ASPERGILLUS SPECIES AND AFLATOXIN CONTAMINATION IN PRE- AND POST-HARVEST PEANUTS IN BARINGO, ELGEYO-MARAKWET AND MERU COUNTIES IN KENYA

WILFRED AYUGI OSAO
B. Sc. (MICROBIOLOGY AND BIOTECHNOLOGY), UNIVERSITY OF NAIROBI

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DECLARATION

This is my original work and has not been submitted for award of a degree in any other University.

Wilfred Ayugi Osao

Signature: ------------------------------------- Date: -----------------------------------

This thesis was submitted for examination with our approval as the University supervisors:

Dr. Maina Wagacha

School of Biological Sciences

University of Nairobi

Signature: -- Date: --------------------------------

Prof. James W. Muthomi

Department of Plant Science and Crop Protection

University of Nairobi

Signature: ------------------------------------ Date: ---------------------------------

DEDICATION

I dedicate this thesis to my family who have been supportive of me, Mr. Parbat; CEO Lalji Meghji Patel and co. ltd who is my mentor and role model. God bless you all.

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LIST OF ABBREVIATIONS

AEZ – Agro-ecological zone

ANOVA - Analysis of Variance

AFS- *Aspergillus flavus* S-strain

AFL - *Aspergillus flavus* L-strain

AP – *Aspergillus parasiticus*

AN – *Aspergillus niger*

CABI – Commonwealth Agricultural Bureau International

CAST – Council for Agricultural Science and Technology

CDC - Centers for Disease Control and Prevention

CFU - Colony Forming Units

CV – Coefficient of Variation

ELISA – Enzyme Linked Immuno-Sorbent Assay

EU – European Union

EC - European Commission

ENP - Equatorial Nut Processors

FAO – Food and Agriculture Organization of the United Nations

GAP - Good Agricultural Practices

ICRISAT - International Crops Research Institute for the Semi-Arid Tropics

KARI – Kenya Agricultural Research Institute

KEBS – Kenya Bureau of Standards

LSD – Least Significant Difference

MDRB - Modified Dichloran Rose Bengal Agar

NDP – National Development Plan

nm - nanometer

PPB - Parts per Billion (equivalent to μg/kg)

RH - Relative Humidity

UoN - University of Nairobi

USAID -United States Agency for International Development

WFP - World Food Program

WHO – World Health Organization

ABSTRACT

Aflatoxin contamination is a major challenge in peanut (*Arachis hypogaea* L.) production and trade besides posing health challenges to humans and animals. This study monitored the population of *Aspergillus* spp. and aflatoxin contamination of peanuts at pre- and post-harvest stages to establish the critical aflatoxin contamination points during peanut production so as to recommend interventions to promote compliance with market standards. Field monitoring was carried out in 40 farms in Meru, Baringo and Elgeyo-Marakwet Counties during the long and short rains of 2013 and 2014, respectively. A semi structured questionnaire was used to obtain information on peanut production practices. Soil was sampled at seedling stage, podding stage, two weeks pre-harvest and at harvest while plant debris was sampled at planting. Below ground peanut parts were sampled at podding stage, two weeks pre-harvest and at harvest. Peanut kernels were sampled two weeks pre-harvest, at harvest, from farmer stores, buyer collection point and at the processing factory after shelling. *Aspergillus* spp. were isolated from soils, kernels, plant parts and plant debris on modified Dichloran Rose Bengal (MDRB) agar medium and identified to species level. Total aflatoxin in peanut kernels was analyzed by indirect competitive Enzyme Linked Immuno-Sorbent Assay (ELISA). Aflatoxin levels were grouped into three categories based on the standards set by the Kenya Bureau of Standards (KEBS) and the European Commission (EC).

Majority (70%) of the peanut farmers were small scale producers who did not apply soil amendments, practiced mixed cropping and ploughed their fields manually or used animal ploughs. Maize was the main rotation crop with 35% of the farms having maize, only farmers in Meru North and Meru South making 62% of the farms used irrigation with splinkler and furrow being the main irrigation methods. Prevalence of peanut diseases significantly ($P \leq 0.05$) varied in the different regions and agro-ecological zones (AEZ) with 40% of the farmers saying they were aware of the recommended method of drying peanuts. However, most (60%) farmers had

no previous knowledge on aflatoxin and those who did, think it is only a problem in maize. *Aspergillus flavus* S-strain, *A. flavus* L-strain, *A. parasiticus* and *A. niger* were isolated from soil, crop debris, below ground peanut parts and kernels sampled at various stages pre-and post-harvest. *Aspergillus flavus* L-strain was the most prevalent fungus in kernel samples from Baringo and Elgeyo-Marakwet Counties with 350 to 4,000 CFU/g kernel whereas *A. flavus* S-strain was highest in kernel samples from Meru County with 50 to 60,000 CFU/g kernel. Aflatoxin levels varied depending on sampling time with the levels being significantly ($p \leq 0.05$) lower in samples taken two weeks before harvest in all the regions during the long rains of 2013. Overall, 80% of kernels sampled from Meru at all stages did not meet the European Commission aflatoxin limit of ≤4 μg/kg while 73% did not meet the KEBS limit of ≤10 μg/kg. All the kernel samples from Baringo Central and Keiyo South were within the safe limits set by the EC and KEBS. There was a positive significant ($p \leq 0.05$) correlation between the population of *A. flavus* S-strain and aflatoxin levels in kernels sampled from Meru County. Although *A. flavus* L-strain was the dominant fungus in samples from Baringo and Elgeyo-Marakwet Counties, there was no correlation between the population of the *A. flavus* L-strain and aflatoxin levels. The high population of *A. flavus* S-strain in peanuts sampled from Meru County implied a health risk to consumers of peanuts because the pathogen produces high amount of aflatoxin especially aflatoxin B1. Dominance of *A. flavus* S-strain, off season rains during harvesting and challenges in drying peanuts in windrows in Meru County could explain the high aflatoxin contamination of peanuts in the County. It is recommended that a control strategy of the population of major aflatoxin producing *Aspergillus* spp. and aflatoxin levels be put in place to target critical pre- and post-harvest stages of peanuts. Raising awareness on implications of aflatoxin on trade, human and livestock health and possible management strategies is also recommended.

Key words: Aflatoxin, *Aspergillus* spp., peanut.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Peanut (*Arachis hypogea* L.) also known as groundnut is one of the species in the legume family (Fabaceae). It is a high value crop nutritionally and economically and is the sixth most important oil seed crop in the world (FAO, 2005). It contains up to 50% oil, 28% protein, and is a rich source of dietary fibre, minerals and vitamins (Nigam *et al*., 2004). With the increasing cost of animal protein, peanuts have become an important source of protein in Africa (Okello *et al*., 2010). Most of the crop is produced where rainfall ranges between 600 to 1,200 mm per year, and mean daily temperatures are over 20°C. According to FAO (2008) statistics, the world peanut production rose from 14 million tons in 1961 to 47 million tons in 2006, representing a 3.2% annual growth rate. The global peanut harvested area increased at an annual rate of 0.7%, from about 17 million hectares in 1961 to about 22 million hectares in 2006.

One of the major challenges in peanut production worldwide is aflatoxin contamination, which is of great concern as this toxin has teratogenic and carcinogenic effects in humans and animals. Studies have demonstrated that chronic exposure to aflatoxins in animals can also cause growth inhibition and immune suppression (Khlangwiset *et al*., 2011). Maize and peanuts are the main sources of human exposure to aflatoxin because they are highly consumed worldwide and unfortunately are also the most susceptible crops to aflatoxin contamination (Wu and Khlangwiset, 2010). For example, 13.3 million tons of peanuts were consumed in Kenya in 2001-2003 with a projected consumption of 16.32 million tons in 2030 (Wright *et al*., 2002: Wu *et al*., 2011). Factors responsible for the high incidence of aflatoxin contamination of peanuts include poor agricultural practices during planting, harvesting, drying, transportation and storage of the product which favor fungal growth (Coulombe, 1991).

Aflatoxins are a group of extremely toxic metabolites produced by fungi, specifically *Aspergillus flavus* and *Aspergillus parasiticus* (Passone *et al*., 2010). Williams *et al.* (2004) reported that over five billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins. In 2004, 125 people died in Kenya from consuming aflatoxin-contaminated maize (CDC, 2004). Aflatoxin is also associated with several health conditions including jaundice, liver cancer, and even death (Jolly *et al*., 2007). *Aspergillus* are ubiquitous but are commonly found in warm and humid climates (Dohlman, 2003) hence most agricultural commodities from tropical countries, especially peanuts and maize, are easily contaminated with aflatoxin. Infection of peanuts by *Aspergillus flavus* which are the molds responsible for the production of aflatoxins occurs both under pre- and post-harvest conditions (Liang *et al*., 2006; Guo *et al*., 2008; Passone *et al*., 2009).

1.2 Problem statement and justification

It is estimated that more than five billion people in developing countries are at risk of chronic exposure to aflatoxin through contaminated foods (Strosnider *et al*., 2006). Aflatoxins are potent carcinogens and have many adverse effects on their consumers (Hoogenboom *et al*., 2001; Wild and Gong, 2010). High-level exposure may cause instant death while long-term chronic effects include cancer, mutagenicity and nervous disorders (KEPHIS, 2006). At the farm level, the real problem is that contaminated peanuts may appear just like the normal kernels without any outward physical signs of fungal infection. Destruction of aflatoxin by conventional food processing is difficult because they are typically resistant to heat and detection is complicated due to limitations in analytical capacity. Aflatoxins exist in different forms; B1, B2, G1, and G2 with aflatoxin B1 being the most common. The health concerns associated with aflatoxin (and

other mycotoxins) have led many countries to set standards on acceptable limits of total aflatoxin as well as different aflatoxin types.

peanuts grown and marketed in Kenya do not meet aflatoxin standards set by the European Commission (EC) (Mutegi *et al*., 2013;Wagacha *et al*., 2013). High levels of aflatoxin contamination have also been reported in peanut products traded in retail markets with 44% of the samples exceeding 10 mg/kg (Mutegi *et al*., 2013). This has broadly affected the export of peanut products to the European markets.

This study aimed at monitoring the population of *Aspergillus* species in soil, below ground peanut parts and peanut kernels from planting to harvesting. Additionally, aflatoxin levels were monitored in peanuts from two weeks pre-harvest through storage and shelling. The study also aimed at identifying pre and post-harvest stages critical for aflatoxin contamination of peanuts. Upon identification of these stages, intervention measures would be recommended to assist peanut farmers and traders achieve regulatory measures regarding aflatoxin levels set by the Kenya Bureau of Standards (KEBS, 2007) for the local market and the European Commission (EC, 2006) for the European Union market.

1.3 Objectives

The main objective of the study was to monitor the population of *Aspergillus* spp. and aflatoxin levels in pre and post-harvest peanuts to determine critical aflatoxin infection points and recommend interventions to promote compliance with statutory standards.

The specific objectives were:

i. To determine the role of peanut production practices on the population of *Aspergillus* species in soil, peanut kernels and plant parts at various stages pre-and post-harvest.

ii. To determine aflatoxin levels in peanut kernels at different pre- and post-harvest stages.

iii. To determine the effects of seasonal variation on the population of *Aspergillus* species and aflatoxin contamination of peanuts pre and post-harvest.

1.4 Hypothesis

Production practices influence the population of *Aspergillus* spp. in soil and peanut plant parts leading to infection of kernels and subsequent aflatoxin contamination.

CHAPTER TWO: LITERATURE REVIEW

2.1 Peanut production and consumption in Kenya

Peanut (*Arachis hypogaea* L.*)* is a four-foliate legume of the family *Fabaceae* although it is widely identified as a nut and has similar nutrient profile with tree nuts (Ros, 2010). Native to South America, peanut is mainly produced in China, India, the United States of America and many Sub-Saharan African countries. According to (FAO, 2002), developing countries account for approximately 95 percent of world peanut production. In Kenya, the crop is mainly grown in parts of the Nyanza and Western regions, and to a lesser extent in the Rift valley, Coast and Eastern regions (USAID, 2010). Of the 25,098 hectares planted with peanuts in 2008, Nyanza accounted for 75.6%, Western for 16.3%, Eastern for 4.1% and Rift Valley for 4% of the total area. Nyanza and Western regions account for 89% of peanuts output while 11% is produced in the rest of the country (USAID, 2010).

In these regions, peanut is significant both as a cash and food crop, and has at least two harvest seasons per year except in some parts of Rift valley where there is only one season. Most commercially available peanuts are processed by small and micro-enterprises, a sector that contributes approximately 18 per cent of Kenya's gross domestic product (Mitullah, 2003). Peanuts are processed at home and marketed through informal marketing systems where products are seldom tested for aflatoxin contamination (Wu, 2004). Common types of peanuts grown include, Homa bay local, Valencia Red, ICGV 12988, ICGV 12991, JL24 and CG7, the latter four being improved varieties introduced to Kenya by ICRISAT (Mutegi *et al*, 2010).

Systems of production include intercrops with maize, sugar cane, sorghum, finger millet, and small pockets of pure stand. Due to the small scale nature of production of peanuts, the use of

fertilizers and pesticides is not common (Mutegi *et al*., 2009). The crop is used for subsistence, cash-income and provides raw materials for agro-based industries. As human food, peanut is used in the form of raw, boiled or roasted nuts, as edible oil and protein by being pounded and used as a vegetable oil for cooking, or made into paste and eaten with sweet potatoes, cassava, banana among other dishes. For cash, peanut is sold in the local market as boiled unshelled and shelled roasted nuts while some is sold in the confectionery trade (Kiriro, 1993). As a legume, peanuts improve soil fertility in the farming systems by fixing atmospheric nitrogen and also as trap-catch crop in the management of Striga weed in cereal crop (Kiriro, 1993). Production of peanuts in Kenya in 2010 was 19,000 tons (FAOSTAT, 2012). With an estimated per capita consumption of 0.5 kg/ca (USAID, 2010). Peanut is considered a highly nutritious food, in terms of protein (38.6 %), and oil (47 %) contents (Atasie *et al*., 2009). It has also been shown to have other health benefits such as reduced risk in developing type II diabetes (Jiang *et al*., 2002) and cardio vascular disease (Kris-Etherton *et al*., 1999). With the increasing cost of animal protein, peanuts have become an important source of protein in Africa (Okello *et al*., 2010).

2.2 Aflatoxins and their occurrence in peanuts

Aflatoxins are naturally occurring secondary metabolites that are produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (CAST, 2003; Passone *et al.,* 2010) which mostly infect peanuts as a complex (Varga *et al.,* 2012). They were first discovered in England in the early 1960s when more than 100,000 young turkeys on poultry farms died within a few months (Blout, 1961) after consuming an *A. flavus* contaminated peanut meal that had originated from Brazil. The disease contracted by these animals was called Turkey X disease (Klich *et al*., 2000; Papp *et al*., 2002).

The name aflatoxin was derived from the fact that it was originally found to be produced by *Aspergillus flavus* (Agrios, 1978), but currently is known to be produced by other *Aspergillus* species in the section *Flavi* like *A. flavus* and *A. parasiticus* (Bennett and Klich, 2003) on a number of food commodities. *Aspergillus flavus* and *A. parasiticus* are the most important species in agriculture as they are found throughout the world being present in both soil and air (Klich, 2002). When the spores of these species find a suitable nutrient source and favorable environmental conditions they rapidly colonize, establish and produce aflatoxins (Payne, 1992) on susceptible crops. The optimum growth condition for *A. flavus* during post-harvest are between $25^{o}C$ and $30^{o}C$ and humidity levels of 0.99_{aw}, with production of aflatoxin occurring optimally at $25^{o}C$ and 0.99_{aw} (Giorni *et al*., 2009). Several types of aflatoxin exist, but the four main types are aflatoxin B1, B2, G1 and G2, with aflatoxin B1 being the most toxic (Olaru *et al*., 2008). A common metabolite of Aflatoxin B1 and B2 is aflatoxin M1 and M2 found in the milk of animals that have consumed contaminated feed (Bahout and El-Abbassy, 2004). While both *A. flavus* and *A. parasiticus* can produce the B toxins, *A. parasiticus* which is more prevalent in peanuts than in other crops, also produces the G toxins (Diener *et al*., 1987).

Aflatoxin is found in many food commodities, but common substrates are maize and peanuts. Peanuts in Kenya are frequently infected by *A. flavus* and *A. parasiticus* (Mutegi *et al*., 2012; Wagacha *et al*., 2013) and this infection can occur at all stages in the peanut value chain (Waliyar *et al*., 2005). Humans and animals come into contact with aflatoxin through several channels such as direct ingestion of contaminated products (Wagacha and Muthomi, 2008), transmission through milk as M1 and M2 metabolites (Bahout and El-Abbassy, 2004), and through consuming the meat of animals reared on contaminated feed. The toxin can also pass through human skin (Wagacha and Muthomi, 2008) through direct contact with contaminated

produce. Aflatoxin can also pass through the respiratory system, especially in people engaged in peanut harvesting, shelling, storage, marketing and transportation (Mehan *et al*., 1991). Peanuts and maize are the main sources of human exposure to aflatoxin due to their high level of consumption e.g. 13.3 million tons of peanuts were consumed in Kenya in 2001-2003 with a projected consumption of 16.32 million tons in 2030 (Wu *et al*., 2011). Pre-harvest and post-harvest management strategies for reducing aflatoxin in food result in lower productivity, but better quality. This is confirmed by Hell *et al*. (2008), who state that commodities contaminated with aflatoxin have a lower market value and cannot be exported. In past studies, there has been an increased level of contamination in peanuts further up the commodity chains (Kaaya *et al*., 2006), possibly due to poor post-harvest management practices.

Contamination of many dietary staple crops with *Aspergillus* section *Flavi* (Cotty *et al*., 1994) and the subsequent production of aflatoxins is considered to be one of the most serious food safety problems worldwide (Williams *et al*., 2004). Presence of aflatoxin in human food cause acute and chronic health effects depending on the duration and level of exposure. Aflatoxins cause the most concern due to their carcinogenic, immune-suppressing and growth-retardation effects in both humans and animals (Williams *et al*., 2004). They also cause economic losses in international trade when toxin contamination exceeds permissible levels (Wu, 2006). Food contamination with aflatoxins depends on environmental conditions, particularly temperature and water activity (Weidenborner, 1998), as well as *Aspergillus* strain composition (Probst *et al*., 2007).

2.4 The genus *Aspergillus*

The genus *Aspergillus* is a deuteromycetes, composed of about 180 anamorphic species with teleomorphs described in nine different genera (Pitt *et al.,* 2000). Their teleomorphs can be found in the Ascomycete (Pelczar *et al*., 1998) structures that produce sexual spores (ascospores), endogenously in a well differentiated ascocarp. According to Klich, (2002), the genus is subdivided into seven subgenera, which in turn are further divided into sections.

Aspergillus subgenus *Circumdati* section *Flavi*, also refered to as the *Aspergillus flavus* group, has attracted worldwide attention for its industrial use and toxigenic potential. Section *Flavi* is divided in two groups of species. One include the aflatoxigenic species *A. flavus, A. parasiticus* and more recently *A. nomius* which cause serious problems worldwide in agricultural commodities. The other includes the non-aflatoxigenic species *A. oryzae*, *A. sojae* and *A. tamarii*, traditionally used for production of fermented foods in Asia (Kumeda and Asao, 2001). Four species not in section *Flavi* are known to produce aflatoxin: *A. ochraceoroseus*, *A. rambellii, Emericella venezuelensis* and *E. astellata* (Frisvad *et al*., 2005). The latter two species have *Aspergillus* anamorphs (asexual states).

Aspergillus section *Flavi* are a group of fungi that are found throughout the world and are present in soil and the air, hay, decaying vegetation and grains under microbial deterioration (Klich, 2002). Although aflatoxin producing fungi occur in three sections of the genus *Aspergillus*, section *Flavi* contains the greatest number of potential aflatoxin producers (Pildain *et al*., 2008). *Aspergillus flavus* and *A. parasiticus* are the two fungal species in the *Aspergillus* section *Flavi* group that are most important in agriculture. These species spend most of their life growing as saprophytes in the soil (Accinelli *et al*., 2008). Based on morphological, genetic and physiological criteria (Ehrlich *et al*., 2003). *Aspergillus flavus*, the main aflatoxin producing

species, can be divided into two morphotypes, the S and L strains (Cotty, 1994). The S-strain produces high levels of B-aflatoxins and also produce many small sclerotia (average diameter <400 µm) with some strains producing both B- and G-aflatoxins (Barros *et al*., 2006), while the L-strain produces less B-aflatoxins (Barros *et al*., 2006; Probst *et al*., 2011) and generate fewer but larger sclerotia. *Aspergillus parasiticus* and *A. nomius* produce both B- and G-aflatoxins (Cardwell and Cotty, 2002). In culture, members of the section *Flavi* group grow as yellow green molds.

2.5 Factors that influence fungal colonization and aflatoxin production

Peanut contamination by aflatoxin producing fungi and subsequent production of toxin can occur both at pre- and post-harvest stages of the production chain (Holmes *et al*., 2008). Aflatoxin contamination of peanuts is widespread where the crop is grown under rain fed conditions (Reddy *et al*., 2003). End-season drought stress and elevated soil temperatures common in Sub-Saharan Africa also promote aflatoxin contamination (Bankole *et al*., 2006). Attack of peanut pods by pests and diseases contribute to aflatoxin contamination (Waliyar *et al*., 2003). Poor seed storage, mechanical damage during harvesting, poor drying, and poor transportation provides conducive environment for contamination (Waliyar *et al*., 2005). *Aspergillus flavus*, the main producer of aflatoxin, only grows in peanuts when the moisture content exceeds 9% and has optimum growth conditions of between 25 and 30°C, and water activity of 0.99 with a minimum of 0.83_{aw}. Production of aflatoxin occurs optimally at 25°C and 0.99aw with a minimum of 0.87_{aw} (Ribeiro *et al*., 2006). The warm and humid environmental conditions in Africa are ideal for growth of *A. flavus* making aflatoxin contamination of food, including

peanuts, a widespread problem across the continent (Gordon, 2003; Bankole *et al*., 2006; Wagacha and Muthomi, 2008).

2.6 Effects of aflatoxin on trade, human and animal health

Aflatoxins in crops can have direct economic effects resulting in loss of market value. Presence of high levels of aflatoxins in peanuts may make it unacceptable for marketing, causing financial loss to the farmer or retailer (Okello *et al.*, 2010). Depending on the market, economic losses may reach 100%, when the entire produce/product is rejected if aflatoxin levels are higher than acceptable limits (Ntare *et al.*, 2008). It is estimated that Africa loses over 670 USD million annually due to failure to meet the requirements of European Union aflatoxin standards for all food exports. Globally billions of dollars are lost by farmers and traders due to aflatoxin contamination (Otsuki *et al*., 2001; Guo *et al*., 2009).

Aflatoxins are known to be hepatotoxic, carcinogenic, and teratogenic A positive correlation has been established between the consumption of aflatoxin-contaminated foods and the increased incidence of liver cancer worldwide (Aly, 2002). Intake of low, daily doses of the toxins over long periods may result in chronic aflatoxicosis expressed as impaired food conversion, stunting in children (Gong *et al*., 2004), immune suppression, cancer and reduced life expectancy (Cardwell and Henry, 2004; Gong *et al*., 2004; Williams *et al*., 2004; Farombi, 2006). Chronic dietary exposure to aflatoxins is a major risk factor for hepatocellular carcinoma, particularly in areas where hepatitis B virus infection is endemic. Ingestion of high concentrations of aflatoxin results in rapid development of acute aflatoxicosis which manifests as hepatotoxicity or, in severe cases, fulminant liver failure (Fung and Clark, 2004). Aflatoxicosis is characterized by severe liver damage leading to jaundice, hepatitis, mutagenicity and nervous disorders (KEPHIS, 2006) and when most severe, death (Williams *et al*., 2004).

A severe outbreak of acute aflatoxicosis occurred in Kenya in 2004 as a result of ingestion of contaminated maize (Lewis, *et al.,* 2005). This was one of the most severe episodes of human aflatoxin poisoning in history, with a case-fatality rate (CFR) of 39 percent. A study conducted in various parts of Kenya (Nagindu *et.al*., 1982) established a positive relationship between exposure to aflatoxin and prevalence of hepatitis infections. After entering the body, aflatoxins may be metabolized by the liver to a reactive epoxide intermediate or be hydroxylated and become the less harmful aflatoxin M1. No animal species is immune to the acute toxic effects of aflatoxins and chronic, sub clinical exposure does not lead to symptoms as dramatic as acute aflatoxicosis. Children, however, are particularly affected by aflatoxin exposure which leads to stunted growth and delayed development (Abbas *et al*., 2005). In humans, therefore, the aflatoxin exposure and the toxic effects of aflatoxins on immunity and nutrition may combine to negatively affect health factors, including HIV infection, that account for more than 40 percent of the burden of disease in developing countries where a short lifespan is prevalent (Williams *et al*., 2004). Other health conditions in which aflatoxin has been implicated are Kwashiorkor and Reye‘s syndrome, sicknesses that are more prevalent in Africa than in other parts of the world (Wild *et al*., 1991). From liver biopsy of 27 Sudanese children suffering from Kwashiokor, Coulter *et al*. (1986) reported the detection of aflatoxin B1, B2 and aflatoxicol in the organs of the children. In a different study, Stora *et al*. (1983) found aflatoxin B1 levels of between 120 and 180μg^{-g} in the livers of 5 infants suffering from Reye‘s syndrome.

Toxic and especially carcinogenic effects of aflatoxins have been reported in several different animals, but susceptibility to these toxins varies greatly with sex, age, species and strain within a species (Busby, 1984; CAST, 2003). Aflatoxins cause liver damage, decreased milk and egg production, recurrent infection as a result of immunity suppression, in addition to embryo

toxicity in animals consuming low dietary concentrations (Saad, 2007). Aflatoxin B1 is the most potent mycotoxin to affect cattle. When significant quantities of B1 are consumed, the metabolite M1 appears in milk within 12 hours. Young animals of all species are more susceptible than mature animals to the effects of aflatoxin. Pregnant and growing animals are less susceptible than young animals, but more susceptible than mature animals. Feed refusal, reduced growth rate and decreased feed efficiency are the predominant signs of chronic aflatoxin poisoning. In addition, listlessness, weight loss, rough hair coat and mild diarrhoea may occur (Cassel, 2001).

2.7 Prevention of aflatoxin contamination

At community level, mycotoxin formation in crops can be limited before harvest through good agricultural practices (GAP) such as rotating crops, irrigating to eliminate drought stress, controlling weeds, cultivating mold-resistant varieties and introducing biocontrol agents such as non-mycotoxigenic fungal strains. Postharvest measures include drying rapidly by mechanical means and keeping crops dry, sorting out contaminated nuts by physical means, sorting by colour, and washing with water will also reduce mycotoxins. Previous studies recommended farmers to embrace practices that improve peanut quality and safety through proper drying, grading and storage. Such practices have consistently been shown to reduce the level of contaminated peanuts (Gowda *et al*., 2002; Turner *et al*., 2005; Waliyar *et al.,* 2008).

2.8 Methods of aflatoxin analysis

Three types of assays have been developed for the detection of aflatoxins. These include biological, chemical and immunochemical techniques (Okello *et al*., 2010). Biological assays are qualitative or at best semi-quantitative, and often are non-specific. Pascale and Visconti (2008)

have summarized the various methods available for mycotoxin analysis including Thin Layer Chromatography (TLC), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Liquid Chromatography/Mass Spectrometry (LC/MS), Enzyme-Linked Imunosorbent Assay (ELISA), and rapid tests.

Liquid Chromatography, TLC and HPLC are the most used quantitative methods in research and routine analysis of aflatoxins (Vosough *et al*., 2010). These techniques offer excellent sensitivities but require skilled operators, extensive sample pretreatment and expensive equipment (Sapsford *et al*., 2006). Thin Layer Chromatography has been used to analyze agricultural products and plants. It has advantages that include simplicity of operation; availability of many sensitive and selective reagents for detection and confirmation without interference of the mobile phase; ability to repeat detection and quantification; and cost effectiveness analysis, because many samples can be analyzed on a single plate with low solvent usage. The time that TLC employs to analyze the sample is less than LC method (Sherma, 2000; Fuchs *et al*., 2010). Some of the differences between TLC and HPTLC are: the different particular size of stationary phase, the care used to apply the samples and the way to process the obtained data (Fuchs *et al*., 2010).

Enzyme-Linked Imunosorbent Assay (ELISA) procedures are the most widely used serological tests for aflatoxin analysis due to their simplicity, adaptability and sensitivity (ICRISAT, 2007) and ability to detect very low levels. ELISA procedures allow for analysis of multiple samples which is ideal for screening purposes. HPLC has the advantage of being highly sensitive and has good selectivity, and is easily automated. However, HPLC’s major challenge is its high cost, making it unsuitable for routine analysis.

2.9 Food policy and market standards for mycotoxins in peanuts

The risk of contamination by aflatoxins is an important food safety hazard for field crops (Dolman, 2003). In order to protect consumers from health risks, regulatory limits have been imposed on field crops intended for use as food and feed, and have significant impact on world export market. The World Health Organization (WHO) has set a maximum level for aflatoxin at 20 ppb in human foods and 100 ppb in animal feed. Likewise, the Food and Drug Administration (FDA) set a tolerance limit for peanut at 15 ppb for human (FDA, 1978). The upper limit set by European commission (EC) for total aflatoxin in peanuts is 4 µg/kg and 2 µg/kg for Aflatoxin B1 (AFB1) (EC, 2006), while the corresponding limits by the Kenya Bureau of Standards (KEBS) are 10 and 5 µg/kg, respectively (KEBS, 2007) (Table 1).

To minimize potential human exposure, the aflatoxin content of food and feed is strictly regulated in most of the world (Shephard, 2008). However, the standard set has little relevance to poor, small-scale farmers in Africa; these farmers produce and sell their products at the local markets and avoiding any inspection that is common in large scale production. Even though heavily contaminated foods are not permitted in the market places in developed countries, concerns still remains for the possible adverse effects resulting from long term exposure to low levels of aflatoxins in the food supply.

Table 1: Maximum level of total aflatoxin in foodstuffs

Country	Product	Maximum tolerable limit (ppb)
EU	Peanuts – Ready to eat	4
	Peanuts – for further processing	15
Kenya	Peanuts (all products)	10
USA	Peanuts (all products)	20
India	Peanuts (all products)	30
Uganda	Peanuts (all products)	10

Okello *et al*., 2010

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area and selection of farms

The study was conducted in Baringo (Baringo Central), Elgeyo- Marakwet (Keiyo South) and Meru (Meru South, Meru Central and Meru North regions) Counties. Baringo is one of the 47 Counties in Kenya; covering an area of 8655km^2 of which about 140.5 is covered by water surface. The County lies between latitudes 00°13’’S, 1° 40’’N and longitude 35°36’’, 36° 30’’E. Elgeyo-Marakwet County is located in the former Rift Valley province. It covers an area of 3029.8 km^2. Meru County is located along the East side of Mt. Kenya, located in the former Eastern province. It covers an area of 6936.9 Km^2 and lies between latitudes 0° 5.28’’N and longitude 38° 11.39’’ E. Agro-ecological zones where peanuts were grown were lower midland zone 4 (LM4) and lower midland zone 3 (LM3) (Ministry of Agriculture, 1987).

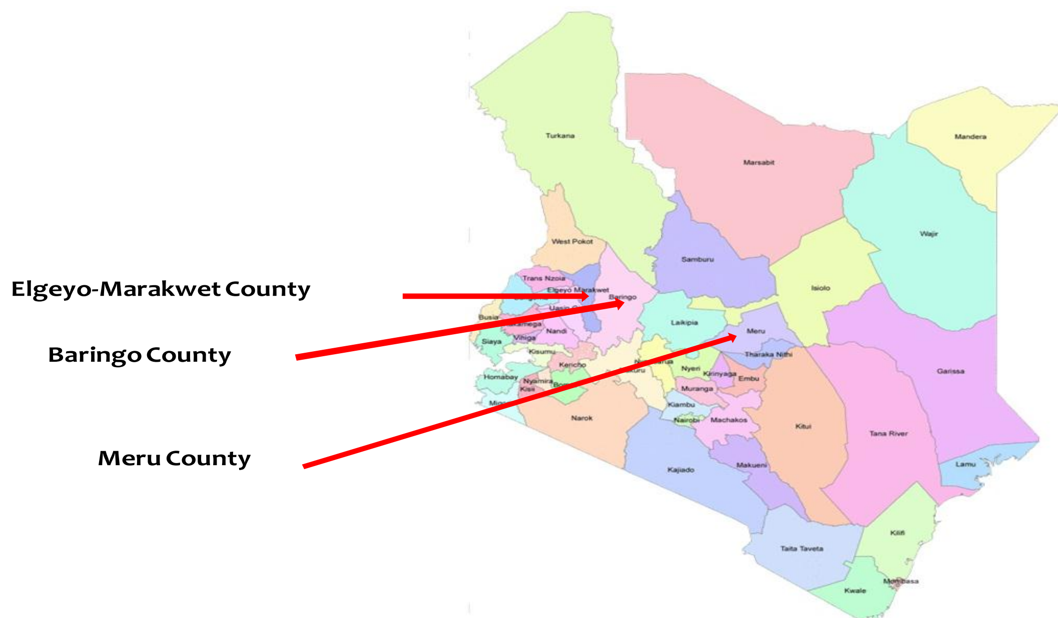

Figure 1: A map of Kenya showing the regions where the study was carried out.

Table 2: Characteristics of peanut growing agro-ecological zones of Baringo, Elgeyo-Marakwet and Meru Counties

AEZ	Description	Annual average rainfall (mm)	Altitude (m)
LM3	Cotton zone/Maize/Peanuts	900-1100	1070-1280
LM4	Marginal cotton zone/Maize/Peanuts	800-900	980-1220

AEZ-Agro-ecological zone, LM4- Lower midland zone 4 (Baringo and Elgeyo-Marakwet counties), LM3- Lower midland zone 3(Meru County)

Source: Ministry of Agriculture and GTZ (2007); Ministry of Agriculture, 1987

3.2 Survey and sampling design

Field survey was conducted in 40 peanut farms between June 2013 and March 2014. Of the 40 farms, 20 were covered in the long rains of 2013 between June to October 2013; 12 from Meru County (Meru South - 5 farmers; Meru North - 5 farmers; Meru Central - 2 farmers), 4 from Baringo Central (Baringo County) and 4 from Keiyo South (Elgeyo-Marakwet County). The remaining 20 farms were covered in the short rains of 2014 between November 2013 to March 2014; 5 from Kiorimba, 5 from Giaki, 5 from Gaitu and 5 from Kathuene. Sampling was not done in Baringo and Elgeyo-Marakwet Counties in season two because there is a single cropping season in the two Counties per year. A semi structured questionnaire (Appendix 1) was used to obtain information on peanut production practices. Information which was gathered included availability of irrigation, farm size, peanut varieties, sources of planting seeds, field preparation practices, rotation programs, knowledge on aflatoxin, willingness to upscale and diseases of peanuts among others.

3.3 Sample collection

3.3.1 Sampling of soil

In each farm, a minimum of five sampling points were identified. Approximately 100g of soil was collected from the top five centimetre layer of each sampling point using a table spoon. The same procedure was repeated in four different randomly selected points in the same farm and at least four meters apart until five sub-samples were collected. Table spoons were surface sterilized using 70% ethanol to avoid cross contamination when sampling fresh farms. In a paper bag, the samples were thoroughly mixed to make a composite sample. The 0.5 kg composite sample was thoroughly mixed in a paper bag, labelled, put in a zip lock bag, stored in a cool box and transported to the laboratory where they were dried on benches until mycological analysis to determine the population of aflatoxin producing fungi.

3.3.2 Sampling of crop debris and below ground peanut parts

In each farm debris were collected at random from the entire field, scissors used during sampling were surface sterilized using 70% ethanol to avoid cross contamination after every farms. The samples were labelled, put in a Kraft bag, stored in a cool box and transported to the laboratory where they were stored at $4^{o}C$ in a cold room until mycological analysis.

Peanut plant parts (stems and roots) were collected from a minimum of five sampling points identified in each farm. Scissors used during sampling were surface sterilized using 70% ethanol to avoid cross contamination. The samples were labelled, put in a Kraft bag, stored in a cool box and transported to the laboratory where they were stored at $4^{o}C$ in a cold room until mycological.

3.3.3 Pre and post-harvest sampling of peanut kernels

Peanut kernels were sampled pre and post-harvest to determine the population of *Aspergillus* spp. and aflatoxin levels. Samples were collected from multiple sampling points in each farm with the number of sampling points being based on the size of the farm. Approximately 100g of un-shelled peanuts was sampled from each point. The same procedure was repeated in a minimum of 10 other randomly selected points in the same farm and at least four meters apart until a minimum of 10 sub-samples were collected. The samples were thoroughly mixed to make a 1kg composite sample which was labelled, put in a Kraft bag and transported to the laboratory where they were oven dried for 48 hours at $45^{o}C$. Kernel samples were then shelled manually under sterile laboratory conditions; and then mixed thoroughly and ground in the laboratory using a dry mill kitchen blender (BL335, Kenwood, UK). The sample was divided into two parts for microbial and aflatoxin analysis.

3.4 Isolation and identification of *Aspergillus* species

3.4.1 Media preparation

Dichloran Rose Bengal Agar (Horn and Dorner, 1998), a semi selective medium that inhibits growth of other fungi and bacteria while favouring the growth of *Aspergillus* species section *Flavi* was used. The medium was prepared by adding 10g glucose, 2.5g peptone, 0.5g yeast extract, 1.0g potassium phosphate monobasic, 0.5g $MgSO_4.7H_2O$, 20.0g agar, 25mg rose Bengal in 1L distilled water. The pH was adjusted to 5.5 with 0.01M Hydrochloric acid before autoclaving. Autoclaving was done at $121^{o}C$ and 15psi pressure for 20 minutes and cooling to $60^{o}C$. To inhibit bacterial growth and ensure the medium was semi-selective for *Aspergillus*

section *Flavi*, five ml of four mg/L dichloran (in acetone), 40 mg/L streptomycin and 1 mg/L chlortetracycline was added to the medium through a sterile 0.25 mm syringe filter.

3.4.2 Isolation of *Aspergillus* spp. from soil and kernels

The 0.5kg soil sample from each farm was thoroughly mixed and then sieved with No. 20 standard testing sieve (0.833mm opening). The soil was then air dried, stored at room temperature and processed within three weeks of collection. kernel samples were shelled manually under sterile laboratory conditions; and then mixed thoroughly and ground in the laboratory using a dry mill kitchen blender (BL335, Kenwood, UK). The sample was divided into two parts for microbial and aflatoxin analysis. Two sub-samples of 1g each were taken for serial dilution. Fungi were isolated from the soil samples using the dilution plating technique on a modified dicloran rose Bengal agar (MDRB), (Probst *et al*., 2007). One gram of the sample was suspended in 9 ml of sterile water forming a stock solution (10^{-1} dilution), vortexed for 30 seconds and 1ml of the dilution transferred to another 9ml of sterile water making a 2nd dilution (10^{-2}). Two hundred microliter aliquot of the 2nd dilution was then plated on MDRB agar in four replicates. Plated cultures were incubated at 30°C for seven days. The number of *Aspergillus* spp. isolates in the soil and kernel samples was expressed as the number of colony forming units (CFU) per gram of sample (Jaime-Garcia and Cotty, 2004). The number of colony forming units per gram (CFU/g) was calculated as follows:

$$\text{Number of Fungi/g Substrate} = \frac{\text{No. of colonies}}{\text{Amount plated} \times \text{Dilution Factor}}$$

3.4.3 Isolation of *Aspergillus* species from crop debris and below ground peanut parts

Isolation was made from crop debris (~1cm long) and below ground peanut parts (stems and roots) on MDRB Agar medium. Crop debris and below ground peanut parts were washed with running tap water to remove soil particles and then dried on paper towels. The tissue were surface sterilized by putting them in porous glass tube, immersed in 1.3% sodium hypochlorite (NaOCl) for two minutes followed by two consecutive two minutes rinsing using sterile distilled water. The samples were then dried on sterile paper towels. Using sterile forceps, 5 plant sections were plated in each Petri dish containing approximately 25mL MDRB agar and each treatment replicated 4 times. The plates were incubated at 30°C for seven days.

3.4.4 Identification of *Aspergillus* species

Aspergillus spp. were distinguished based on colony colour, shape, margins, elevation, pigmentation, texture, pattern of growth and conidial morphology characteristics (Klich, 2002). Microscopic examination of *Aspergillus* spp. was done and identification done according to taxonomic schemes proposed by Pitt and Hocking (1997) and Klich and Pitt (1994). Slide cultures of *Aspergillus* spp. were made by placing MDRB agar squares on a microscope slide raised with a V-shaped glass rod in a sterile glass Petri plate covered with a sterile filter paper at the bottom. Some of the *Aspergillus* mycelia or spore was transferred from the isolate to the four edges of the agar block using a sterile inoculating needle. A clean cover slip was placed on the surface of the agar block, the filter paper wetted with sterile distilled water and the plate partially sealed with parafilm™ and incubated at 25°C for 14-21 days. Slides for light microscopy were prepared by removing the agar block and then adding a drop of lactophenol cotton blue on the

slide and cover slip added to cover the growth on the slide. The prepared slides were used for identification and taking photos of morphological characteristics of the commonly isolated *Aspergillus* species. Microscopy was done at ×1000 of the light microscope (LEICA DM 500, Leica Microsystems, Wetzler, Germany). The microscope was fitted with a camera (LEICA ICC 50, Leica Microsystems, Wetzler, Germany).

3.5 Determination of aflatoxin levels in peanut kernels sampled at different pre- and post-harvest stages

A 200g sub-sample was drawn from each sample and divided into two equal portions. The powder was triturated in 70% methanol (70 ml absolute methanol in 30 ml distilled water, v/v) containing 0.5% potassium chloride (w/v) until thoroughly mixed. The extract was transferred to centrifuge tubes and shaken for 30 min at 300 rpm. The extract was filtered through Whatman No. 41 filter paper and diluted 1:10 in phosphate buffered saline containing 500 µl/l Tween-20 (PBS-Tween) and analyzed for aflatoxin levels using indirect competitive ELISA as described by Waliyar *et al.* (2005). This method has a detection limit of 1.75µg/kg.

Detection and quantification of aflatoxin in the kernel samples was done by preparing an aflatoxin-bovine serum albumin conjugate in carbonate coating buffer at 100 ng/mk concentration and dispensing 150 µl in each well of the Nunc-Maxisorp® ELISA plates (Thermo Fisher Scientific Inc). Absorbance was measured at 405 nm in an ELISA plate reader (Multi skan Plus, Lab systems Company, Helsinki, Finland). Sufficient number of microtiter wells was inserted in to micro well holder for all standards and samples to be run in duplicate, standard and sample positions were then recorded. Fifty microliters (50µl) of the standard solution or prepared

sample was added to separate duplicate wells, 50µl of the diluted enzyme conjugate was also added to each well, then 50µl of the diluted antibody solution added to each well. The contents were then mixed gently by shaking the plate manually and incubated for 30min at room temperature (20-25°C) in the dark. The liquid was then poured out of the well and the microwell holder tapped upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of the liquid from the wells. All the wells were then filled with 250 µl distilled water and again poured out. The washing procedure was repeated twice. Fifty microliters (50µl) of the substrate and 50µl of chromogen were added to each well, mixed gently by shaking the plate manually and incubated for 30min at room temperature (20-25°C) in the dark. Hundred microliters (100µl) of the stop solution was added to each well, mixed gently by shaking the plate manually and absorbance measured using spectrophotometer ELISA reader (RIDA®SOFT Win) at 450nm. Reading was done within 30 minutes after addition of the stop solution.

3.6 Data analysis

Survey data were reported in percentage form. Data obtained from isolations was subjected to Analysis of Variance (ANOVA) using the PROC ANOVA procedure of GENSTAT version 15 and differences among the treatment means were compared using Fisher's Protected (Least significant difference) LSD at 5% probability level. Frequency data that was not normally distributed was transformed to arcsine before analysis whereas the CFU data that were not normally distributed were transformed as $\log_{10}X+1$.

Aflatoxin samples were grouped into three categories based on their aflatoxin content: samples with: <4µg/kg, >4µg/kg to 10µg/kg, or >10µg/kg. The <4µg/kg category represents the European

Union regulatory limit for total aflatoxin (Wu, 2004); peanuts in the second group would be rejected in the European Union but accepted under the Kenya Bureau of Standards (KEBS) limits (KEBS, 2007), while nuts in the third category would be rejected both under the KEBS and EU standards.

CHAPTER FOUR: RESULTS

4.1 Agronomic practices in peanut production

4.1.1 Farm sizes, history of peanut production, irrigation facilities and land preparation

Seventy percent of the farmers were small scale (Figure 2A) with the rest (30%) large scale. There was variability in the duration that the farmers had practiced peanut production (Figure 2B). From 5 to 30 years, with most (35%) of the farmers having grown peanuts for less than 5 years. Farmers in Baringo County, Elgeyo-Marakwet County and Meru Central had no irrigation facilities unlike their counterparts in Meru North and Meru South who used sprinkler irrigation benefitting from community irrigation schemes (Table 3). Most (79%) farmers used animals to plough their farms (Table 3) with only a few (21%) using tractors in their fields. Animal ploughing was the exclusive method of land preparation in Baringo central and in Meru central.

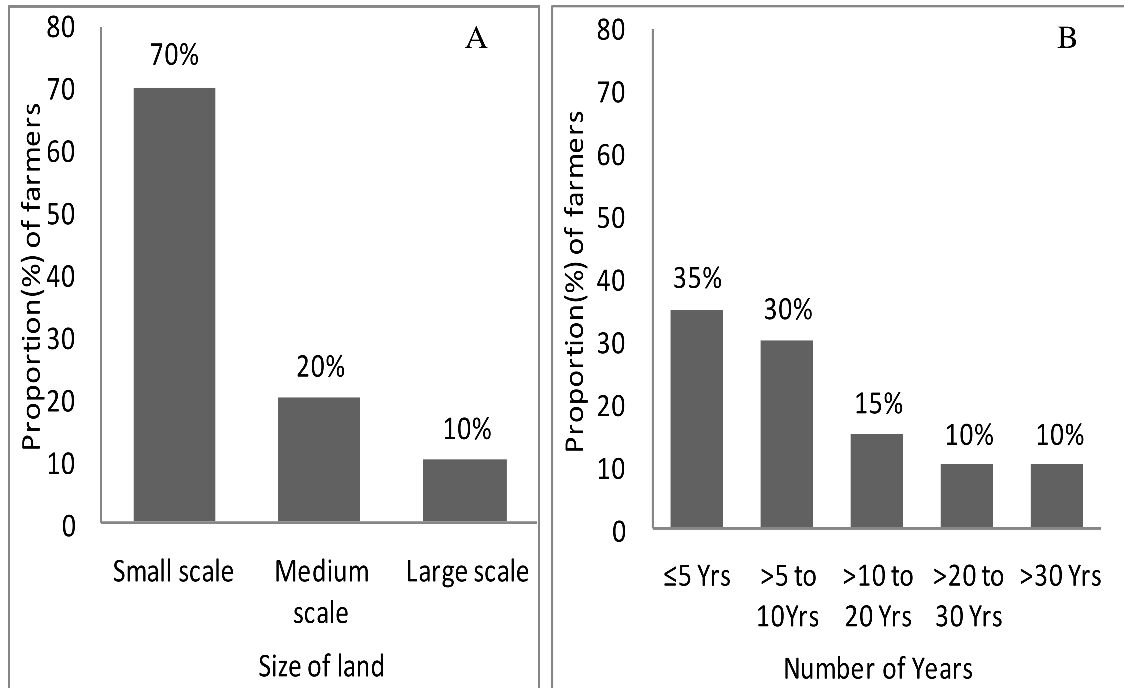

Figure 2: Categories of farm sizes (A) and duration in years that the farmers have practiced peanut production (B)

Small scale: <8 hectares; medium scale: 8 – 20 hectares; large-scale: >20 hectares, Yrs-years

Table 3: Availability of irrigation facilities and proportion of farmers undertaking different land preparation methods in different regions in Meru, Baringo and Elgeyo-Marakwet Counties

AEZ	Region	Irrigation facilities	Land preparation (%)	
			Animal ploughing	Tractor ploughing
LM3	Meru North	Sprinkler irrigation	60.0	40.0
	Meru Central	Not available	100.0	0.0
	Meru South	Sprinkler irrigation	60.0	40.0
LM4	Baringo Central	Not available	100.0	0.0
	Elgeyo-Marakwet	Not available	75.0	25.0
Mean			79.0	21.0

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3

4.1.2 Cropping systems and soil amendments

There was variation in cropping systems in the regions, with most (70%) farmers practicing mixed cropping especially in Meru central (Table 4). All the farmers in the two agro-ecological zones practiced crop rotation. Only farmers in Meru North applied soil amendments eg fertilizer application in peanut production. Besides peanuts, the farmers also grew maize, beans sorghum, green grams, bananas, sweet potatoes, rice, tobacco and mangoes (Figure 3). Ninety percent of the farms where peanuts were grown also grew maize both in rotation and mixed cropping with the remaining farms growing sorghum and green peas. In the 18 farms where maize was grown, the stovers were fed to livestock. In previous seasons, the farmers used their own peanut seeds but during the study period, certified seeds were supplied by Equatorial Nut Processors (ENP).

Table 4: Proportion (%) of peanut farmers practicing different cropping systems and applying soil amendments in various regions in Baringo, Elgeyo-Marakwet and Meru Counties during the long rains of 2013

AEZ	Region	Cropping systems			Application of soil amendments
		Mixed cropping	Mono cropping	Crop rotation	
LM3	Meru North	60.0	40.0	80.0	20.0
	Meru Central	100.0	0.0	60.0	0.0
	Meru South	40.0	60.0	60.0	0.0
LM4	Baringo Central	75.0	25.0	50.0	0.0
	Keiyo South	75.0	25.0	75.0	0.0
Mean		70.0	30.0	65.0	4.0

AEZ - Agro-ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3.

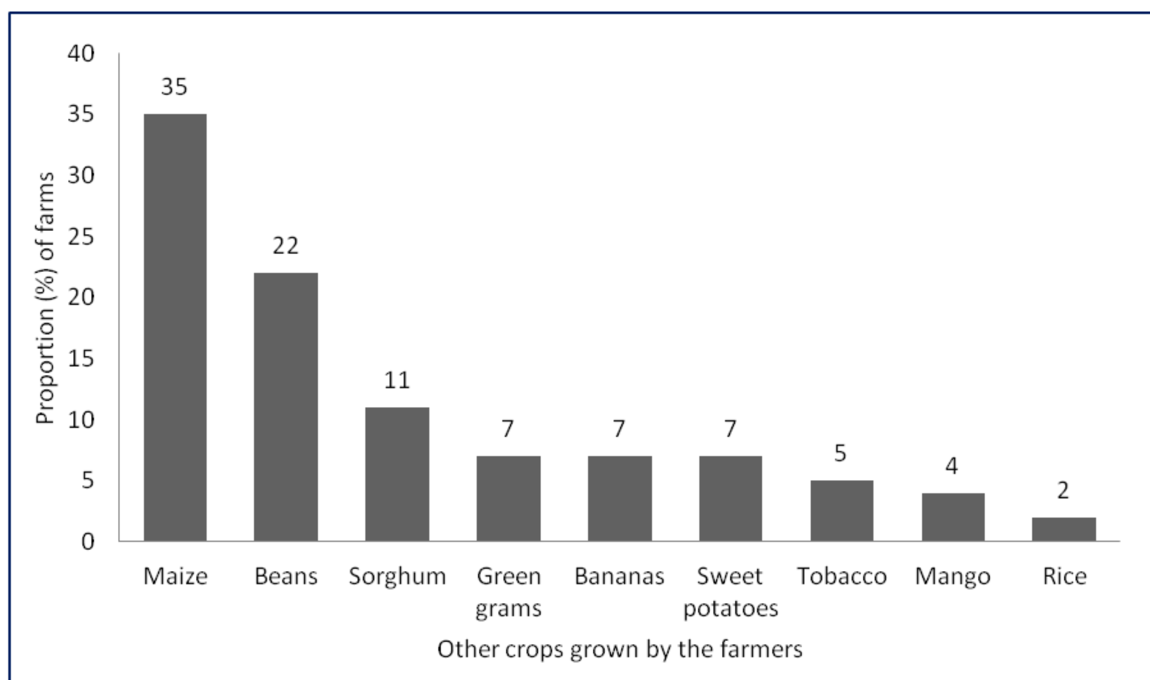

Figure 3: Other crops grown by the peanut farmers both in rotation and mixed cropping in Baringo, Elgeyo-Marakwet and Meru Counties Kenya

4.1.3 Diseases associated with peanuts

The common diseases of peanuts were late blight, root rot, Alternaria leaf blight, Aschochyta blight and early blight (Table 5). Aschochyta blight was the most (87%) prevalent while Alternaria leaf spot was the least (57%) prevalent. There was significant ($P \leq 0.05$) variation in the prevalence of the diseases in the different regions in the two agro-ecological zones. The farmers did not use fungicides in the management of peanut diseases. Only one farmer in Meru North used insecticides (Karate® and Score®) to manage insect pests.

Table 5: Prevalence of peanut diseases in various regions in two agro- ecological zones in Baringo, Elgeyo-Marakwet and Meru Counties during the long rains of 2013

AEZ	Region	Aschochyta blight	Late blight	Early blight	Root rot	Alternaria leaf spot	Mean
LM3	Meru North	80.0	80.0	80.0	75.0	60.0	75.0b
	Meru Central	100.0	100.0	100.0	50.0	50.0	80.0a
	Meru South	80.0	60.0	80.0	75.0	60.0	71.0c
LM4	Baringo Central	75.0	75.0	75.0	50.0	50.0	65.0d
	Keiyo South	100.0	75.0	75.0	50.0	50.0	70.0c
Mean		87.0	82.0	82.0	60.0	54.0	72.2
LSD (P≤0.05)Region*disease							3.0
CV (%)							5.7

AEZ - Agro-ecological zone, LM4- Lower midland zone 4, LM3- Lower midland zone 3, LSD-Least significant difference, CV-Coefficient of variation. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

4.1.4 Harvesting and postharvest handling practices

Majority (96%) of farmers determined the peanut harvesting stage through visual observation (Table 6). The crop was considered to be ready for harvesting when leaves started dropping or when there was change in color of the pods. Some of the farmers in Meru South based harvesting

time on duration recommended for the variety planted. The recommendation is by the manufacturers of the variety.

Table 6: Percentage of farmers using different criteria for determining peanut harvesting time in different agro-ecological zones in Baringo, Elgeyo-Marakwet and Meru Counties during the long rains of 2013

AEZ	Region	Visual observation	Duration in the field
LM3	Meru North	100.0	0.0
	Meru Central	100.0	0.0
	Meru South	80.0	20.0
LM4	Baringo Central	100.0	0.0
	Keiyo South	100.0	0.0
Mean		96.0	4.0

AEZ-Agro-ecological zone, LM4- Lower midland zone 4, LM3- Lower midland zone 3

4.1.4.1 Drying, storage and grading of the peanuts

Most (70%) of the farmers dried their peanuts for 7 days with the duration varying from 7 to 12 days. Only forty five percent of the farmers were aware of the recommended method of drying peanuts in windrows. All the peanut farmers used jute bags for storage of their peanuts. Fifty percent of the farmers stored their peanuts in the family living house with 50% of farmers storing their produce in traditional granaries (Table 7). All the farmers stored their peanuts in shells. Farmers sorted and graded their peanuts based on color and size. Before engagement of farmers with Equatorial Nut Processors, they used to produce peanuts for consumption by the household as well as selling to the local market. Ninety six percent of farmers in all the regions showed willingness to upscale peanut production.

Table7: Percentage of farmers using different storage facilities and their views on up scaling of peanut production in Baringo, Elgeyo-Marakwet and Meru Counties Kenya during the long rains of 2013

AEZ	Region	Peanut storage structure		Willingness to up-scale peanut production
		Family house	Granary	
LM3	Meru North	60.0	40.0	100.0
	Meru Central	50.0	50.0	100.0
	Meru South	40.0	60.0	80.0
LM4	Baringo Central	50.0	50.0	100.0
	Keiyo South	50.0	50.0	100.0
Mean		50.0	50.0	96.0

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone

4.1.4.2 Shelling methods and knowledge on aflatoxin

All peanut farmers in Baringo Central and Keiyo South shelled their pods manually while 10% of farmers in Meru County used an improvised manual shelling machine (Table 8). The farmers used to shell their peanuts before the introduction of Equatorial Nut Processors in their regions to whom they sell their peanuts unshelled. On average 61% of the farmers had no information on aflatoxin (Figure 4) and the few who were informed only related the toxins with maize not peanuts.

Table 8: Percentage of farmers using different peanut shelling methods in two agro-ecological zones in Baringo, Elgeyo-Marakwet and Meru Counties during the long rains of 2013

AEZ	Region	Shelling method	
		Manual shelling	Improvised machine
LM3	Meru North	60.0	40.0
	Meru Central	50.0	50.0
	Meru South	60.0	40.0
LM4	Baringo Central	100.0	0.0
	Keiyo South	100.0	0.0
Mean		74.0	26.0

AEZ-Agro- ecological zone, LM4- Lower midland zone 4, LM3- Lower midland zone

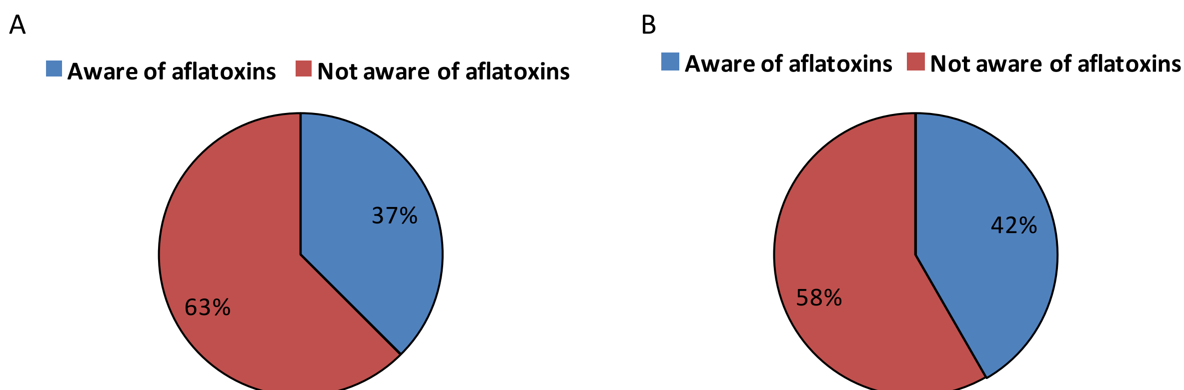

Figure 4: Proportion of peanut farmers in Baringo and Elgeyo Marakwet Counties (A), and Meru County (B) knowledgeable about aflatoxin

4.2 Population of *Aspergillus* spp. in soil and debris at planting

4.2.1 Population of *Aspergillus* spp. in soil at planting

Various *Aspergillus* spp. were isolated from the soil sampled from Meru County at planting stage (Table 9). The species included *Aspergillus flavus* S-strain, *Aspergillus flavus* L-strain,

Aspergillus niger and *Aspergillus parasiticus* (Figure 5). There was no significant ($p \geq 0.05$) variation in the population of the species in the soil sampled from the different regions in Meru County except for *A. flavus* S-strain, which was isolated in the highest incidence with a mean population of 6 $x10^3$ CFU/g soil. The highest mean population (1.1 x 10^4 CFU/g soil) of *A. flavus* S-strain was in soil sampled from Giaki region while the population of *A. flavus* L-strain was highest (7.5 x 10^3 CFU/g soil) in soil sampled from Gaitu region.

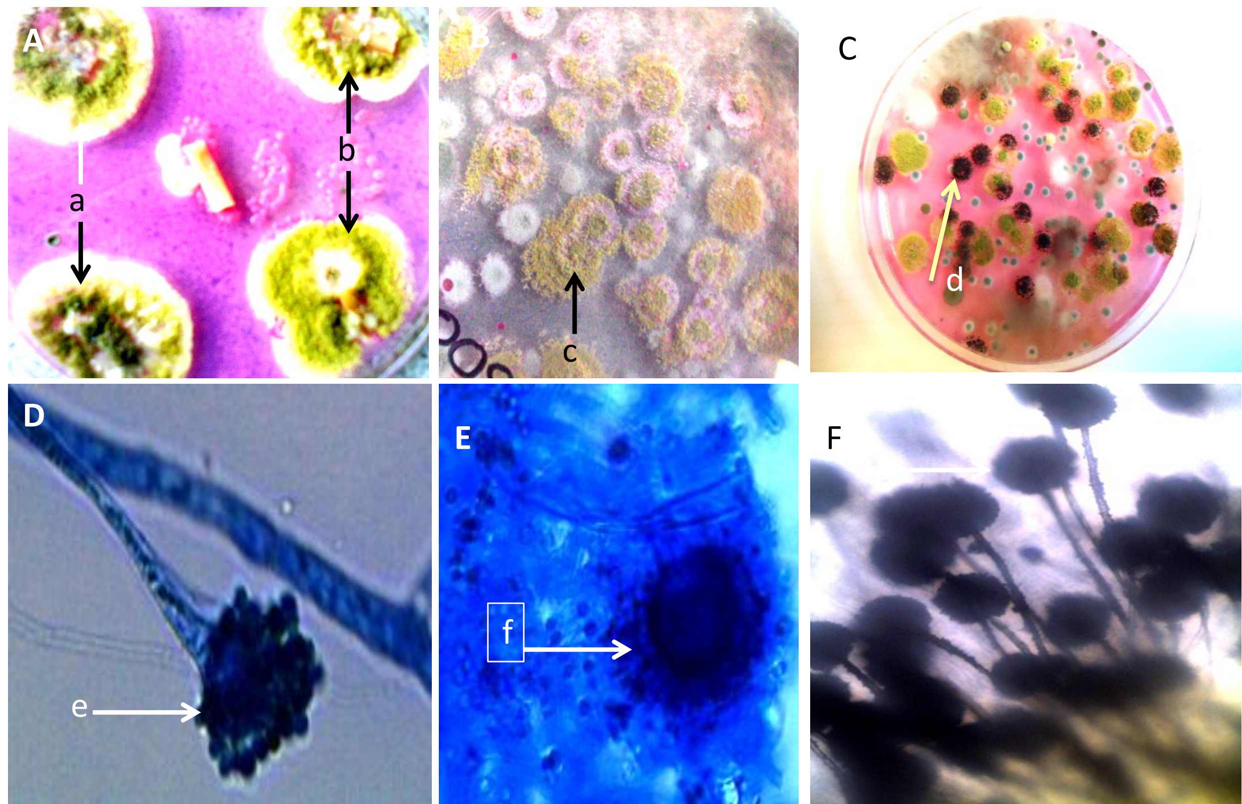

Figure 5: Morphological characteristics of different *Aspergillus* spp. isolated from soil sampled from Meru County. A: *Aspergillus flavus* L-strain (a), *A. parasiticus* (b), B: *A. flavus* S-strain (c), C: *A. niger* (d), D: sporangium of *A. flavus* L-stain (e), E: sporangiospores of *A. flavus* S-stain (f), F: sporangium of *A. niger* (g) growing on MDRA.

Table 9: Population (CFU/g soil) of *Aspergillus* spp. in soil sampled from various regions of Meru County at planting during the short rains of 2014

AEZ	Region	AFL	AFS	AN	AP
LM3	Kathuene	850.0a	1625.0b	1400.0a	225.0a
	Gaitu	7100.0a	700.0b	7575.0a	550.0a
	Giaki	1150.0a	10975.0a	1250.0a	675.0a
	Kiorimba	925.0a	9750.0a	925.0a	375.0a
Mean		2506.0	5762.0	2788.0	456.0
LSD ($p \leq 0.05$)		6137.9	7800.7	6097.5	390.8
CV (%)		55.6	48.4	40.9	92

AEZ - Agro-ecological zone, LM 3- Lower midland zone 3, LSD-Least significant difference, CV-Coefficient of variation, AF-L- *A. flavus* L-strain, AF-S- *A. flavus* S-strain, AP- *A. parasiticus*, AN- *A. niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

4.2.2 Incidence of *Aspergillus* spp. in crop debris at planting

Aspergillus spp. were isolated in low incidence from crop debris sampled from the various regions of Meru County at planting stage (Table 10). *Aspergillus* species isolated from the crop debris included *A. flavus* L-strain, *A. flavus* S-strain, *A. niger*, and *A. parasiticus* . There was no significant ($p \geq 0.05$) variation in the incidence of *Aspergillus* spp among the regions in meru county. *Aspergillus niger* was isolated in the highest incidence from crop debris followed by *A. flavus* S-strain then *A. flavus* L-strain and lastly *A. parasiticus* with a mean incidence of 15, 10, 3 and 1, respectively. *Aspergillus parasiticus* was not isolated from crop debris sampled from Gaitu and Giaki regions.

Table 10: Incidence (%) of *Aspergillus* spp. in crop debris sampled from various regions in Meru County at planting during the short rains of 2014

AEZ	Region	AFL	AFS	AN	AP
LM3	Kathuene	3.0a	15.0a	8.0a	1.0a
	Gaitu	1.0a	7.0a	14.0a	0.0a
	Giaki	3.0a	5.0a	15.0a	0.0a
	Kiorimba	2.0a	5.0a	19.0a	1.0a
Mean		2.3	8.0	14.0	0.5
LSD ($p \leq 0.05$)		5.0	7.6	10.6	2.0

AEZ-Agro- ecological zone, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AF-L- *A. flavus* L-strain, AF-S- *A. flavus* S-strain, AP- *A. parasiticus*, AN- *A. niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

4.3 Population of *Aspergillus* spp. in soil and peanut plant parts at various pre-harvest and harvest stages

4.3.1 Population of *Aspergillus* spp. in soil at different peanut growth stages

Aspergillus species were isolated from soil sampled from Baringo, Elgeyo-Marakwet and Meru Counties at different growth stages during the two seasons (Table 11). The species included *Aspergillus flavus* S-strain, *A. flavus* L-strain, *A. parasiticus, A. niger* and *A. tamarii*. *Aspergillus tamari* was isolated in low incidence in the soil samples from LM3. In both seasons, *A. flavus* L-strain and *A. flavus* S-strains were isolated from soil sampled during most of the peanut growth stages and in most regions in the two agro-ecological zones. There was a significant ($P \leq 0.05$) variation in the population of the two strains in the soil sampled at different growth stages. However, in the short rains of 2014 only *A. flavus* S-strain had its population varying significantly ($P \leq 0.05$) in soil during different growth stages unlike *A. flavus* L-strain which had no variation in its population during podding stage and two weeks before harvest (Table 12). *Aspergillus flavus* L-strain was isolated in the highest incidence from the soil at podding stage and was the most prevalent in soil sampled from Baringo Central in season one. However, in season two *A. flavus* S-strain was isolated in the highest incidence from soil sampled at podding stage and was the most prevalent in samples from Kiorimba with a mean population of 5000 CFU/g of soil (Table 12). In season one, *A. flavus* S-strain was isolated in the highest frequency in soil at two weeks before harvest while in season two the highest population of this pathogen was in samples taken at podding stage during the two seasons (Table 11, Table 12).

Table 11: Population (CFU/g) of *Aspergillus flavus (*L and S-strains) in soil sampled from two agro-ecological zones at different growth stages during the long rains of 2013

AEZ	Region	Podding Stage		Two weeks pre-harvest		At harvest	
		AFL	AFS	AFL	AFS	AFL	AFS
LM3	Meru North	312b	1625 a	375 c	12250 a	62b	1438 ab
	Meru Central	375b	1050 a	250 bc	1275 b	150b	625 bc
	Meru South	100b	250 b	0 c	300 b	425ab	1500 a
LM4	Baringo Central	39719a	62 b	593 b	31 b	1406a	406 bc
	Keiyo South	1469b	406 b	1562 a	0 b	844ab	62 c
Mean		8395	678	556	2771	577	806
LSD (p ≤ 0.05)		26149	770	691	2582	873	1143
CV (%)		89	93	98	82	95	79

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AFL- *Aspergillus flavus* L-strain, AFS- *Aspergillus flavus* S-strain. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at p ≤ 0.05).

Table 12: Population (CFU/g soil) of *Aspergillus flavus* (L and S-strains) in soil sampled from various regions of Meru County at different growth stages during the short rains of 2014

Region	Podding		Two weeks pre-harvest		Harvest	
	AFL	AFS	AFL	AFS	AFL	AFS
Kathuene	925a	1250 b	300 ab	675 a	375 b	275 b
Gaitu	2550a	600 b	525 a	975 a	1075 a	2225 a
Giaki	875a	900 b	150 b	425 a	550 b	850 b
Kiorimba	1050a	4600 a	50 b	675 a	1025 a	1975 a
Mean	1350	1838	256	688	756	1331
LSD (p ≤ 0.05)	1725	1844	275	425	397	1111
CV (%)	48	50	129	98	83	58

AEZ-Agro- ecological zone, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AFL- *Aspergillus flavus* L-strain, AFS- *Aspergillus flavus* S-strain. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at p ≤ 0.05).

Aspergillus parasiticus was isolated from the soil samples from various regions though in low frequency both in season one and season two. There was significant ($P \leq 0.05$) variation in the

population of *A. parasiticus* isolated from soil sampled two weeks before harvest among different regions during the long rains of 2013 (Table 13). In season one the highest isolation frequency of *A. parasiticus* from soil was at two weeks before harvest while in season two the highest frequency was during podding stage. *Aspergillus parasiticus* was present in the soil sampled from all the regions and during all the growth stages in season two unlike in season one were the isolation of *A. parasiticus* was in most soil sampled and in most growth stages.

Aspergillus niger was also isolated from soil sampled from the various regions during different peanut growth stages in both seasons. There was a significant ($P \leq 0.05$) variation in the population of *A. niger* in soil sampled within different growth stages and among regions in seasons one (Table 13) and two (Table 14). In season one, the population of *A. niger* was highest in samples collected during podding stage with a mean frequency of 24,000 CFU/g soil (Table 13) while in season two, the population of the fungus was highest in samples collected two weeks before harvest with a mean frequency of 4,000 CFU/g soil (Table 14).

Table 13: Population (CFU/g) of *A. parasiticus* and *A. niger* in soil sampled from two agro-ecological zones at different growth stages during the long rains of 2013

AEZ	Region	Podding		Two weeks pre-harvest		Harvest	
		AP	AN	AP	AN	AP	AN
LM3	Meru north	125a	21375 ab	100 bc	4250 ab	0a	750 bc
	Meru central	375a	57950 a	125 bc	6800 a	75a	250 c
	Meru south	50a	2750 b	0 c	3200 b	325a	2000 a
LM4	Baringo Central	125a	1281 b	281 b	1594 b	250a	1250 ab
	Keiyo south	250a	36312 a	562 a	2656 b	188a	156 c
Mean		219	23933	267	3700	128	881
LSD ($p \leq 0.05$)		334	36893	328	3724	349	1026
CV (%)		102	51.6	107	36	99	93

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AP- *Aspergillus parasiticus*, AN- *Aspergillus niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

Table 14: Population (CFU/g soil) of *A. parasiticus* and *A. niger* in soil sampled from various regions of Meru County at different growth stages during the short rains of 2014

Region	Podding		Two weeks pre-harvest		Harvest	
	AP	AN	AP	AN	AP	AN
Kathuene	350.0 a	5700.0 a	150.0 a	2625.0 b	50.0b	2975.0 b
Gaitu	625.0 a	1500.0 b	200.0 a	1400.0 b	400.0a	1925.0 b
Giaki	625.0 a	2800.0 b	175.0 a	2250.0 b	425.0a	2200.0 b
Kiorimba	325.0 a	2000.0 b	225.0 a	9350.0 a	400.0a	8100.0 a
Mean	481.0	3000.0	188.0	3906.0	319.0	3800.0
LSD ($p \leq 0.05$)	327.6	1560.2	192.8	2843.4	206.7	2510.9
CV (%)	76.0	82.6	147.9	70.9	88.1	42.2

AEZ-Agro- ecological zone, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AP- *Aspergillus parasiticus*, AN- *Aspergillus niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

4.3.2 Incidence of *Aspergillus* spp. in below ground peanut parts (stems and roots) sampled at different growth stages.

Various *Aspergillus* spp. were isolated from below ground peanut parts sampled from various regions both during the long rains of 2013 and short rains of 2014 (Tables 15, 16, 17 and 18). The *Aspergillus* spp. isolated were; *Aspergillus flavus* L-strain, *A. flavus* S-strain, *A. niger* and *A. parasiticus.* During the long rains of 2013, *A. flavus* L-strain and *A. flavus* S-strains were isolated from below ground peanut parts sampled from all the regions in the two agro-ecological zones during all the stages (Table 15). There was significant variation ($p \leq 0.05$) in the incidence of *A. flavus* L-strain in plant parts sampled from different regions among various growth stages. Unlike at two weeks before harvest and at harvest, there was no significant variation in the incidence of *A. flavus* S-strain in plant parts at podding stage. The highest incidence of *A. flavus* L-strain was in peanut plant parts sampled from Baringo central at two weeks before harvest while *A. flavus* S-strain was isolated in highest incidence from samples collected from Meru

north both two weeks before harvest and at harvest. *Aspergillus flavus* L-strain was isolated in higher incidence in LM4 while *A. flavus* S-strain was isolated in higher incidence in LM3.

Table 15: Incidence (%) of *A. flavus* (L and S-strains) in below ground peanut parts sampled from various regions in two agro-ecological zones at different growth stages during the long rains of 2013

AEZ	Region	Podding		Two weeks pre-harvest		Harvest	
		AFL	AFS	AFL	AFS	AFL	AFS
LM3	Meru North	12.5b	20.0 a	30.0ab	40.0 a	5.0 b	40.0 a
	Meru Central	14.0b	13.0 a	23.0bc	24.0 abc	7.0 b	20.0 b
	Meru South	14.0b	17.0 a	15.0c	32.0 ab	14.0 b	33.0 a
LM4	Baringo Central	38.8a	11.4 a	41.3a	20.0 bc	32.5 a	7.5 b
	Keiyo South	20.0b	12.5 a	36.3a	12.5 c	29.0 a	10.0 b
Mean		19.86	14.8	29.1	27.2	17.5	22.1
LSD ($p \leq 0.05$)		13.2	9.6	14.9	17.8	12.2	16.8
CV (%)		79.3	80.4	63.7	87.3	81.1	97.2

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AF-L- *A. flavus* L-strain, AF-S- *A. flavus* S-strain. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

During the short rains of 2014, *A. flavus* S-strain and *A. flavus* L-strain were isolated from below ground peanut plant parts sampled from the various regions at all the growth stages (Table 16). There was significant ($p \leq 0.05$) variation in the incidence of *A. flavus* L-strain in plant parts sampled during most growth stages and among regions. However, there was no significant ($p \leq 0.05$) variation in the incidence of *A. flavus* S-strain from samples collected two weeks before harvest and at harvest time. During podding, there was significant ($p \leq 0.05$) variation in the incidence of *A. flavus* S-strain in plant parts among regions. *Aspergillus flavus* L-strain was isolated in the highest incidence from plant parts collected at harvest and in the lowest incidence

at podding while *A. flavus* S-strain was isolated in the highest incidence in plant parts collected at harvest and lowest two weeks before harvest. *Aspergillus flavus* L-strain and *A. flavus* S-strain were isolated in the highest incidence from the samples collected from Kiorimba at harvest (Table 16) with mean isolation frequency of 23 and 22, respectively.

Table 16: Incidence (%) of *A. flavus* (L and S-strains) in below ground peanut parts sampled from various regions in Meru County at different growth stages during the short rains of 2014

Region	Podding		Two weeks pre-harvest		Harvest	
	AFL	AFS	AFL	AFS	AFL	AFS
Kathuene	5.0 a	7.0 a	15.0ab	0.0 a	6.0b	15.0 a
Gaitu	1.0 a	5.0 b	18.0a	2.0 a	20.0a	18.0 a
Giaki	3.0 a	2.0 ab	6.0bc	3.0 a	15.0ab	14.0 a
Kiorimba	7.0 a	7.0 a	4.0c	3.0 a	23.0a	22.0 a
Mean	4.0	5.3	10.8	2.0	16.0	17.2
LSD ($p \leq 0.05$)	3.7	1.1	10.9	4.3	10.7	9.5
CV (%)	92.0	92	99.8	98.2	94.4	87.8

AEZ-Agro- ecological zone, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AF-L- *Aspergillus flavus* L-strain, AF-S- *Aspergillus flavus* S-strain. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

Aspergillus niger were isolated in below ground peanut plant parts from every region in the two agro-ecological zones at every growth stage in the long rains of 2013 (Table 17). There were significant ($p \leq 0.05$) differences in the incidence of *A. niger* in plant parts within different peanut growth stages and amongst different regions. The highest incidence of *A. niger* in below ground peanut plant parts was in the samples which were collected from Meru north at harvest time with an isolation incidence of 75% and lowest incidence in those which were from Meru south at podding time with an isolation incidence of 10% (Table 17). *Aspergillus niger* was isolated in the highest incidence from samples which were collected from Meru during all the

peanut growth stages with an isolation frequency of up to 90, 60 and 80 during podding, two weeks before harvest and at harvest, respectively (Table 17). In season one; *A. parasiticus* was isolated in low incidence from the samples collected in Meru South and Keiyo South with a mean isolation incidence of 1%, *A. parasiticus* was not isolated from samples from the other regions.

Table 17: Incidence (%) of *A. niger* in below ground peanut parts sampled from various regions in two agro-ecological zones at different growth stages during the long rains of 2013

AEZ	Region	Podding	Two weeks Pre-Harvest	Harvest
LM3	Meru north	87.5 a	55.0 a	72.5a
	Meru central	32.0 b	30.0 b	32.0bc
	Meru south	10.0 d	15.0 c	44.0b
LM4	Baringo Central	12.5 cd	15.6 c	23.8bc
	Keiyo south	22.5 bc	12.5 c	26.3bc
Mean		32.9	25.6	39.7
LSD ($p \leq 0.05$)		15.1	16.9	24.3
CV (%)		68.8	90.9	80.5

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AN- *Aspergillus niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

During the short rains of 2014, there were no significant ($p \leq 0.05$) variation in the incidence of *A. parasiticus* in below ground peanut plant parts within different growth stages and amongst different regions (Table 18). The highest incidence of *A. parasiticus* in the plant parts was during harvest and lowest at two weeks before harvest. *Aspergillus parasiticus* was most prevalent in samples from Kiorimba with an incidence of up to 20% at harvest (Table 18). Unlike in the long rains of 2013, there was no significant ($p \leq 0.05$) variation in the incidence of *A. niger* in below ground plant parts sampled at most growth stages and amongst the different regions; the

variation was only at two weeks before harvest . As in season one, *A. niger* was isolated in higher incidence than *A. parasiticus* with the highest incidence being in plant parts sampled at podding stage with incidence of up to 30% and lowest incidence being at two weeks before harvest with incidence of as low as 20% (Table 18). *Aspergillus niger* was isolated in the highest incidence in the samples from Kathuene with an incidence of up to 40%.

Table 18: Incidence (%) of *A. parasiticus* and *A. niger* in below ground peanut parts sampled from various regions in Meru County at different growth stages during the short rains of 2014

Region	Podding		Two weeks pre-harvest		Harvest	
	AP	AN	AP	AN	AP	AN
Kathuene	3.0 a	39.0 a	0.0a	36.0 a	9.0 a	14.0 a
Gaitu	0.0 a	24.0 a	1.0a	21.4 b	9.0 a	30.0 a
Giaki	3.0 a	25.0 a	2.0a	5.0 c	6.0 a	19.0 a
Kiorimba	1.0 a	19.0 a	3.0a	14.0b c	13.0 a	24.0 a
Mean	1.8	26.8	1.5	19.1	9.3	21.8
LSD ($p \leq 0.05$)	1.5	15.4	1.0	14.6	10.5	15.1
CV (%)		91.1		99.0	102.0	89.1

LSD-Least significant Difference, CV-Coefficient of variation, AP- *Aspergillus parasiticus*, AN- *Aspergillus niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

4.4 Population of *Aspergillus* species in peanut kernels sampled pre- and post-harvest

Aspergillus flavus L-strain, *A. flavus* S-strain, *A. niger* and *A. parasiticus* were isolated from peanut kernels sampled from different regions of Baringo Central, Elgeyo-Marakwet and Meru Counties pre and post-harvest stages in the long rains of 2013 and short rains of 2014 (Tables 19, 20, 21 and 22). In the long rains of 2013 there was a significant ($p \leq 0.05$) variation in the population of *A. flavus* L-strain and *A. flavus* S-strain in kernels within different pre and post-harvest stages and among different regions in the two agro-ecological zones (Table 19).

Aspergillus flavus S-strain was isolated in high frequency in kernels sampled during different pre-and post-harvest stages, with the highest incidence in samples collected from Meru north throughout the different pre-and post-harvest stages with a frequency of up to 60,000 CFU/g kernel (Table 19). *Aspergillus* spp. were isolated in higher frequency from peanut kernels sampled from LM3 regions than kernels sampled from LM4. *Aspergillus flavus* L-strain and *A. flavus* S-strain were isolated in highest incidence from peanut kernels sampled during harvest in the different regions with the isolation frequency of up to 2500 CFU/g kernel and 20,000 CFU/g kernels respectively.

Table 19: Population (CFU/g) of *Aspergillus flavus* (L- and S-strains) in peanut kernels sampled from various regions in two agro-ecological zones at different pre-and post-harvest during long rains of 2013

AEZ	Region	Two weeks pre-harvest		Harvest		Farmer store		Collection point	
		AFL	AFS	AFL	AFS	AFL	AFS	AFL	AFS
LM3	Meru North	188b	56875a	4000a	38000a	2000ab	22688a	1500bc	20625a
	Meru Central	450ab	375b	2250ab	9475b	675b	1225b	575c	1175c
	Meru South	225b	225b	2825a	34900a	3325a	24025a	3150a	10850b
LM4	Baringo Central	668a	3781b	1188b	9594b	2156a	125b	2750ab	438c
	Keiyo South	344b	2344b	938b	2406b	3156a	94b	1344c	688c
Mean		379	12720	2240	18875	2262	9631	1863	6755
LSD ($p \leq 0.05$)		366	15761	1773	11074	1769	8466	1614	7309
CV (%)		90	82	39	76	49	55	42	63

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AF-L- *Aspergillus flavus* L-strain, AF-S- *Aspergillus flavus* S-strain. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

Aspergillus flavus L-strain and *A. flavus* S-strain were isolated in peanut kernels from various regions of Meru County during the short rains of 2014 (Table 20). There was significant ($p \leq 0.05$) variation in the population of *A. flavus* S-strain in peanut kernels sampled during most pre and post–harvest peanut stages amongst regions. *Aspergillus flavus* S-strain was isolated in highest frequency in kernels sampled from the different regions at harvest with a mean isolation frequency of up to 7 x 10^3 CFU/g kernels. *Aspergillus flavus* L-strain was isolated in highest frequency in kernels sampled from various regions at collection point with a mean isolation frequency of 3000 CFU/g kernels and lowest frequency in samples at two weeks before harvest with a mean isolation frequency of 700 CFU/g kernels (Table 20).

Table 20: Population (CFU/g kernels) of *Aspergillus flavus* (L- and S-strains) in peanut kernels sampled from various regions in Meru County at different pre-and post-harvest stages during the short rains of 2014

Region	Two weeks pre-harvest		Harvest		Farmer store		Collection point	
	AFL	AFS	AFL	AFS	AFL	AFS	AFL	AFS
Kathuene	540a	200bc	300c	175c	3475 a	5625 a	3750a	4200b
Gaitu	720a	50c	3500a	4600bc	2125 b	5325 a	2325a	13600a
Giaki	800a	500ac	1425b	5250b	1650 b	3175 a	2775a	3800b
Kiorimba	400a	700a	1925b	17000a	1300 b	4000 a	2675a	2500b
Mean	615	362	1788	6756	2138	4531	2881	6025
LSD ($p \leq 0.05$)	416	318	731	4977	1142	2333	1301	2765
CV (%)	73	112	65	30	85	82	72	73

AEZ-Agro- ecological zone, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AF-L- *Aspergillus flavus* L-strain, AF-S- *Aspergillus flavus* S-strain. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

During the long rains of 2013, *A. parasiticus* and *A. niger* were isolated in peanut kernels from most regions during different pre and post harvest stages in the two agro –ecological zones (Table 21). There was a significant ($p \leq 0.05$) variation in the population of *A. parasiticus* and *A. niger* in peanuts from different regions at harvest, from farmer stores and at collection points. However, there were no significant ($p \geq 0.05$) differences in the population of *A. parasiticus* and *A. niger* among the different regions two weeks before harvest. *A. parasiticus* was isolated in highest incidence in kernel samples from Meru south at farmer store with an isolation frequency of up to 2300 CFU/g kernel. *Aspergillus niger* was isolated in highest frequency in kernel samples from Meru central at farmer store with an isolation frequency of up to 6500 CFU/g kernel. *Aspergillus parasiticus* and *A. niger* were isolated at higher frequency in the samples collected from LM3 than from LM4.

Table 21: Population (CFU/g) of *A. parasiticus* and *A. niger* in peanut kernels sampled from two agro-ecological zones at different pre-and post harvest stages during the long rains of 2013

AEZ	Region	Two weeks pre-harvest		Harvest		Farmer store		Collection point	
		AP	AN	AP	AN	AP	AN	AP	AN
LM3	Meru North	0 a	148 a	2250a	750bc	813ab	2500b	500b	2562 ab
	Meru Central	250 a	123 a	1250ab	250c	275b	6150a	325b	5750 a
	Meru South	75 a	752 a	500bc	2000a	2275a	1925b	1000a	925 b
LM4	Baringo Central	125 a	222 a	500bc	1250ab	156b	0b	250b	3844 a
	Keiyo South	31 a	253 a	281c	156c	250b	0b	281b	500 b
Mean		96	300	956	881	754	2115	471	2755
LSD ($p \leq 0.05$)		261	789	1035	1026	1476	2945	448	3399
CV (%)		98	94	88	93	89	89	88	76

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AP- *Aspergillus parasiticus*, AN- *Aspergillus niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

Table 22: Population (CFU/g kernel) of *A. parasiticus* and *A. niger* in peanut kernels sampled from various regions of Meru County at different pre-and post-harvest stages during the short rains of 2014

Region	Two weeks pre-harvest		Harvest		Farmer store		Collection point	
	AP	AN	AP	AN	AP	AN	AP	AN
Kathuene	100 a	1075 a	75 b	5575 a	1525 a	14100a	1075 b	14100a
Gaitu	100 a	2375 a	1025 a	4200 a	875 a	1350c	1125 b	500b
Giaki	50 a	1650 a	800 a	3775 a	925 a	7125b	1075 b	2250b
Kiorimba	50 a	2600 a	1175 a	2725 a	900 a	4975bc	2825 a	4525b
Mean	75	1925	769	4069	1056	6888	1525	5344
LSD ($p \leq 0.05$)	125	1300	404	2667	600	5614	1177	5411
CV (%)	98	58	83	47	90	40	43	45

LSD-Least significant Difference, CV-Coefficient of variation, AP- *Aspergillus parasiticus*, AN- *Aspergillus niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

4.4.1 Population of *Aspergillus* spp. in peanut kernels sampled after shelling

Four *Aspergillus* spp. – *A. flavus* L-strain, *A. flavus* S-strain, *A. niger* and *A. parasiticus* - were isolated from peanut kernels sampled after shelling at the ENP factory (Table 23). There were significant variations ($p \leq 0.05$) in the population of the four species between the two agro-ecological zones. The species isolated in the highest incidence was *A. flavus* S- strain especially from kernels sampled from LM3 while *A. parasiticus* was isolated in the lowest frequency. Kernel samples which originated from LM3 zones had a higher isolation incidence of the various *Aspergillus* spp. than samples from LM4 zones (Table 23).

Table 23: Population (CFU/g) of *Aspergillus* spp. in peanut kernels sampled from two agro-ecological zones at the processing factory after shelling during the long rains of 2013

AEZ	AFL	AFS	AP	AN	Mean
LM3	3800a	32500 a	800 a	3475 a	10143
LM4	1475b	700b	100b	3475 a	1437
Mean	2637	16600	450	3475	579
LSD (p≤0.05)	899	5421	243	1809	
CV (%)	53	51	84	81	

AEZ-Agro- ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3, LSD-Least significant Difference, CV-Coefficient of variation, AF-L- *A. flavus* L-strain, AF-S- *Aspergillus flavus* S-strain, AP- *Aspergillus parasiticus*, AN- *Aspergillus niger*. Means followed by the same letter within columns are not significantly different (Fisher's protected LSD at $p \leq 0.05$).

4.5 Aflatoxin levels in peanut kernels pre- and post-harvest

During the long rains of 2013, there were variations in the total aflatoxin levels in peanut kernels sampled among the different pre-and post harvest stages between the two agro-ecological zones (Table 24). The levels in kernels sampled from LM4 were lower in comparison to those sampled from LM3 among the different stages. Samples from Meru County were the most contaminated

by aflatoxin especially those sampled at harvest and at farmer stores. The levels of total aflatoxin in the kernels were significantly lower ($p \leq 0.05$) in samples from most regions sampled two weeks pre-harvest in the two agro-ecological zones. At collection point, the total aflatoxin levels in the samples which were collected from Baringo Central and Keiyo South were not significantly different and 100% of these samples were within the standards set by both EC and KEBS (Table 24).

During the short rains of 2014, there were variations in the total aflatoxin levels in peanut kernels sampled among the different pre-and post-harvest stages (Table 25). Thirty five percent of the Kernel sampled at collection point had aflatoxin levels which were above the detection limit (>405 µg/kg). Samples from Kathuene region of Meru Central had lower levels of total aflatoxin throughout the different pre-and post-harvest stages. The most contaminated kernel samples were from Gaitu which is also a division in Meru Central sampled at collection point (Table 25). Eighty percent of the kernel samples from Meru in the short rains of 2014 had total aflatoxin levels which were beyond the EC and KEBS limits.

Table 24: Total aflatoxin (µg/kg) in peanut kernels sampled from various regions in two agro-ecological zones at different pre- and post-harvest stages during the long rains of 2013

AEZ	Sample ID	Region	Two weeks pre – harvest	At harvest	Farmer store	Collection point
LM3	009	Meru North	<2.0	123.9	13.3	16.3
	010	Meru North	2.7	>141.8	>141.8	>141.8
	011	Meru North	<2.0	4.9	11.3	12.8
	012	Meru North	<2.0	28.6	41.8	47.1
	013	Meru North	<2.0	2.2	135.5	>141.8
	014	Meru Central	10.1	>141.8	12.8	26.8
	015	Meru Central	>100.0	>141.8	>141.8	>141.8
	016	Meru South	<2.0	<1.8	10.0	27.2
	017	Meru South	<2.0	>141.8	>141.8	>141.8
	018	Meru South	<2.0	>141.8	>141.8	2.8
	019	Meru South	<2.0	>141.8	>141.8	>141.8
	020	Meru South	<2.0	>141.8	>141.8	3.8
LM4	001	Baringo Central	<1.8	<1.8	2.2	<1.8
	002	Baringo Central	2.7	2.0	1.9	<1.8
	003	Baringo Central	<1.8	<1.8	<1.8	<1.8
	004	Baringo Central	1.9	2.0	2.3	<1.8
	005	Keiyo South	3.1	<1.8	<1.8	<1.8
	006	Keiyo South	2.3	2.0	1.9	<1.8
	007	Keiyo South	<1.8	4.0	2.1	<1.8
	008	Keiyo South	<1.8	6.0	2.1	<1.8

AEZ - Agro-ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3

Table 25: Total aflatoxin (μg/kg) in peanut kernels sampled from various regions in Meru County at different pre- and post-harvest stages during the short rains of 2014

Region	Sample ID	Two weeks pre –harvest	At harvest	Farmer store	Collection point
Kathuene	001	11.1	9.5	93.0	12.5
Kathuene	002	8.9	6.8	39.6	43.8
Kathuene	003	9.1	5.1	84.9	13.9
Kathuene	004	8.9	7.1	84.1	19.0
Kathuene	005	11.1	8.8	122.4	17.9
Gaitu	006	9.8	>405.0	>405.0	>405.0
Gaitu	007	6.1	>405.0	52.0	>405.0
Gaitu	008	<1.75	63.1	>405.0	>405.0
Gaitu	009	21.5	101.5	100.9	>405.0
Gaitu	010	12.4	21.3	8.4	>405.0
Giaki	011	11.2	20.5	>405.0	>405.0
Giaki	012	14.5	>405.0	13.4	30.0
Giaki	013	>405.0	43.9	69.5	21.6
Giaki	014	22.5	30.8	55.9	131.2
Giaki	015	6.7	132.7	14.4	>405.0
Kiorimba	016	>405.0	36.5	17.4	39.5
Kiorimba	017	37.6	35.0	5.7	32.0
Kiorimba	018	41.1	>405.0	>405.0	48.1
Kiorimba	019	69.8	>405.0	13.7	50.7
Kiorimba	020	>405.0	>405.0	16.0	63.1

Test detection limit was (1.75 to 405.0) ppb

Total aflatoxin level in some peanut kernels sampled at different pre- and post-harvest stages during the long rains of 2013 were considered acceptable based on European Commission and KEBS aflatoxin regulations while others were above these levels (Table 26). At two weeks pre-harvest, 90% of the kernel samples had aflatoxin levels that were within the acceptable limits by the European Union and also by KEBS regulations. At harvest, farmer store and at collection point, kernel samples from Meru County in LM3 zones were above both the standards set by the EC and KEBS at 75, 91 and 83 of the samples, respectively. All of the kernel samples from Baringo Central and Keiyo South (both in LM4) were within the safe limits set by the EC and KEBS during the different pre- and post-harvest sampling stages.

Table 26: Proportion (%) of peanut samples in different aflatoxin content categories (µg/kg) and sampled at pre- and post-harvest stages from different regions in two agro-ecological zones during the long rains of 2013

AEZ	Region	Two weeks pre-harvest			At harvest			Farmer store			Collection point		
		≤4	4-10	>10	≤4	4-10	>10	≤4	4-10	>10	≤4	4-10	>10
LM3	Meru North	100	0	0	20	20	60	0	0	100	0	0	100
	Meru Central	0	0	100	0	0	100	0	0	100	0	0	100
	Meru South	100	0	0	20	0	80	0	20	80	40	0	60
LM4	Baringo Central	100	0	0	100	0	0	100	0	0	100	0	0
	Keiyo South	100	0	0	75	25	0	100	0	0	100	0	0
Mean		80	0	20	43	9	48	40	4	56	48	0	52

AEZ-Agro-ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3; ≤4 - EU limit for total aflatoxin, ≤10 KEBS limit for total aflatoxin

During the short rains of 2014, most kernel samples had total aflatoxin levels which were above the limits set by the EC and KEBS throughout the different pre-and post-harvest stages (Table 27). Unlike in season one where most of the samples collected two weeks pre-harvest were considered safe according to the regulations set by the EC and KEBS, 65% of the samples collected in season two at two weeks pre-harvest had aflatoxin levels which were above the set limits. At collection point, all the samples had total aflatoxin levels above the safe limits set by EC and KEBS. Total toxin levels in the samples collected in season two were higher than those collected in season one.

Table 27: Proportion (%) of peanut samples in different aflatoxin content categories (μg/kg) and sampled at various pre- and post-harvest stages from different regions in Meru County during the short rains of 2014

Region	Two weeks pre-harvest			At harvest			Farmer store			Collection point		
	≤4	4-10	>10	≤4	4-10	>10	≤4	4-10	>10	≤4	4-10	>10
Kathuene	0	60	40	0	100	0	0	0	100	0	0	100
Gaitu	20	40	40	0	0	100	0	20	80	0	0	100
Giaki	0	20	80	0	0	100	0	0	100	0	0	100
Kiorimba	0	0	100	0	0	100	0	20	80	0	0	100
Mean	5	30	65	0	20	75	0	10	90	0	0	100

<4ug/kg-EU limit for total aflatoxin, 4-10ug/kg-KEBS limit for total aflatoxin, >10ug/kg-above the KEBS limit for total aflatoxin.

Total aflatoxin levels in kernel samples collected after shelling significantly varied between the two agro-ecological zones (Table 28). There were higher levels of total aflatoxin in the samples collected from Meru County in LM3 than those which originated from Baringo and Elgeyo-Marakwet Counties in LM4. All of the samples from Baringo and Keiyo had aflatoxin levels which were considered safe according to the regulations set by both the EC and KEBS. However, 100% of the kernel samples from Meru were above the safe limit (≤10 µg/kg) for total aflatoxin set by KEBS.

Table 28: Total aflatoxin (µg/kg) in peanut kernels sampled at the factory after shelling during the long rains of 2013

AEZ	Region	Sample ID	Aflatoxin levels (µg/kg)
LM3	Meru	006	87.0
	Meru	007	>141.75
	Meru	008	>141.75
	Meru	009	>141.75
	Meru	010	128.8
LM4	Baringo and Keiyo	001	<1.75
	Baringo and Keiyo	002	2.2
	Baringo and Keiyo	003	2.1
	Baringo and Keiyo	004	1.9
	Baringo and Keiyo	005	3.8

AEZ-Agro-ecological zone, LM 4- Lower midland zone 4, LM 3- Lower midland zone 3.

CHAPTER FIVE: DISCUSSION

This study aimed at monitoring the population of *Aspergillus* spp. and aflatoxin levels in order to determine the critical aflatoxin contamination points in pre-and post-harvest peanuts in Meru, Baringo and Elgeyo-Marakwet Counties of Kenya. Ultimately, data generated from the study would be important in ensuring timely interventions that would ensure production of peanuts that are compliant with aflatoxin limits set by the European Commission and the Kenya Bureau of Standards of 4 µg/kg and 10 µg/kg, respectively.

5.1 Agronomic practices in peanut production

The peanut farmers in Baringo, Elgeyo-Marakwet and Meru Counties were mostly small scale farmers who did not use any soil amendments or even pesticides to control pests and diseases of peanuts, lacked knowledge on key peanut production practices like land preparation methods, crop rotation, common diseases of peanut and their management, among others. Most peanut farmers practiced crop rotation and maize was the main rotation crop. Most of the farmers practiced mixed cropping with 90% of the peanut farms also growing maize. These peanut production practices were in agreement with the findings from a previous study by Kumar (2010) that farmers with large land holdings were high adopters of aflatoxin management practices of peanuts compared to farmers with small land holdings.

Maize and peanuts are highly susceptible to fungal contamination especially aflatoxigenic fungi such *Aspergillus* spp. (Wu and Khlangwiset, 2010). Inclusion of maize in the rotation programs might have been a contributing factor to the high incidence of aflatoxigenic *Aspergillus* spp. in Meru County. In field studies in the USA, *A. flavus* propagules were greater in soils collected

from fields where continuous maize production was practiced compared to soils where either cotton or wheat had been grown (Abbas *et al*. 2004; Reddy *et al*. 2007). Likewise, Griffin *et al.* (1981) observed greater *A. flavus* propagules in soils from fields where continuous maize or peanuts cropping was practiced. The current study also concurred with the findings by Garcia and Cotty (2010) that previous crop influences both the incidence of *A. flavus* and *A. flavus* S-strains. This suggested that it may be possible to manipulate crop rotations in order to reduce aflatoxin contamination particularly in Meru County. If done in an appropriate way, crop rotation may be important in breaking the life cycle of aflatoxin producing fungal pathogens (Strosnider *et al.,* 2006). A study by Mutegi *et al*. (2012) in Western Kenya indicated that the percentage of *A. flavus* and *A. parasiticus* isolates testing positive for aflatoxin B1 and B2 was significantly higher in samples obtained from farmers who did not practice crop rotation compared to those that rotated their crops.

In the current study, most farmers did not use any soil amendments in peanut production. This might be one of the contributing factors to high incidence of fungal pathogens in the study areas. In previous studies, application of lime and farm yard manure as soil amendments have been shown to be effective in reducing *A. flavus* contamination and aflatoxin levels by 50-90% (Waliyar *et al*., 2008). Calcium in lime thickens the cell wall and accelerates pod filling, while manure facilitates growth of saprophytic micro-organisms that suppress pathogenic fungi in soil. Similar findings have been reported by Rajik *et al.* (2011) who reported that soil amendment reduced the populations of soil borne pathogens. Singh and Pathak (2006) also reported that the application of organic compounds reduced the population of soil harmful microflora in potato fields. Amendments like application of gypsum provide sufficient calcium to the plant and offers

tolerance to infection by *A. flavus* (Reddy *et al*., 2003). Some soil amendments should be considered in peanut fields to allow reduction of *Aspergillus* contamination and subsequent production of aflatoxin in the peanut kernels.

Farmers in Baringo, Elgeyo-Marakwet and Meru Central produced their peanut under rain fed conditions unlike in Meru North and Meru South where peanut was produced under sprinkler irrigation. Higher levels of aflatoxin were found in kernels from Meru County than Baringo and Elgeyo-Marakwet Counties. This might have been due to higher population of *Aspergillus flavus* S-strain in Meru County. This contradicts the findings by Kebede *et al.* (2012) who found out that aflatoxin contamination in the maize kernels was higher in non-irrigated fields than the irrigated plots. Although the effect of weather was not investigated in this study, previous studies have demonstrated a relationship between aflatoxin contamination and whether conditions especially temperature and relative humidity. It is therefore important to generate weather models that can predict an aflatoxin contamination outbreak. Kumar *et al*. (2008) also mentioned that aflatoxin contamination of peanuts is a major problem of rain fed agriculture in semi-arid tropical environments. According to Reddy *et al.*, (2003) and Kebede *et al*, (2012), irrigation reduces moisture stress in the farms. Moisture stress fevers production of aflatoxin by the *Aspergillus* spp. Since the quantity of aflatoxin was more under irrigated peanut farms of Meru County than in rain fed regions of Baringo and Elgeyo-Marakwet counties. It could be that frequent sprinkling help to spred *Aspergillus* spp.From the current study *A. flavus* S-strain was isolated in higher frequency in these regions with irrigation facilities.

There was significant ($P \leq 0.05$) variation in the prevalence of peanut diseases in the different regions in the two agro-ecological zones; Meru Central was the most affected generally with early blight, late blight and aschochyta which were all foliar diseases, being the most prevalent

diseases. The farmers did not use fungicides in the management of peanut diseases. Previous studies (Hell *et al*., 2000), show that diseases and pests of peanuts are common in western Kenya smallholder cultivation systems that have minimal investment in inputs including application of chemical pesticides. In this regard, the study also concurs with Mutegi *et al*. (2009) who reported that due to the small scale nature of production of peanuts, the use of fertilizers and pesticides in peanuts is not common and quality aspects such as Good Agricultural Practices (GAP) and appropriate post-harvest handling practices such as sorting, that would have a direct impact on aflatoxin levels in the nuts are disregarded. Stress from diseases could have predisposed peanut plants to aflatoxin contamination.

The farmers determined the harvesting time by visual observation of changes in peanut plants like changes in color, shedding of leaves and also by manual uprooting of the plant to observe if the pods were mature for harvest. The method of determination of harvest time by the farmers could be responsible for the development of fungal pathogens in the study regions since the technique is never precise. This is in agreement with Kaaya *et al*. (2006) who observed that aflatoxin levels increased by about four times by the third week after the recommended harvesting time and more than seven times when maize harvest was delayed for four weeks. However, after early harvested produce has to be dried to safe levels to stop fungal growth.

A clear outcome of the current study is that most farmers were unaware of aflatoxin, and therefore they do not perceive aflatoxin contamination as a problem in peanut production. The lack of awareness could have contributed to the high aflatoxin quantities detected in the various regions since the farmers were not even aware of the recommended practices to control aflatoxin contamination in peanuts (Kumar, 2010).

5.2 Population of *Aspergillus* spp. in soil and plant debris at planting

The soils and plant debris from different regions did not significantly differ in the levels of *Aspergillus flavus* L-strain, *A. flavus* S-strain, *A. parasiticus* and *A. niger*. Previous studies have reported that *Aspergillus* spp. forms sclerotia that allows the fungus to survive saprophytically for extended periods in the soil, maize residue and maize cobs (Scheidegger and Payne, 2003; Jaime-Garcia and Cotty, 2004; Wagacha and Muthomi, 2008; Accinelli *et al*., 2008). Presence of *Aspergillus* spp. in the soil and plant debris predisposed the peanut plants to attack by the *Aspergillus* spp. This concurs with the findings that, propagules in the soil and crop debris act as the primary source of *Aspergillus* inoculum infecting maturing maize crops (Jaime-Garcia and Cotty, 2004; Horn, 2007; Atehnkeng *et al.,* 2008). The study is also in agreement with Payne (1992) who found out that when the spores of *Aspergillus* spp. find a suitable nutrient source and favorable environmental conditions, the fungus rapidly colonize, establish and produce aflatoxin on susceptible crops. The findings also concurs with the reports by Olanya *et al.* (1997) and Strosnider *et al.* (2006) that elimination of inoculum sources such as infected debris from the previous harvest, may prevent infection of the current crop. Soil and debris were the major sources of infection to the kernels, the study proved that peanut debris had capability of transferin infection to the crops in the field.

5.3 Population of *Aspergillus* spp. in soil and peanut plant parts at various pre-harvest and harvest stages

Aspergillus flavus S-strain*, A. flavus* L-strain, *A. parasiticus* and *A. niger* were the *Aspergillus* spp. isolated from soil and below ground peanut plant parts from Baringo, Elgeyo-Marakwet and

Meru Counties. *Aspergillus tamarii* was isolated too although in low incidence in the soil samples from LM3 of Meru County. The findings concur with Accinelli *et al*. (2008) that *Aspergillus* spp. spends most of their life growing as saprophytes in the soil. According to other studies, *Aspergillus flavus* and *A. parasiticus* are ubiquitous fungi that can colonize many crops including maize, peanuts and tree nuts (Hill *et al*., 1985; Diener *et al*., 1987; Klich, 2002; Gachomo *et al*., 2004; Wagacha and Muthomi 2008). The low incidence of *A. tamari* in the current study concurs with the findings by Probst *et al*. (2010) who reported that *A. tamarii* occurred at a relatively low incidence in maize samples from several provinces in Kenya. The fungal species were isolated during all the peanut growth stages. Similar to the current findings, Ferreira de Souza1 *et al.* (2014), detected aflatoxin-producing *Aspergillus* spp. in the peanut samples analyzed at different phenological stages, aerial gynophore, pod filling (seeds), mature pods, and dry fruits (harvest), and added that it indicates the importance of good agricultural practices from the cultivation to storage of peanuts in southern Brazil.

Aspergillus flavus L-strain was isolated in higher frequency in samples from Baringo and Elgeyo-Marakwet while *A. flavus* S-strain was isolated in higher frequency in samples collected from Meru County. *A. parasiticus* and *A. niger,* had similar trends in the study. The high incidence of *A. flavus* S-strain in Meru County positively correlated with the total aflatoxin levels in these regions whereas high incidence of *A. flavus* L-strain in Baringo and Elgeyo-Marakwet Counties did not significantly influence the total aflatoxin levels. *Aspergillus* spp. were isolated in higher frequency in soil samples at podding stage and at two weeks before harvest when peanut plants were still immature. According to Dorner *et al*. (1989) immature

peanuts are more resistant to fungal invasion and contamination with aflatoxins because they produce higher amounts of phytoalexins than the mature peanuts.

5.4 Population of *Aspergillus* species and aflatoxin contamination in peanut kernels sampled pre- and post-harvest

Aspergillus spp. isolated from peanut kernels sampled from the various regions under study at different pre- and post-harvest stages included *Aspergillus flavus* L-strain, *A. flavus* S-strain, *A. parasiticus* and *A. niger.* These findings concurs with those of (Mutegi *et al.,* (2012) who also isolated members of *Aspergillus* section *Flavi* from peanut kernel from Busia and Homa bay districts of western Kenya. The study by Wagacha *et al*. (2013) also reported diverse fungal pathogens from peanuts in Kenya.

Aspergillus flavus L-strain, *A. flavus* S-strain, and *A. niger* were the most isolated species in the different regions of Baringo, Elgeyo-Marakwet and Meru Counties. The findings agree with those of Mutegi *et al*. (2012) that the predominant species in Western (>60% incidence) were *A. flavus* L-strain, *A. flavus* S-strain and *A. niger*. Abdela (2009) also reported contamination of groundnut samples from Sudan by *A. niger* and *A. flavus*, which were isolated at frequencies of 29-60% for *A. niger* and 4-52% for *A. flavus*. Nyirahakizimana *et al*. (2013) also isolated *A. flavus* L strain, *A. flavus* S strain, *A. parasiticus*, *A. tamarii*, *A. caelatus*, *A. alliaceus* (members of *Aspergillus* section *Flavi*) and *A. niger* in 69% of the peanuts sampled from Kericho and Eldoret towns in Kenya.

There were high population of *Aspergillus* spp. in kernels sampled at harvest both in season one and in season two, this predisposed the kernel to Aflatoxin contamination especially in Meru

County. The high populations might have been due to infestation of peanut pods by pests and mechanical damage during harvesting that could have provided avenues for colonization of the nuts by the aflatoxin-producing fungi (Waliyar *et al*., 2008). Peanuts sampled from farmer stores and at collection points, had high population of *A. flavus* S-stain and *A. flavus* L-stain which could be attributed to poor storage practices. In the current study, 50% of the farmers stored their peanuts in their houses, majority of which did not have proper ventilation to allow air flow as recommended in good storage practices.

Aspergillus flavus, the main producer of aflatoxin, only grows in groundnuts when the moisture content exceeds 9% and has optimum growth conditions of between 25 and 30°C, and water activity of 0.99 with a minimum of 0.83_{aw}. Production of aflatoxin occurs optimally at 25°C and 0.99aw with a minimum of 0.87_{aw} (Ribeiro *et al.,* 2006). The warm and humid environmental conditions in Africa are ideal for growth of *A. flavus* making aflatoxin contamination of food, including groundnuts, a widespread problem across the continent (Gordon, 2003; Bankole *et al*., 2006; Wagacha and Muthomi, 2008). Previous studies expected farmers to embrace practices that improve peanut quality and safety through proper drying, grading and storage. Such practices have consistently been shown to reduce the level of contaminated peanuts (Gowda *et al*., 2002; Turner *et al*., 2005; Waliyar *et al.,* 2008). Dry grains keep longer and safe from moulds because the water activity required for their growth is not met (Sanders, *et al.,* 1982). However, high humidity conditions in many tropical countries constrain efforts to dry peanuts to acceptable moisture levels (Mestres *et al*., 2004), thereby increasing the risk of fungal growth and aflatoxin contamination.

The warm and humid environmental conditions in Africa are ideal for growth of *A. flavus* making aflatoxin contamination of food, including peanuts, a widespread problem across the continent (Gordon, 2003; Bankole *et al*., 2006; Wagacha and Muthomi, 2008). *Aspergillus flavus* and aflatoxin have also been reported in peanut butter in Sudan (Eshafie *et al*., 2011). Members of *Aspergillus* section *Flavi* mainly *A. flavus* and *A. parasiticus* are the major producers of aflatoxin in peanuts (Passone *et al.,* 2010). *Aspergillus flavus* produces only B-type aflatoxins and it is the second leading cause of human aspergillosis (Hedayati *et al*., 2007; Pasqualotto and Denning, 2008) while *A. parasiticus* produces both B- and G-type aflatoxins (Ehrlich *et al*., 2004; Frisvad *et al*., 2005). Although *A. flavus* and *A. parasiticus* are the species most frequently implicated in aflatoxin contamination (Cotty, 2006), the wide fungal species diversity poses a health risk of exposing peanut consumers to other secondary metabolites.

The high incidence of *A. niger* is particularly of great health concern since they too produce a wide spectrum of secondary metabolites. *Aspergillus niger* produces ochratoxin A and malformins among others (Frisvad *et al.,* 2007; Weidenbörner, 2008). The population of *A. flavus* S-strain was found to significantly influence aflatoxin production for example Meru County samples had high frequencies of *A. flavus* S-strain and the total aflatoxin levels in these samples too were found to be high. This concurs with the findings by Wagacha *et al*. (2013) who reported that the incidence and population of *A. flavus* S-strain significantly and positively correlated with the levels of total aflatoxin in peanuts sampled from different markets in Kenya. 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 B1 (Mutegi *et al.,* 2012) which is also classified as class 1 carcinogen (IARC, 1987). Garcia and

Cotty (2010) reported *A. flavus* S-strain to be the primary cause of aflatoxin contamination events in North America and Africa.

The current study also investigated the levels of total aflatoxin in peanut samples from Meru, Baringo and Elgeyo-Marakwet Counties at different pre-and post-harvest stages. The total aflatoxin levels significantly varied among the different pre- and post-harvest stages and among regions in the two seasons. This is contrary to the findings by Gonçalez *et al*. (2008) that there is no difference in levels of contamination for samples collected at different stages of maturity.. Total aflatoxin levels in peanut kernels sampled from Baringo and Elgeyo-Marakwet Counties were significantly lower compared to total aflatoxin levels in kernels collected from Meru County.

Eighty percent of the peanut samples collected two weeks before harvest met the EU standards for total aflatoxin (≤ 4 µg/kg) while 85% of the samples met the Kenyan regulatory threshold of ≤10 µg/kg at two weeks before harvest during season one. At harvest, in farmer store, at the collection point and after shelling, there was a steady increase in the total aflatoxin in samples collected from Meru County, most samples exceeded both EU and KEBS limits. All of the samples collected from Baringo and Elgeyo-Marakwet Counties during the different stages were within the safe limits by EU and KEBS. It is clear that Meru County faces a great risk of aflatoxin contamination and this may be of great health concern in the region.

Aflatoxin contamination of peanuts should be a public health concern not only in Meru County but also in other parts of Kenya as well as other tropical countries. High aflatoxin contamination

levels (above the 10 μg/kg limit set by KEBS) have been reported in raw and processed peanuts sampled from different regions of Kenya (Gachomo *et al*., 2004; Mutegi *et al*., 2012; Mutegi *et al*., 2013; Wagacha *et al*., 2013). In a recent study Mutegi *et al*. (2012), reported that 37% of groundnuts and their products including peanut butter and peanut flour sampled from Nairobi, Nyanza and Western Kenya did not meet the 10 μg/kg total aflatoxin limit set by the Kenya Bureau of Standards (KEBS, 2007). Diop *et al.* (2000) reported a mean content of 40 μg/kg of aflatoxin B1 in over 85% of peanut oil samples from Senegal. Different levels of aflatoxin were also reported in peanuts collected from processors, stockers, farmers and traders in Benin. Oliveira *et al*. (2009) too reported mean total aflatoxin level of 56 μg/kg in unprocessed peanuts in Brazil.

Aflatoxin contamination can occur during crop development when the crop is damaged by insects, or stressed by heat and drought and after maturation when the crop is exposed to high moisture either before harvest or in storage (Payne, 1992; Atehnkeng *et al*., 2008). The problem escalates when peanuts are stored in a facility where there is poor air circulation in the immediate environment (Mutegi *et al*., 2013). Efforts should be made to prevent moisture migration into stored grains through leaking roofs and condensation resulting from inadequate ventilation (Bankole and Adebanjo 2003; Hell and Mutegi, 2011).

The detected *Aspergillus* spp. and aflatoxin contaminations in the samples collected from various regions could be due to the moderate temperature, rainfall and relative humidity in the Meru, Baringo and Elgeyo-Marakwet counties during the 2013 growing season (Appendix II and III). The weather pattern in Meru Baringo and Elgeyo-Marakwet Counties were not significantly different (Appendix II and III). The temperature and humidity ranges were 11-28°C and 58-80

respectively, for Baringo whereas in Meru County the temperature and humidity ranges were 11-26°C and 55-87, respectively (Appendix II and III).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Peanut production practices in Baringo, Elgeyo-Marakwet and Meru counties predisposed peanuts to infection by *Aspergillus* spp. and subsequently to aflatoxin contamination. The farmers did not apply soil amendments or pesticides to control pests and diseases and lacked awareness on key peanut production practices like land preparation methods, proper crop rotation, common diseases of peanut and their management, among others. Although most peanut farmers practiced crop rotation, maize was the main rotation crop. These agronomic practices are likely to have contributed to accumulation of propagules of *Aspergillus* spp. in the soil that acted as the primary source of the pathogens inocula.

The results showed that peanuts are at risk of aflatoxin contamination both pre and post harvest and that intervention strategies should aim at managing the pathogens both pre and post harvest. Another possible indication of high risk of aflatoxin contamination of peanuts is the diversity of aflatoxin producing fungi. Wide fungal species diversity poses a health risk of exposing peanut consumers to other secondary metabolites. The high incidence of *A. niger* is particularly of great health concern since they too produce a wide spectrum of secondary metabolites.

There was higher aflatoxin contamination of peanuts sampled from Meru County than from Baringo and Elgeyo-Marakwet Counties. The aflatoxin level in most peanut samples from Meru County exceeded the acceptable aflatoxin limits set by the EC and KEBS whereas all the samples from Baringo and Elgeyo-Marakwet Counties were within the safe limits set by the EC

and KEBS. High aflatoxin contamination in Meru is a health concern and may impact negatively on peanut trade locally, regionally and internationally. Therefore, there is a high risk of aflatoxin contamination of peanuts in Meru County which could be attributed to the dominance of *A. flavus* S-strain.

6.2 Recommendations

i. In order to reduce aflatoxin contamination of peanuts, farmers should be trained on good agronomic practices particularly on crop rotation, disposal of plant residues after harvesting, application of soil amendments, timely weeding, avoiding water stress during critical growth stages and timely harvesting.

ii. Training farmers on the use of appropriate drying and storage methods, for example, drying on windrows or the Mandela-cock method in case of rains during harvesting, storage in cool, dry structures with proper aeration should be practiced to reduce the likelihood of contamination with aflatoxigenic fungi and aflatoxin.

iii. Inclusion of maize as a rotation crop in peanut production should be avoided. Besides acting as an alternative host to *Aspergillus* spp., maize stovers are difficult to decompose remaining in soil for up to two years thereby playing a critical role as source of primary inocula.

iv. Raise awareness on the aflatoxin contamination of peanuts, its implications on human and livestock health and trade and on possible management strategies.

v. Further studies to generate weather models and mapping of aflatoxin hot spots are necessary to mitigate aflatoxin contamination in crops.

vi. Further research should be conducted to investigate the dominance of *A. flavus* S-strain in Meru County on long term trends in aflatoxin contamination of peanuts produced in the County.

vii. Further research should also be conducted to determine whether the *A. flavus* L-strain which was dominant in Baringo and Elgeyo-Marakwet Counties are atoxigenic.

viii. Continuous monitoring of *Aspergillus* and aflatoxin contamination in peanut production, handling and marketing to ensure that the aflatoxin limits set by the EU and KEBS are adequately and effectively adhered to by all the stakeholders. Incentives may be given to farmers who produce peanuts that meet the set safe standards.

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LIST OF APPENDICES

Appendix I: Survey questionnaire: Aflatoxin contamination of peanuts

Section I: Background information

Farmer ID: ------- Name of the farmer: -- Date: ----/----/2013

Age: -------- Gender: (M) (F) Village: -------------------------- Agro-Ecological Zone: -------------
Latitude: ----------------------- Longitude: ---------------------- Elevation (m): -----------------------

Section II: Information on farming practices

1. Total land size (Acres) -------------- Area under peanut production (Acres): --------------------
2. How many years have you practiced peanut production? ---------------------------------------
3. Varieties of peanuts grown: a) -------------------------------------- b) ------------------------------
   c) ------------------------------------ d) ------------------------------------
4. Sources of seeds: a) Own b) Neighbor c) Local Market d) Agro-shop e) Other (specify) ---
5. What method(s) of field preparation do you practice? --
   ----
6. Other crops grown on the farm a) -------------------------------------- b) ------------------------
   c) --------------------------------- d) -------------------------------- e) ---------------------------------
7. Do you mix peanut crop with other crops? (Yes) (No)
8. If yes, with what crops? a) --- b) ------------------------------
   c) --------------------------------- d) -------------------------------- e) ---------------------------------
9. Do you practice crop rotation in peanut production? (Yes) (No)
10. If yes, with what crops? a) --- b) ------------------------------
    c) --------------------------------- d) -------------------------------- e) ---------------------------------
11. What crop(s) did you plant the previous season on the area currently under peanuts?
    a) ---------------------------------- b) ------------------------------c) ---------------------------------
12. If one of the crops is maize, how did you use the maize stovers? a) ------------------------------
    b) ------------------------------- c) --------------------------------- d) ----------------------------------
13. Do you use any soil amendments in peanut production? (Yes) (No)
14. If yes, which amendments and at what growth stage? a) ---
    b) --- c) ---
15. Do you practice irrigation? (Yes) (No)
16. If yes, by which method(s)? a) Sprinkler b) Drip c) Furrow d) Manual (Bucket) e) Others (specify) ---

17. At what growth stage do you weed your peanut crop? a)Emergence b)Vegetative growth c) Flowering d) Pegging e) Podding f) Pod filling f) Maturity
18. What are the most common peanut diseases in your farm? a) ------------------------------------ b) ------------------------------------- c) --------------------------------- d) ---------------------------
19. Do you employ any methods of pest and disease control in peanuts? (Yes) (No)
20. If yes, which ones? a) -------------------------------------- b) ------------------------------------- c) --- d) --
21. How do you know that your peanut crop is ready for harvesting? --------------------------------- --
22. How do you dry your peanuts after harvesting? --- ---
23. For how long do you dry them? -- --
24. How do you shell your peanuts? a) Manually b) Use of machine c) Others (specify) -----------
25. If you use machine, what kind of machine? ---
26. How do you store your produce? a) Jute bag b) Polypropylene bag c) Polyethylene bag d) Bucket e) Reed basket f) Tin g) Others (specify) --
27. Where do you store your produce? a) Traditional granary b) In the house c) Modern store d) Others (specify) --
28. On average, how long do you store your peanuts before: a) Consumption ----------------------- b) Planting ----------------------- c) Selling ---------------------------
29. Why do you produce peanuts? a) Subsistence b) Commercial c) Others (specify) --------------
30. If commercial production, where do you market your peanuts? a) -------------------------------- b) --------------------------------- c) --------------------------------- d) -------------------------------
31. What challenges do you face as a farmer in your peanut production process?
    a) -------------------------------- b) ---------------------------------- c) --------------------------------
32. Would you accept up-scaling peanut production in your farm? (Yes) (No)
33. Have you heard of Aflatoxin contamination of peanuts (Yes) (No)
34. If yes, what practices do you put in place to reduce Aflatoxin contamination of your peanuts?

Thank you for your time and cooperation

Appendix II: Monthly precipitation (mm), temperature (°C), and relative humidity (%) data recorded at the Meru Weather Stations for the year 2013.

Month	Total Precipitation	Minimum temperature	Maximum temperature	Relative humidity 06Z	Relative humidity 12Z
January	75.2	12.0	24.8	79.0	63.0
February	1.4	11.5	26.2	75.0	52.0
March	115.7	14.7	26.1	79.0	58.0
April	321.4	15.0	24.1	85.0	73.0
May	6.2	13.9	23.5	81.0	64.0
June	7.2	13.2	20.8	86.0	68.0
July	8.1	12.7	20.7	87.0	65.0
August	8.9	12.6	21.5	85.0	59.0
September	2.6	12.7	24.7	77.0	49.0
October	30.1	14.3	26.0	74.0	45.0
November	494.4	14.4	23.4	86.0	69.0
December	88.8	12.7	23.3	81.0	71.0

Relative humidity 06Z – relative humidity taken at 9.00am; relative humidity 12Z - relative humidity taken at 3.00pm

Source: Ministry of Environment, Water and Natural Resources, State Department of Environment, Meteorological Service, Kenya (2014)

Appendix III: Monthly precipitation (mm), temperature (°C), and relative humidity (%) data recorded at Baringo and Elgeyo-Marakwet Weather Stations for the year 2013.

Month	Total Precipitation	Minimum temperature	Maximum temperature	Relative humidity 06Z	Relative humidity 12Z
January	47.7	10.0	25.9	65.8	37.2
February	0.0	9.0	27.5	53.0	25.4
March	80.3	11.3	27.9	59.8	37.1
April	290.5	13.4	25.3	80.8	58.4
May	122.5	12.3	25.0	74.9	47.8
June	123.2	12.8	24.0	75.6	51.6
July	135.1	11.2	24.0	75.3	49.8
August	123.5	11.8	23.7	74.6	53.2
September	179.4	11.7	25.6	71.5	46.4
October	63.9	10.7	25.4	64.9	44.5
November	32.4	11.0	23.4	73.8	58.3
December	183.2	10.9	23.9	70.1	46.8

Relative humidity 06Z – relative humidity taken at 9.00am; relative humidity 12Z - relative humidity taken at 3.00pm

Source: Ministry of Environment, Water and Natural Resources, State Department of Environment, Meteorological Service, Kenya (2014)