1000°C. Charcoal made at 300°C (572 °F) readily inflames at 380 °C (716 °F); the one made at higher temperatures does not fire until heated to about 700 °C (1,292 °F) (Chisolm, 1910). However, in this study, roasting did not reduce peanuts contamination by aflatoxigenic fungi in Kericho. The exact factors contributing to this were not established in this study. Nevertheless, possible causes could include weather fluctuations, handling practices and long time interval between roasting and selling. In Kericho, some peanuts stayed between 1 month and 6 months before they were sold. According to Okello *et al*., (2010), peanuts purchased should not be stored for more than three months.

Among the isolated species, *A. tamarii* produces $AFB_1$and AFB_2 and cyclopiazonic acid (Goto *et al.*, 1996) while *A. alliaceus* produces ochratoxin (Bayman *et al.*, 2002).

Ochratoxin is reported to be nephrotoxic, hepatotoxic, immunotoxic and possibly neurotoxic (MacLauchlin and Little, 2007). *Fusarium* spp. produce fumonisins, zearalenone and trichothecenes among other mycotoxins while *Penicillium* spp. produce ochratoxin A and patulin (Samson *et al.,* 1995). Although the above toxins were not the subject of investigation in this study, the presence of fungal species known to produce them implies a greater health risk to consumers of peanut products. In addition, this observation reveals the need for management strategies that target the control of both aflatoxin-producing fungi and those fungi that produce other types of mycotoxins.

5.3. Aflatoxin Contamination Levels in Peanut Products

The sample type, market type, population of aflatoxin producing fungi significantly influenced aflatoxin contamination levels in peanuts. Aflatoxin levels ranged from 0 to 684.8 µg/kg and 0 to 2344.8 µg/kg in samples from formal and informal markets, respectively. These findings are in agreement with the findings of Mutegi *et al.* (2012) who reported aflatoxin levels ranging from 0 to 2687.6 µg/kg and 0 to 1838.3 µg/kg in peanuts from Busia and Homa bay regions in Western Kenya. The process of peanut packaging reduces exposure to environmental conditions that influence fungal proliferation and subsequent aflatoxin production. In addition, fungi and aflatoxin are not homogeneously distributed in peanuts; sorting which involves removal of defective kernels and foreign materials also reduce the risk of aflatoxin contamination (Bankole and Adebanjo, 2003; CAC/RCP, 2004; Kaaya *et al.*,2006; N'dede; 2009; Battilani, 2010; Okello *et al.*, 2010; Filbert and Brown, 2012). Filbert and Brown (2012) reported that hand sorting peanuts reduced aflatoxin concentration by 98% and that should be done

before storage and on kernels before processing into other food products. These practices are applied on peanuts marketed in formal markets and may have been responsible for low level of detected aflatoxin as compared to high levels found in informal markets.

About 43% of peanut samples from Kericho and Eldoret towns contained unsafe levels of total aflatoxin based on KEBS standards. This proportion compares favourably with the findings of Mutegi *et al.* (2010) who reported contamination levels of 38% of peanut samples from Nairobi and western Kenya. It is now evident that aflatoxin contamination of peanuts should be a public health concern not only in Eldoret and Kericho towns but also in other parts of Kenya, as well as in other tropical countries such as Botswana (Mphande *et al.*, 2004), Senegal (Diop *et al.*, 2000), Benin (N'Dede, 2009), Sudan (Omer *et al.*, 1998) and Brazil (Oliveira *et al.*, 2009).

Mphande *et al.*, (2004) reported that 49% of raw peanut samples purchased from retail outlets in Botswana contained aflatoxin level above 20 µg/kg limit set by the World Health Organization, while Diop *et al.*, (2000), found a mean content of about 40 µg/kg of aflatoxin B_1 in over 85% of peanut oil samples from Senegal. In Sudan, Omer *et al.*, (1998) found high aflatoxin content of 25 to 600 µg/kg in peanuts. Different levels in aflatoxin contamination were also reported in peanuts collected from processors, stockers, farmers and traders in Benin (N'Dede, 2009) while Oliveira *et al.*, (2009) reported total aflatoxin level of 56 µg/kg in unprocessed peanuts in Brazil. High incidence of contamination in raw peanuts (83% of raw peanut samples having the highest levels of aflatoxin averaging 340.2 µg/kg) corroborated findings from Botswana

by Mphande *et al.*, (2004) who reported a contamination incidence of 78% of raw samples, with aflatoxin concentration ranging from 12 to 329 µg/kg.

Generally, there was higher risk of exposure to aflatoxin through raw than roasted peanuts. Roasting contributes to the killing or reduction of fungal population on and/or in peanut kernel hence reducing the potential of aflatoxin production (CAC/RCP, 2004; Jacobsen *et al.*, 2007). However, roasted de-coated peanuts also had high percentage (74%) of contaminated samples but the concentration of aflatoxin was relatively low with an average of 7.9 µg/kg. Previous studies have established that exposure of humans to high levels of aflatoxins leads to acute aflatoxicosis and that long-period of exposure to aflatoxins, even in low concentration, may lead to liver cancer, stunted growth in children and to immune system disorders through chronic aflatoxicosis (Wild and Gong, 2010; Wu and Khangwiset, 2010).

A part from the type of peanut products, the current study revealed that the level of contamination of peanuts marketed in Kericho and Eldoret towns was influenced by the location (town), type of market (formal versus informal) and product processing (roasting and decoating).

5.4. Relationship Between Population of Aflatoxin-producing Fungi and Aflatoxin Contamination Level

There was strong positive correlation between aflatoxin-producing fungi and total aflatoxin levels detected in raw peanuts. However, total aflatoxin levels in roasted (coated

and de- coated) peanuts from both formal and informal markets were not significantly correlated with the population of aflatoxigenic fungal species. Previous studies have reported that roasting and de-coating processes reduce fungal population and aflatoxin contamination (Jacobsen *et al.*, 2007; Mutegi, 2010).

The population of *A. flavus* S strain was found to significantly influence aflatoxin production. This concurred with the findings by Mutegi *et al.* (2012) who found that the incidence and the number of colonies of *A. flavus* S strain significantly and positively correlated with levels of total aflatoxin in peanuts. The presence of *A. flavus* S strain implies a major health problem to consumers of peanuts because it has been reported to produce greater amount of aflatoxin especially aflatoxin B_1 (Mutegi *et al.,* 2012) which is also classified as class 1 carcinogen (IARC, 1987). *Aspergillus flavus* S strain produces greater quantities of aflatoxin than *A. flavus* L strains (Cotty and Jaime- Garcia, 2007). Hedayati *et al.*, (2007) identified two types of S strain isolates termed SB and SBG that produce B aflatoxins and both B and G aflatoxins, respectively. Jaime-Garcia and Cotty (2010), reported S strain to be the primary cause of contamination events in North America and Africa. Humans are exposed to aflatoxin through diet (Bommakanti and Waliyar, 2000). Care must therefore be exercised to avoid poor conditions that support growth of fungal and aflatoxin contamination during storage of food commodities. Peanut traders and consumers should be aware of aflatoxin and the health risks associated with consumption of contaminated products.

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The incidence of aflatoxin producing fungi (*Aspergillus flavus* L strain, *A. flavus* S strain, *A. parasiticus)* in peanut products marketed in Eldoret and Kericho towns was high (up to 76%) but the levels of contamination differed between formal and informal markets. The highest population of aflatoxin-producing fungi was recorded in raw peanuts sampled from informal market outlets. The high incidence of aflatoxigenic fungi in peanuts and peanut products implies poor quality of peanuts marketed in Eldoret and Kericho towns, a high risk for aflatoxin production and health risk to consumers of peanut products. There is therefore need to improve quality standards of marketed peanuts.

Forty three percent of the peanut samples contained aflatoxin levels beyond the 10 µg/kg regulatory maximum limit set by KEBS, thus unsafe for human consumption. This implies that consumers of peanuts and peanut products in Kenya are at a health risk as a result of chronic exposure to aflatoxin levels higher than the recommended limits set by KEBS.

The significantly higher aflatoxin contamination of raw peanuts (mean = 146.8 ppb) compared to roasted de-coated peanuts (mean = 19.9 ppb) implies that processing - combining roasting and decoating - potentially reduces the incidence of aflatoxin-producing fungi and aflatoxin production in peanuts.

6.2. Recommendations

a) Roasting of peanuts by vendors should be promoted as it reduces fungal and aflatoxin contamination.

b) Awareness raising is important among peanut vendors (traders) in Kericho and Eldoret on proper handling of peanut products and the health risks posed to consumers as a result of consuming unsafe peanut products.

c) Awareness should be raised among consumers of peanut products on the health risks associated with consuming products which do not meet the standards set by the Kenya Bureau of Standards.

d) Controlled experiments should be conducted to establish other specific factors that may be influencing fungal and aflatoxin contamination of peanuts during storage.

e) There is need for Kenya Bureau of Standards to periodically monitor and check the safety (quality) of peanuts being marketed in various market outlets in Kenya.

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APPENDICES

APPENDIX 1: SAMPLING AND PEANUT SAMPLE SIZES

Appendix 1.1. Sampling source of raw peanut samples from formal market outlets in Kericho and Eldoret towns, Rift Valley, Kenya.

Groundnuts Packaging agencies	Kericho	Eldoret
Kamili Packers	4	0
Tuskys Nairobi	11	5
Transmat Kitale	0	5
Transmat Eldoret	0	5
Total no. samples*	**15**	**15**
Quantity (kg)	**7.5**	**7.5**

* 1 sample = 0.5 kg of peanuts

Appendix 1.2. Number of coated roasted peanuts and respective processing companies for samples collected from formal market outlets (Supermarkets and retail Shops) in Eldoret and Kericho towns, Rift Valley, Kenya

Peanuts processing industries / Enterprise	**Kericho**	**Eldoret**
Pioneer Food	3 (SM)	3(SM)
Kibagare Nuts	-	-
Gnomis	-	3(SM)
Weatbee	-	3(SM)
Leakims	-	3(SM)
Munii	-	2(SM)
Deepa	3(SM)	3(SM)
Pisani Snacks(Couger)	-	3(SM)
Deluxe food	3(SM)	3(SM)
Chirag	3(SM)	3(SM)
Elly Products	3(SM)	-
T.G.S(Kericho)	3(SM)	-
Gravir	3(SM)	-
Galaiya Food	3(SM)	-
Primarava Picnick Snacks	3(SM)	-
Ricky's(Saima)	3(SM)	-
Locally sealed air tight plastic papers	0	10 (RS**)
Total no. samples	**30 (SM)+0 (RS) =30**	**26 (SM) + 10 (RS) =36**
Quantity (kg)	**15 (SM) + 0 (RS) = 15**	**13 (SM) + 5 (RS) =18**

- No peanuts were available at the time of sampling; SM - supermarkets outlet; RS = Retail shop

Appendix 1.3. Number of de-coated roasted peanuts and respective processing companies for samples collected from formal market outlets (Supermarkets and retail Shops) in Eldoret and Kericho towns, Rift Valley, Kenya

Peanuts processing industry / Enterprise	**Kericho**	**Eldoret**
Pioneer Food	3(SM*)	3(SM)
Kibagare Nuts	-	1(SM)
Gnomis	-	3(SM)
Weatbee	-	3(SM)
Leakims	-	1(SM)
Munii	-	-
Deepa	3(SM)	3(SM)
Pisani Snacks(Couger)	-	3(SM)
Deluxe food	3(SM)	3(SM)
Chirag	3(SM)	3(SM)
Elly Products	3(SM)	-
T.G.S(Kericho)	-	-
Gravir	3(SM)	-
Galaiya Food	-	-
Primarava Picnick Snacks	3(SM)	-
Ricky's(Saima)	-	-
Locally sealed air tight plastic papers	-	-
Total No. of samples	**21 (SM) + 0 (RS**) =21**	**23 (SM)+0 (RS) = 23**
Quantity (kg)	**10.5 (SM) + 0 (RS) = 10.5**	**11.5 (SM) + 0 (RS) = 11.5**

- No peanuts were available at the time of sampling; SM - supermarkets outlet; RS = Retail shop.

Appendix 1.4. Types of packaging materials for coated roasted peanuts and the respective number of samples collected from informal market outlets (Streets hawkers) in Eldoret and Kericho towns, Rift Valley, Kenya

Packaging mode	Kericho	Eldoret
Sealed air tight plastic papers	10	10
Wrapped in used newspapers	5	10
Total samples*	**15**	**20**
Quantity (kg)	**7.5**	**10**

* 1 sample = 0.5 kg of peanuts

Appendix 1.5: Types of packaging materials for de-coated roasted peanuts and the respective number of samples collected from informal market outlets (Streets hawkers) in Eldoret and Kericho towns, Rift Valley, Kenya

Packaging mode	**Kericho**	**Eldoret**
Sealed air tight plastic paper (samples*)	5	-
Wrapped in used newspapers (samples)	-	-
Total samples	**5**	**0**
Quantity (kg)	**2.5**	**0**

* 1 sample = 0.5 kg of peanuts

- means that the product was not found on sale during the sampling period

APPENDIX 2: ANOVA OUTPUTS FOR CORRELATION BETWEEN POPULATION OF AFLATOXIN PRODUCING SPECIES AND AFLATOXIN CONTAMINATION LEVELS IN DIFFERENT PEANUT PRODUCTS

Appendix 2.1. Analysis of Variance for Raw peanuts

Source	DF	SS	MS	F	P
Regression	1	8860886	8860886	171.35	0.000
Residual Error	76	3930177	51713		
Total	77	12791062			

Appendix 2.2. Analysis of Variance for Roasted coated peanuts

Source	DF	SS	MS	F	P
Regression	1	3409	3409	0.46	0.501
Residual Error	75	560343	7471		
Total	76	563753			

Appendix 2.3. Analysis of Variance for Roasted de-coated Peanuts

Source	DF	SS	MS	F	P
Regression	1	1391	1391	0.58	0.455
Residual Error	20	47904	2395		
Total	21	49295			