



University of Nairobi

**ASSESSMENT OF LOCALLY CULTIVATED GROUNDNUT (*Arachis hypogaea*)
VARIETIES FOR SUSCEPTIBILITY TO AFLATOXIN ACCUMULATION IN
WESTERN KENYA**

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DECLARATION

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DEDICATION

I dedicate this thesis to my parents Mr. and Mrs. Richard Okeko and the rest of my family who have been supportive of me.

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LIST OF ABBREVIATIONS

%.....	Percentage
µg	Microgram
µg/ml.....	Microgram per milliliter
µl -.....	Microliter
µM.....	Micrometer
AFPA.....	<i>Aspergillus flavus / parasiticus</i> Agar
°C	Degree Celsius
DNA.....	Deoxyribonucleic acid
g	Grams
GYES.....	Glucose Yeast Extract Soytone
Min.	Minutes
ml	Milliliters
mm.....	Millimeters
mM.....	Millimole
PCR.....	Polymerase Chain Reaction
PDA.....	Potato Dextrose Agar
pH.....	Hydrogen ions concentration
ppb	parts per billion
U.....	Units
UV.....	Ultra violet
WHO.....	World Health Organization
aw	water activity

ABSTRACT

Aflatoxins are secondary metabolites produced mainly by *Aspergillus* spp especially *A. flavus* and *A. parasiticus* in crops. Consumption of groundnuts contaminated with aflatoxins can lead to serious health complications characterized by immune suppression, liver failure, stunted growth in children and even death under severe contamination. The study was aimed at determining susceptibility of locally grown groundnuts varieties to *Aspergillus flavus* and to determine the influence of household pre- and post-harvest practices on *Aspergillus* strain occurrence in the soil and subsequent aflatoxin accumulation in groundnuts in western Kenya. A survey using pretested questionnaires was done on 75 households in Ndhiwa, Nyarongi and Kobama divisions of Homa Bay County to find out about groundnut varieties, yields and pre- and post-harvest agronomic practices. Seventy five Soil samples of 100gm per sample were randomly collected from each of the farms belonging to the surveyed households at harvesting time and *Aspergillus* sp isolated on Modified Rose-Bengal Agar and morphologically identified. 500gm of groundnuts was sampled from each of the 75 households at harvest followed by isolation and identification of *Aspergillus* species from the groundnut kernels and subsequent extraction and quantification of the aflatoxins using direct competitive ELISA. Aflatoxin levels in groundnuts were grouped into three categories based on the standards set by the Kenya Bureau of Standards (KEBS) and the European Commission (EC). Eight groundnut varieties were identified as Homa Bay local (1%), ICGV-9991(10%), Red Valencia (40%), CG07 (48%), ICGV-12991, CG2, CG3, SM99568 (1%). Majority (48%) 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 43% of the farms having maize. Groundnut chlorotic rosette affected majority of the groundnut farms (93.9%) but only 26% of the farmers practicing any form of pest and disease control. Majority of the farmers (95%) stored harvested groundnuts in polypropylene bags. Most (93%) farmers had no previous knowledge on aflatoxin and those who did, think it is only a problem in maize. *A. flavus* L-strain and S-strain, *A. parasiticus* and *A. niger* were isolated from soil and kernels sampled at harvest. *Aspergillus parasiticus* was the most prevalent fungus in soil samples from the three divisions with 2160 CFU/g of soil. The highest mean population (1780 CFU/g soil) of *A. flavus* S-strain was found in soil sampled from Nyarongi region while the population of *A. flavus* L-strain was highest (240 CFU/g soil) in soil sampled from Ndhiwa division whereas *A. parasiticus* was highest in kernel

samples from all the three divisions with 1393 CFU/g kernel. A significant association ($t = 2.652$; $P = 0.010$) was found between storage state of the peanuts and levels of aflatoxin in peanuts. As by KEBS standards the proportion of safe samples was higher in samples stored in shelled state (94%) compared to nuts stored in unshelled state (84%). A strong association ($t = 2.026$; $P = 0.047$) was also found between aflatoxin levels and whether the households considered drought as a problem in production with 94% of samples from household whom considered drought as a problem having aflatoxin concentrations below ppb 200ppb. There was no significant correlation between the *Aspergillus* populations from the kernels and soil with aflatoxin concentrations. Overall, only 6.7% of kernels sampled from all the divisions did not meet the European Commission aflatoxin limit of ≤ 4 ppb while 4% did not meet the KEBS limit of ≤ 10 ppb. There was no significant inter-variety difference in term of aflatoxin accumulation ($p=0.744$). A control strategy should be put in place to target critical pre- and post-harvest stages of groundnuts when they are most vulnerable to fungal colonization and aflatoxin accumulation. It is also recommended that awareness be raised on implications of aflatoxin on human and livestock health, trade, and possible management strategies be put in place at the local farm level.

Keywords: Groundnuts, Aflatoxins, *Aspergillus flavus*

CHAPTER ONE

1: INTRODUCTION

1.1 GENERAL INTRODUCTION

Groundnuts (*Arachis hypogaea* L.) are oilseeds grown by farmers throughout the world. The crop can be consumed raw, as peanut butter, roasted and mixed with other dishes. Groundnut is rich in proteins, fats, carbohydrates and vitamins. With the animal protein becoming more expensive every single day coupled with the health benefits of plant proteins, the demand for groundnuts is on the increase as an alternative source of protein. According to Okello *et al.*, (2010), a kilogram of groundnuts has approximately similar nutritional value as 2 kilograms of beef or 4 liters of milk. Groundnuts are also rich in vitamins, potassium, zinc, and iron. These qualities make groundnuts an important nutritional supplement in the diets of thousands throughout the world.

The high nutritional value of groundnuts is a predisposing factor making it susceptible to attack and colonization by microbes' especially molds which can colonize it during handling, transportation, and storage. The molds can produce mycotoxins making the groundnuts unfit for consumption. Mycotoxins contamination can occur both before and after harvesting and depending on how the groundnuts are handled and processed the toxin levels can increase along the marketing chain (Ndung'u *et al.*, 2013). Aflatoxin is the most common mycotoxin contamination in groundnuts and it poses a health risk to vertebrates once consumed (Mutegi *et al.*, 2009).

Consumption of foods with high levels of aflatoxin can result in liver failure and consequently death (Groopman, 1988). For example, in 2004 in Eastern Province in Kenya occurring there was a great aflatoxicosis outbreak ever to be reported that resulted in 317 cases claiming 125 lives, a case fatality rate (CFR) of 39%. Of the 308 patients, 51 % were children below 14 years of age. It

was therefore presumed that children have a greater predisposition to aflatoxicosis risk (CDC, 2004). Aflatoxin B1 is also carcinogenic and an immune suppressor. Aflatoxin contamination can lead to loss of market value as well as financial loss to farmers. Farmers may also incur economic losses indirectly from increased cost of human healthcare and veterinary services. Under severe contaminations, total losses may be experienced when the produce is rejected by the market (Okello *et al.*, 2010).

Fungal contamination, growth and the degree of toxin production depends on environmental factors and the handling practices of the farmer. Poor agronomic practices during planting, weeding, and harvesting coupled with poor drying, storage, processing, and rainy spells at harvesting are known to promote mycotoxins production and accumulation (Oliveira *et al.*, 2009; Okello *et al.*, 2010). It is also known that some varieties are more susceptible to colonization than others. This project was aimed at evaluating selected agronomic practices and post-harvest management practices carried out by farmers in Ndhiwa, Nyarongi and Kobama divisions and their effect on aflatoxin accumulation. The various groundnut varieties were also assessed for their susceptibility to fungal colonization for control of aflatoxin production and accumulation.

1.2 STATEMENT OF THE PROBLEM

Mycotoxins contamination in groundnuts has been reported in many parts of the World. In Kenya, there have been several reports on aflatoxicosis (Mutegi *et al.*, 2009). A study by Mutegi *et al.*, (2013) indicated aflatoxin levels as high as 2377.1 ppb in groundnut and peanut butter from traders and farmers in Nyanza and Nairobi areas. Consumption of groundnuts contaminated with mycotoxins can result in stunted growth in children, immune suppression and even death among others (Bhat and Vasanthi, 2003; Okoth and Ohingo, 2005; Mutegi *et al.*, 2009). Currently, there is no widely accepted local groundnut variety that shows resistance to fungal colonization and

aflatoxin accumulation together with control strategies in place to mitigate mycotoxins proliferation in groundnuts in the developing world and it is possible that people in the affected areas are consuming doses of mycotoxins in their daily consumption of groundnuts.

1.3 JUSTIFICATION OF THE STUDY

In some parts of Kenya, groundnuts are consumed almost with every meal of the day as vegetables, in weaning formulas and as snacks. This is by extension the case in most parts the developing world where approximately five billion people are continually exposed to mycotoxins through consumption of maize and groundnuts contaminated with aflatoxin (Wagacha *et al.*, 2013). Reports indicate that aflatoxin levels in groundnuts in Kenya, range from 0 to 7525ppb, and this puts the people in the region at risk of aflatoxicosis (Mutegi *et al.*, 2009).

Studies by various individuals have indicated that there is human exposure to aflatoxins in Western Kenya. High levels of stunting have been reported the region in children, a trait attributed to consumption of small quantities of aflatoxin over a long period of time (Bhat and Vasanthi, 2003; Okoth and Ohingo, 2005; Mutegi *et al.*, 2009). Further, groundnuts are produced by resource-limited farmers characterized by poor pre and post-harvest farming practices, factors linked to fungal contamination and aflatoxin accumulation in groundnuts (Waliyar *et al.*, 2005). The climatic conditions in the region characterized by seasonal rainfall, high temperatures, and high rainfall do not only favor the growth of groundnuts but also their colonization by molds and aflatoxin accumulation (Kaaya and Kyamuhangire, 2006).

Due to heavy consumption of groundnuts in the region both by adults and children; a large proportion of the population is exposed to aflatoxins. It is difficult to completely eliminate aflatoxins from food once they are produced (Okello *et al.*, 2010). Mitigation methods are therefore focused on controlling fungal contamination. One of the major susceptibility factors is groundnut

variety. Currently, there is no well known locally cultivated groundnut variety that shows resistance to fungal colonization and aflatoxin accumulation and related agronomic practices which have been accepted and integrated into farming systems by farmers in the developing world, therefore there is an urgent need to study and identify a locally grown variety that shows low susceptibility to aflatoxin accumulation and related acceptable and easy to use technologies and pre- and post-harvest agricultural activities which can be applied by the farmers so as to combat the aflatoxin menace and alleviate the impending health implications should the entire population be exposed to the aflatoxins.

1.4 OBJECTIVES

1.4.1 Overall objective:

To assess the susceptibility of locally cultivated groundnuts (*Arachis hypogaea*) varieties and the effect of associated agronomic practices to aflatoxin accumulation in Homa Bay County, Kenya.

1.4.2 Specific objectives:

- i. To identify groundnut varieties grown in Ndhiwa area, Homa Bay county
- ii. To establish pre- and post-harvest management practices of groundnut cultivation in Ndhiwa area, Homa Bay county
- iii. To determine the correlation between pre- and post-harvest practices and aflatoxin accumulation in groundnuts
- iv. To determine the occurrence of toxigenic *Aspergillus flavus* strains in selected farms in Ndhiwa area, Homa Bay County
- v. To determine the susceptibility of groundnut varieties to aflatoxin contamination

CHAPTER TWO

2: LITERATURE REVIEW

2.1 The groundnut crop

Groundnut (*Arachis hypogaea* L.) is a legume found in the family Fabaceae, .the origins of groundnut crop can be traced back to South America. The crop is currently cultivated in USA, India, China and many countries in the Africa. Developing countries account for 92% of the total worldwide groundnut production. (Talawar *et al.*, 2005). There are 4 common types grown worldwide, these include Spanish, Runner, Virginia, and Valencia (Edinformatics, 2005). Groundnuts are nutritionally rich being high in protein (26-39%), carbohydrates (11%) and healthy fat (47-59%)The nuts also contains assortment of minerals such as Magnesium (3.98 mg/100g), Potassium (705.11 mg/100g), Sodium (42.0 mg/100g), Calcium (2.28 mg/100g), Phosphorus (10.55 mg/100g), Iron (6.97 mg/100g) and Zinc (3.2 mg/100g),, (Atasie *et al.*, 2009), as well as several vitamins such as vitamins B, E and K. These properties make groundnuts have a high nutritional value hence have several uses such as a confectionary, weaning meals for children and also in animal feeds.

2.2 Groundnuts in South Western Kenya

Groundnut is one of the most commonly grown legume crops in semi-arid regions of West and Eastern Africa. In South-Western Kenya, it is one of the major oil crops grown by smallholder farmers within the Lake Victoria region (KARI, 1996, MoA, 1996) that is in the lower midland (LM1-4) agro-ecological zones (AEZ) (Jaetzold and Schmidt, 1982). These zones have the suitable climate for groundnut production.

The most common varieties grown in western Kenya include Nyanza local (Njugu machon), Red Valencia (Nyahela), Minipintar, Asiriye mwitunde, Serere and Makulu red. Groundnuts is grown as a source of both food and cash and is produced with other major crops, such as maize, sorghum, beans, and millet. It is regarded as a high-value crop and a source of protein, vitamins and high-quality edible vegetable oil. Groundnut has been ranked fourth in importance for arid and semi-arid areas (Andima *et al.*, 2006). The growing area for groundnuts in western Kenya can be to some extent described as semi-arid. Reasons for the fourth place in ranking is due to low yields of groundnuts in the area resulting from lack of improved high yielding disease tolerant varieties, organized seed production system, poor agronomic practices, pests and diseases, low producer prices, access to credit, lack of markets and market information and low adoption of developed technologies (Rees *et al.*, 1997, Okoko *et al.*, 1998). There was also poor pre- and post-harvest handling techniques that resulted in the loss of value due to aflatoxin (Kaaya *et al.*, 2006 and 2007). In addition, there was the lack of value addition technologies along the groundnut value chain.

Western Kenya is the major growing area with at least two growing seasons per year. The other groundnut growing regions in Kenya include parts of Rift valley, Coast, and Eastern provinces.

2.3 Mycotoxins and their occurrence in food substances

Mycotoxins are secondary metabolites produced by some species of fungi naturally occurring in foodstuff. Consumption of food contaminated with mycotoxins causes a wide variety of toxic effects in animals including man. (Coulombe, 1991). Contamination of food can occur at different levels in the chain of production i.e. cultivation, transport or storage. Mycotoxins are very chemically stable hence are not denatured or removed by common practices in the food processing line, (Chu, 1991). Some mycotoxins such as aflatoxins, ochratoxin, patulin, fumonisin, sterigmatocystin, and penicillic acid have shown potency to cause cancer in experimental animals.

Aflatoxins, especially Aflatoxin B1 have been classified by International Agency for Research on Cancer as a Group 1 human carcinogen. In the developing, there are cases of co-occurrence of mycotoxins in food substances and in such cases, the chances of multiplicative and additive toxic effects are very high if consumed.

2.4 Aflatoxin producing fungi

Aflatoxins are produced by fungi in the genus *Aspergillus*, especially in the species such as *A. flavus*, *A. parasiticus* and *A. nomius* (Moss, 1998). These fungi are found all over the globe, the optimal growth conditions for these fungi are temperature around 30°C and relative humidity of 80-85% (Coulombe, 1991) for instance, the. Optimum growth conditions for *A. flavus* in post-harvest storage are between 25⁰C and 30⁰ C and humidity levels of 0.99aw, with optimal aflatoxin production occurring at 25⁰C and 0.99 aw. (Giorni *et al.*, 2009).

Currently, there are 18 related compounds called aflatoxins. With the medically important ones being the types B1, B2, G1 and G2 (Coulombe, 1991).Of the four, AFB1 has the highest frequency of occurrence in many substrates and is the most toxic. The others i.e. B2, G1, and G2 having 50, 20 and 10% of AFB1 toxigenic power, respectively (Leeson *et al.*, 1995).

Animals and humans come into contact with aflatoxin via many ways such as direct consumption of contaminated products through milk as M1 and M2 metabolites, consumption of the flesh of animals fed on contaminated feed and in rare cases through human skin by handling contaminated produce (Wagacha and Muthomi, 2008), through the respiratory system, especially in individuals involved in harvesting, shelling, storage, transportation and marketing of the groundnuts (Mehan *et al.*, 1991). Poor health and nutritional status are likely to predispose one to aflatoxin poisoning (Hendrickse, 1984).

2.5 Aflatoxin in groundnuts

Aflatoxins are produced in groundnuts when soil borne saprophytic fungi such as *Aspergillus flavus*, *A. parasiticus*, and *A. nomius*) contaminate groundnuts pre-harvest, during harvest or during post-harvest handling. While both *A. flavus* and *A. parasiticus* can produce the B toxins, *A. parasiticus* which also produces the G toxins (Diener *et al.*, 1987). *A. nomius* produces both B and G toxins and is similar morphologically to *A. flavus* (Vaamonde *et al.*, 2003). Contamination of the groundnut crop varies annually and also within fields. Contamination tends to be high when plants are exposed to environmental stresses toward the end of the growing season. The groundnuts can be infected and contaminated with aflatoxin when the plant is exposed to heat and moisture stress during pod development, when pods are damaged by nematodes or insects or when they are mechanically damaged during cultural operations like weeding and maturity checks. These conditions commonly occur due to heavy reliance on rainfall for watering of the plants. Postharvest infection in groundnuts is influenced by temperature and relative humidity of the storage conditions, insect damage, and shelling methodology.

2.6 Status of the study of aflatoxin contamination in groundnuts

In as much as very little work has been done in Kenya. A good number of key studies has been conducted in other producer countries to establish the baseline data on the extent of aflatoxin contamination in groundnuts. Toyofuku *et al.* (2009) for example studied the distribution of aflatoxin in groundnuts cultivated under rain fed conditions in United States. With a particular focus on total aflatoxins and aflatoxin B1. From the study, all the three lots showed probability density peaks at concentrations of up to 5×10^2 ng/g in a single kernel.

Studies by Horn (2007), has also indicated high genetic diversity in the species of aflatoxigenic *Aspergillus* species, especially those in the section flavi. Another study conducted by Okano *et*

al.(2008) while investigating AFB1 and G1 contamination in imported groundnuts into Japan found that contamination in groundnuts imported from China was mainly from *A. parasiticus* while AFB and G toxin in groundnuts from South Africa were due to both *A. parasiticus* and *A. flavus*.

In India, extensive research has been conducted on groundnuts since it is one of the major components of the countries livestock and poultry feed. For instance, studies by Ahamad *et al.* (2009) indicated high concentrations of AFB1 in groundnut oil cake and broiler finisher mash from samples collected in Namakkal in Tamil Nadu. In Sudan in the Khartoum state, a survey conducted by Elzupir *et al.* (2009) on aflatoxin contamination in animal feeds, over 64 %of all samples analyzed were contaminated with aflatoxin with more than 80% samples having between. 54.41 and 579.87 ppb aflatoxin. In all the samples analyzed Aflatoxin B1 was the most common contaminant. In Nigeria, a study by Odoemelam and Osu (2009) on aflatoxin contamination of edible grains marketed in the Niger Delta region found that the highest levels of Aflatoxin B1 were in groundnuts.

In Egypt, studies by Youssef *et al.* (2008) on mycobiodata have shown regular contamination of *A. flavus*, *A. niger*, *A. ficuum*, *Fusarium* sp and *Penicilliums* sp in groundnuts kernels. *A. flavus* was found to predominate Soil samples in major groundnut growing areas of Gujarat in India (Kumar *et al.*, 2008), with *A. flavus* soil population being positively correlated with aflatoxin contamination in groundnut kernels. In Sao Paulo state in Brazil, Gonzalez *et al.* (2008), found *A. flavus*, *Rhizopus* sp. and *Fusarium* sp. as the commonly occurring fungi in groundnut hulls.

2.7 Health aspects of Aflatoxins

Contamination of groundnuts by aflatoxins can occur at different places along the production chain i.e. during production transportation, and storage, (Nigam *et al.*, 2009). Health effects from

consumption of aflatoxins vary widely and range from a minor irritation to death. studies reveal that exposure to large doses of aflatoxin(>6000mg) may cause acute toxicity and is lethal whereas continual exposure to small doses for over time causes cancer (Groopmann *et al.*, 1988) Therefore the adverse effects of aflatoxins on animals can be classified into two categories; acute and chronic toxicity. Acute toxicity is caused when large doses of aflatoxin are ingested at once or within a short period of time by humans or animals humans and animals (Nyikal *et al.*, 2004) The various cases of aflatoxin outbreaks reported in several parts of the world. Can be linked to acute toxicity. The effect on humans and animals depends on which include; species type, age, gender susceptibility, ingestion levels, aflatoxin concentration, and duration of exposure. Among animals, ruminants have been observed to be more resistant to the aflatoxicosis compared to monogastric species (Hussein and Brasel, 2001).

Studies reveal that acute aflatoxicosis may be accompanied by symptoms such as; edema jaundice, vomiting acute hepatitis and in extreme cases death (Nyikal *et al.*, 2004). The key organ for aflatoxins is the liver. Once in the liver, the toxin molecules infiltrate hepatocytes and lead to necrosis or liver cell death. This is because aflatoxin metabolites react negatively with different cell proteins, leading to inhibition protein synthesis, lipids, and carbohydrates. As the liver function decreases, there is an imbalance in blood clotting mechanism, jaundice and a decrease in essential serum proteins synthesized by the liver. Other symptoms of acute aflatoxicosis include abdominal pain, edema of the lower extremities, and vomiting. The most severe case of acute aflatoxicosis was reported in Kenya, July 2004 leading to the death of 125 people among the 317 reported cases due to consumption of aflatoxin contaminated maize.

Chronic toxicity is caused by exposure to small quantities of aflatoxin over long periods of time and can have carcinogenic and immunosuppressive effects and stunted growth in children

(Hendrickse, 1997, Gong *et al.*, 2002,). In a majority of the cases, liver cancer has been related to AFB1. Over time, Liver damage becomes apparent as jaundice manifests, the gall bladder becomes as the aflatoxins react with the T-cells, as the Vitamin K activities decrease, and as there is a decrease in phagocytic activity in macrophages decreases. Immunosuppression becomes apparent. Other complications may include liver cirrhosis and reproductive problems (Cousin *et al.*, 2005). There is an increased risk of primary hepatocellular carcinoma in cases where there is a concurrent infection with hepatitis B virus during aflatoxin exposure (Williams, 2004). Both hepatitis B virus and aflatoxins act synergistically in causing liver cancer (Montesano *et al.*, 1997, Groopman *et al.*, 1996).

2.8 Factors that influence fungal colonization and aflatoxin production

Colonization of groundnut by aflatoxigenic fungi and subsequent aflatoxin production can occur at pre- and post- harvest. A number of factors therefore influence fungal colonization and toxin production, these include: end-season drought stress, elevated soil temperatures (Bankole *et al.*, 2006), attack of groundnut pods by pests and diseases (Mehan *et al.*, 1991), susceptible varieties (Reddy *et al.*, 2003), poor seed storage conditions, mechanical damage on the nuts during harvesting, poor transportation and poor or inadequate drying. (Duncan, 1981 Bilgrami and Choudhary, 1990, Waliyar *et al.*, 2005).

Various varieties of groundnuts show different levels of resistance to *Aspergillus* colonization and subsequent aflatoxin accumulation, for instance in a study carried out to test the groundnut seed resistance to colonization by *Aspergillus flavus* in tests at ICRISAT Center, 1981/82. In as much as aflatoxin was produced in all cultivars, tested variation was observed in the amount of toxin produced among the cultivars. There was no correlation between resistance to seed colonization by *A. flavus* and the ability of seed to support aflatoxin production. Based on the small proportion

of the varieties that have been tested, there is hope that a cultivar that combines a good resistance to aflatoxigenic fungus invasion with resistance to aflatoxin production will be found.

2.9 Methodologies for aflatoxin determination

A number of methods have been put forward for testing levels of aflatoxin and the choice of methodology relies on factors such as precision, cost-effectiveness, and a number of samples being analyzed. Sampling methodology should also be considered as it affects the margin of error when it comes to result analysis.

(ICRISAT, 2007). the methodologies currently in use in mycotoxin analysis include:, Liquid Chromatography/Mass Spectrometry (LC/MS); Thin Layer Chromatography (TLC)High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC),, Enzyme-Linked Immunosorbent Assay (ELISA), and rapid tests based on lateral flow platforms (Pascale and Visconti, 2008). Of all the methods above ELISA methodology are the most widely used method for aflatoxin analysis since it is adaptable, simple and has high sensitivity (ICRISAT, 2007). It also allows analysis of multiple samples hence ideal for screening purposes. HPLC is the gold standard in aflatoxin analysis due to its high sensitivity and is easily automated. But due to its high cost, it is unsuitable for routine analysis Other emerging technologies in the field of mycotoxin analysis include Fluorescence Polarization Immunoassay (FPIA), capillary electrophoresis, Infrared Spectroscopy, molecularly imprinted polymers and fiber-optic immunosensors. Regardless the type of method chosen for mycotoxin analysis, it should be able to detect the maximum acceptable limits so as to enable monitoring programs and thereby ensure safety in the international trade(Pascale and Visconti, 2008).

2.10 Management strategies for aflatoxin in groundnuts

Management includes all practices that either prevent fungal colonization or subsequent aflatoxin production or both. The management strategies can either be pre- or post-harvest. Pre-harvest strategies include: breeding groundnut cultivars so as to come up with resistant varieties this has been extensively researched by ICRISAT, so far no single variety that is totally resistant has been identified (Waliyar *et al.*, 1994), there has not been much progress in this because of the process is time-consuming, the complications within the reproduction cycle of groundnuts, and emergence of new yet mycotoxigenic fungal species. Another strategy being used is the use of Bio-control agents. These have been shown to reduce contamination in the field by up to 77-98%. Some of the bio-control agents under use currently include non-toxigenic strains of *A. flavus* and *A. parasiticus* (AFLASAFE) (Horn and Dorner, 2009). *Streptomyces* sp. (strain ASBV-1) has also been able to inhibit *A. parasiticus* in groundnuts, as they reduce the viability of *A. parasiticus* spores by up to 85% (Zucchi *et al.*, 2008). *Trichoderma harzianum* and *Trichoderma viride* have also been found to have the ability to substantially inhibit growth of molds in groundnuts and reduce Aflatoxin B1 and B2 significantly (Gachomo and Kotchoni, 2009).

Another strategy involves the use of soil amendments. These include Soil treatments such as application of manure (10 t/ha) lime (0.5 t/ha), and cereal crop residue (5 t/ha) at the time of planting. These have been shown to have the ability to reduce seed infection by *A. flavus* and aflatoxin contamination in groundnuts by up to 90% in studies conducted in research stations in Mali and Niger by ICRISAT (Waliyar *et al.*, 2008). Other traditional practices such as summer ploughing, selecting planting dates to take advantage of periods of higher rainfall, maintaining good plant density in the fields, removing prematurely dead plants, managing pests and diseases,

timely harvesting and excluding damaged and immature pods, as control strategies for aflatoxin contamination (Waliyar *et al.*, 2008).

Irrigation has also been used effectively as a control strategy in pre-harvest control as irrigation eliminates drought stress. The suitability of this method in many African regions, however, remains uncertain as most of the groundnut farming is done by small-scale farmers under rain-fed conditions.

Quick drying of pods or kernels to less than 10% moisture content, control of storage pests, use of mechanical threshers, are some of other suggested post-harvest control strategies (Waliyar *et al.*, 2008). Sorting of the threshed kernels has been shown to reduce aflatoxin levels. This can either be manual sorting) or using electronic sorting machines at a commercial level (Whitaker *et al.*, 2005, Kaaya *et al.*, 2006).

Using machinery such as shellers, threshers, and hermetic packaging protects groundnuts from mold colonization during processing and consequently reducing aflatoxin in groundnuts (Pramawati *et al.*, 2006). Physical cleaning and sorting have been shown to be able to reduce aflatoxin levels by 40-80% (Park, 2000). Other technologies that have been used also include Gamma irradiation (up to 70% reduction of Aflatoxin B1 in groundnut kernels in Brazil (Prado *et al.*, 2003)). Treatment of groundnuts with 10% sodium peroxide has also been shown to reduce aflatoxin levels in groundnuts significantly (Conzane *et al.*, 2002). Detoxification is another postharvest strategy that has been with some success used in control of aflatoxin. One of such proposed means of detoxification of groundnuts is gaseous ozonation (Proctor *et al.*, 2004). Integrated approaches have been proposed by ICRISAT in the control of aflatoxins, these include a combination of resistant cultivars, soil amendments with lime, organic soil supplements to enhance water-holding capacity, use of bio-control agents, and raising awareness within the

farming communities this could be the most effective way of reducing aflatoxin levels in groundnuts (Waliyar *et al.*, 2008).

In the developing world, aflatoxins continue to be one of the big challenges with regard to food safety and security, especially. Lack of substantial data in many parts of Kenya makes it challenging to establish facts about the scope of the problem. This study was to establish the extent of aflatoxin contamination of groundnut samples from farms in Homa Bay County in western Kenya regard to groundnut varieties and associated aflatoxin-producing *Aspergillus* species and pre- and post-harvest agronomic practices.

CHAPTER THREE

3: MATERIALS AND METHODS

3.1 Study area

The study was conducted in Homa Bay County, one of the 47 Counties in Kenya covering an area of 4,267.1 Km². this includes the water surface (Lake Victoria) which itself covers an area of 1,227 km² and is a major source of livelihood The county is located in South Western parts of Kenya along the south eastern shores of Lake Victoria The county borders boarders Siaya and Kisumu and counties to the North, Migori County to the South , Lake Victoria and the Republic of Uganda to the West and Kisii and Nyamira counties to the East, The county lies between latitudes 0° 15' South and 0° 52' South, and longitudes 34° East and 35° East. The population of the county by 2009 census was at 963,794 with 206,255 households, with females comprising 52% and males being 48%. Agro-ecological zones where peanuts were grown were lower midland zone 4 (LM4) and lower midland zone 3 (LM3) (Ministry of Agriculture, 1987). The mean annual temperatures in the county range from a mean f 17.1°C to34.8°C, with rainfall amounts ranging between 250mm and 700mm per annum. Farming is the key source of household income (52%). With other economic activities being fishing and rearing of livestock.

Most farms in the county are small scale with an average size of 2.0 ha. Main food crops include maize, sorghum and cassava with sugar cane, groundnuts rice and cotton being the main cash crops. Sugar cane is the only cash crop grown on a larger scale in the county, groundnut is mainly grown under small scale conditions.

Ndhiwa area lies to the Southern parts of the county and is one of the major farming areas within the county due to heavier rains experienced in these parts in comparison to other parts of the

country. The population density is, therefore, higher in Ndhiwa area thus the available farmlands are smaller and are interspersed with homesteads.

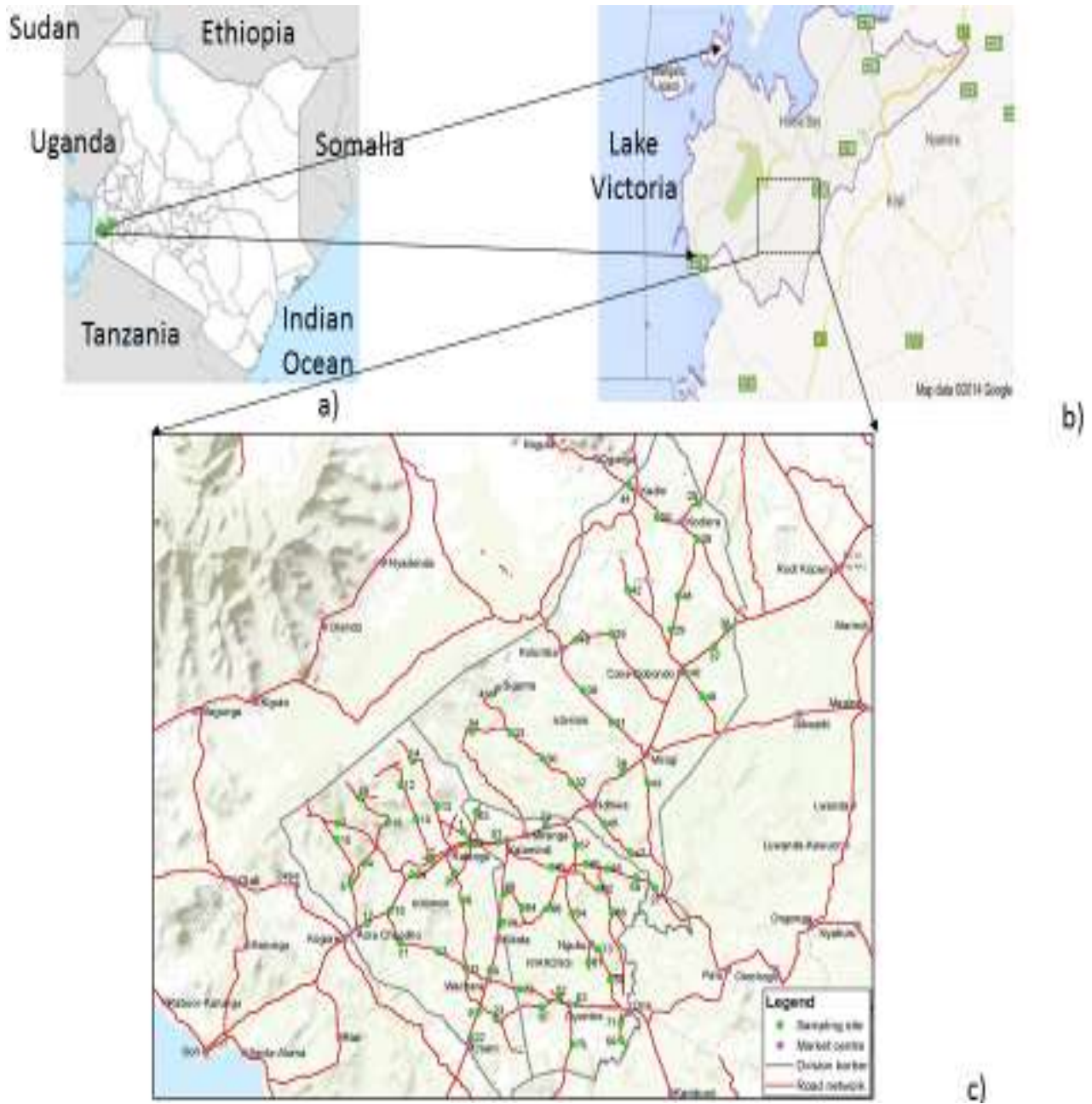


Figure 1: The study area (a) Map of Kenya showing Homa Bay County (in green), (b) Homa Bay County showing the study area, (c) the study area showing the sampling points

3.2 Household Survey to determine the agronomic activities by farmers

A household survey was conducted within Homa Bay County in Kobama, Nyarongi and Ndhiwa divisions. The choice of the county and the divisions was based on their being the major groundnut production zone in the area.

The survey was based on a total of 75 groundnut-growing households. The respondents were the farm owners of the farms mapped out using GIS after adjustments on the ground.

3.2.1 Questionnaire development

The questionnaires were developed based on information collected after conducting focus group discussion involving 20 participants drawn from one of the major farmers' co-operative societies in the area. The developed questionnaire was then tested on 20 randomly selected households 10 from Ndhiwa division and 10 from Nyarongi Divisions. Each of the 20 households was selected by randomly generated GIS coordinates in the two divisions. The responses were then compared and the questions adjusted to enable achieve the set objectives. Data to be collected during the survey were as shown in Table 1.

Table 1: Information collected using the questionnaire during the survey on pre- and post-harvest agronomic practices by farmers in Nyarongi, Ndihiwa and Kobama divisions in Homa Bay County

Pre-Harvest activity	Farmer practice
Farm properties	Size, Area under groundnut production
Farm preparation	Type of land preparation(plowing, furrowing)
Groundnut seeds	Source, Type, Variety
Planting practices	Intercropping, other crops planted, crop rotation, crops planted last season, crop residue handling
Soil amendments/fertilizers	Type, Time and frequency of application
Weed control	Type, Time and frequency
Pest control	Type, time and frequency of application
Other production problems	Drought, Rats
Maturity Checks	Type and frequency
Harvest methodology	Equipment used
Post-Harvest practices	
Drying	Duration, location
Storage	Containers, Location, Duration
Shelling	Equipment used
Value addition	Shelling, sorting, roasting, boiling, grinding
Market	Location, size, logistics
Knowledge on aflatoxin	Depth, utilization, control

The questionnaire was designed to obtain data on groundnut pre- and post-harvest agronomic practices, handling, processing, and value-addition practices employed by various farmers. Information from the questionnaire was analyzed using principal component analysis (PCA) so as to obtain agricultural practices which reduce pre- and postharvest losses in groundnuts.

3.3 Groundnut Sample size determination

The sample size was based on the Fischer formulae for sample size determination:

$$N = \frac{Z^2 pqD}{d^2}$$

N-sample size, Z- 1.96, P- proportion of target population (use p= 0.5 if p is not known), q=1-p, d=α=0.05, D- Design effect (D=1)

Hence sample size $N = \frac{1.96^2 \times 0.5 \times 0.5 \times 1}{0.05^2}$

= 384.16 \approx 385 samples, this translated to 75 samples (5 samples per farm)

Soil samples were collected from 5 points in each of the 25 farms selected in the 3 divisions, the farm owners also served as households for survey and groundnut sample collection. The farms were randomly picked using GIS mapping followed by confirmation and adjustments on the actual ground to the nearest Household.

3.4 Sampling of groundnut kernels from the households

Groundnut kernels were sampled at harvest to determine the population of *Aspergillus* sp. and aflatoxin levels. Samples were collected from multiple sampling points in each farmer's storage bags with the number of sampling points being based on the volume of the storage material. Approximately 100g of un-shelled groundnuts was sampled from each bag/storage container. The same procedure was repeated in a minimum of 5 other randomly selected storage bags in the same household until a minimum of 5 sub-samples were collected. The samples were thoroughly mixed to make a 500gm composite sample which was labeled, put in a Kraft bag and transported to the laboratory where they were oven dried for 48 hours at 45°C. Kernel samples were then stored at 4°C awaiting microbial and aflatoxin analysis.

3.5 Collection of soil samples

Soil samples were collected from each of the 75 farms, whose positions had been randomly generated using GIS mapping technology. Soil sampling was done in about two weeks to harvesting. In each field (= plot), 5 soil samples (100 g each) was collected from four corners of the field and the middle area was taken from 0-5 cm depth. Collected samples were then mixed to

form a composite. The soil composite was then air-dried for 2 days. Soil sub-sample weighing 100g was taken from the soil composite and sieved through a 2mm mesh size. The sieved soil samples were stored at room temperature in the laboratory waiting for fungal isolation and characterization.

3.6 Isolation and identification of *Aspergillus* species

3.6.1 Media preparation

Dichloran Rose Bengal Agar was prepared by adding 20g Agar, 10g glucose, 2.5g mycological peptone, 0.5g yeast extract, 0.5g MgSO₄.7H₂O, 1.0g potassium phosphate monobasic, 0.025g Rose Bengal in 1L distilled water (Horn and Doner 1998). The pH was then adjusted to 5.5 using 0.01M Hydrochloric acid before autoclaving. Autoclaving was done at 121°C and 15psi pressure for 20 minutes. The media was then allowed to cool to 60°C, after which 5 ml of 4mg/L dichloran (in acetone), 1mg/L chlortetracycline and 40mg/L streptomycin was added to the medium to inhibit bacterial growth and make the media semi-selective for *Aspergillus section flavi*.

3.6.2 Isolation of *Aspergillus* species from the soil and groundnut kernels

The soil samples and the groundnut kernels processed within three weeks of collection. Groundnut kernels were shelled manually under sterile laboratory conditions, and then thoroughly mixed and then ground 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 and ground groundnut using the dilution plating technique on a Modified dichloran rose Bengal agar (MDRB), (Probst *et al.*, 2007). 1g of each soil/ground groundnut sample was suspended in 9ml of autoclaved distilled water, vortexed for 30 seconds to form a stock solution (10⁻¹dilution) 1-ml aliquots of a one in ten dilutions of the soil suspension was then transferred to another 9ml sterile distilled water making the second

dilution (10^{-2}). 200 μ l of the second dilution was then spread on the surface of MDRB agar in triplicates and the inoculated plates incubated for 7 days at 30 °C, the number *Aspergillus* sp in each sample was counted and expressed as the number of colony forming units(CFUs) per gram of sample (Jaime-Garcia and Cotty, 2004). CFUs/g was calculated as follows:

$$\text{Number fungi per gram of sample} = \frac{\text{No. of colonies}}{\text{Amount plated} \times \text{dilution factor}}$$

3.6.3 Identification of *Aspergillus* species

Aspergillus sp. were distinguished macroscopically based on colony diameter and soluble pigment, surface and reverse colors and margins texture, shape, elevation, the pattern of growth, pigmentation, and conidial morphology characteristics (Klich, 2002).

Microscopic examination of *Aspergillus* sp. was done and identification done in reference to taxonomic schemes suggested by Pitt and Hocking (1997) and Klich and Pitt (1994). Slide cultures of *Aspergillus* sp. 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 spores 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, the following microscopic characteristics were investigated: Diameter, shape, color and surface texture of conidia, phialides, metulae, vesicle, stipes, sclerotia and seriation (uniseriate or

biseriate). Microscopy was done at $\times 1000$ 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). All isolates were identified at species level.

Strain differentiation between the S and the L strains was done based on the sclerotia number and size with strains having large sclerotia in large numbers classified as L-strain and those with small and few sclerotia classified as S-strains.

3.6.4 Molecular identification of *Aspergillus* isolates

DNA Extraction and PCR amplification

In order to determine the aflatoxigenicity of the isolated and identified species, DNA extraction was done using ZR Fungal/Bacterial DNA MiniPrepTM (Zymo Research) kit according to manufacturer's instructions to extract DNA.

PCR amplifications were performed in 25 μ l of a reaction mixture containing 12.5 μ l One Taq 2x Mastermix with standard buffer (New England BioLabs), 1 μ l of each primer, 8.5 μ l Nuclease-free (double-distilled) water and 2 μ l of the template of the fungal DNA. PCR was done as follows: 1 step at 94 °C for 5 min; the next 40 cycles of the following three steps: 20 sec 94 °C, 20 sec at annealing temp of 57 °C, 1 min 72 °C; and one final 30 min step at 72 °C. PCR products were separated by electrophoresis on a 1% agarose gel with 2 μ l ethidium bromide in 1x TBE buffer and visualized under UV light.

3.7 Determination of levels of aflatoxin in groundnuts

Twenty grams of the ground groundnut was then triturated in 100ml of 80% acetonitrile (v/v 80 ml absolute acetonitrile in 20 ml distilled water) containing 0.5 per cent w/v potassium chloride in a sealed container, until thoroughly mixed (a minimum of 2 minutes). The particulate matter in the

extract was then allowed to settle followed by filtration of 5-10ml through Whatman No.1 filter paper. An aliquot of the extract (1ml) was diluted 1:10 with reconstituted wash buffer and analyzed for total aflatoxin with a direct competitive ELISA using Total Aflatoxin Assay- Low matrix kit (Helica Biosystems Inc.) as follows: one dilution well was placed in a microwell holder for each standard and sample to be tested and equal number of antibody coated microtiter wells placed in another microwell holder and dispensing 200 $\mu\ell$ of sample diluent into each dilution well. This was followed by adding 100 $\mu\ell$ of each standard and prepared sample to appropriate dilution well containing the diluent and mixing by priming pipette at least 3 times. Using a new pipette tip for each, 100 $\mu\ell$ of the contents from each dilution well was transferred to a corresponding antibody coated microtitre well and incubated at room temperature for 30mins. This was followed by decanting the contents from the microwells into a discard basin and washing the microwells 3 times by filling each with PBS-Tween wash buffer and then decanting the wash buffer into a discard basin. The wells were then tapped (face down) on a layer of absorbent towels to remove residual wash buffer. 100 $\mu\ell$ of Aflatoxin HPR-conjugate was then added to each antibody coated well, covered to avoid direct light and incubated at room temperature for 30 minutes. The wash process using PBS-Tween wash buffer and drying was repeated. 100 $\mu\ell$ of the substrate reagent was then added into each microwell and incubated at room temperature for 10 minutes with tops covered using aluminum foil to avoid direct light. 100 $\mu\ell$ of the stop solution then to be added in the same sequence and the same pace as the substrate was added. The optical density of each microwell was then read using a microtiter plate reader using a 450nm filter and recorded accordingly. The kit had a detection limit of 300ppb.

3.8 Statistical Analysis

Analysis of Variance (ANOVA) was performed to determine the frequency of isolation of different strains of *A. flavus* and *A. parasiticus*. Determination of the relationship between toxigenic strains and toxin production was carried out using correlation analysis.

To determine the relationship between the amounts of aflatoxin found in the groundnut kernels, the aspergillus species isolated from the same kernels and the respective agronomic activities carried out by the farmers from whence the samples were collected. The samples were grouped into three categories based on their aflatoxin content in regard to established economic limits used in imposing trade restrictions by Kenya bureau of standards (KEBS) and European Union (EU). The proportion of the samples of samples on each category was calculated. The samples were grouped as follows; samples with <4 ppb (acceptable by both KEBS and EU); ≥ 4 ppb to ≤ 10 ppb (rejected by EA but acceptable under KEBS standards), and >10 ppb (rejected by both EU and KEBS) (Felicia *et al.*, 2004); Categorical data analysis using contingency tables was used to evaluate the association between these aflatoxin categories and fungal populations and agronomic activities. Categorical data analyses (Stokes *et al.*, 2000) are carried out using R Gui version 3.0.2





CHAPTER FOUR





4: RESULTS

4.1 Groundnut varieties grown in Homa Bay county

There were two main families of groundnuts in the study area, the Runner and the Virginia as described by their growth habits. The Runner varieties which matured in 90-100 days while the Virginia varieties which matured in 60 -75 days. Eight different groundnut varieties under these two families were identified as Homa Bay local, ICGV-9991, ICGV-12991, Red Valencia, CG7, CG2, CG3 and SM99568 (Table 2). Apart from Homa Bay local and Red Valencia, the rest of the groundnut varieties grown by these farmers were improved varieties.

Table 2: Characteristics of groundnut varieties grown in Homa Bay County

Variety name	Local name	Family name	Characteristics	Nuts per pod	Picture
Homa Bay local	Nyadongo	Runner	Color: brown Size: big Maturity period: 3.5-4 months Usage: good for both groundnut butter and roasted groundnuts	2-4	
ICGV-9991	Nyaela	Virginia	Color: Red Size: small Maturity period: Early(2-2.5 months Usage: roasted groundnuts	4	
ICGV-12991	AEP matindo	Runner	Color: Brown Size: very Big Maturity period: medium(3-3.5 months) Usage: boiled groundnuts and roasted groundnuts	4	
Red Valencia	Nyaela matindo	Virginia	Color: Red Size: small Maturity: early(2-2.5 months) Usage: roasted groundnuts	2-3	

CG7	Nyaela madongo	Virginia	Color: Red Size: big Maturity: medium(3-3.5months) Usage: roasted groundnuts	2	
CG2	AEP madongo	Runner	Color: white Size: big Maturity period: medium(3-3.5 months) Usage: boiled groundnuts and groundnut butter	2	
CG3	Rachar madongo	Virginia	Large White	3-4	
SM 99568	Nyakingi	Runner	Color: Brown Size: medium Maturity period: Medium (3/3.5months) Usage: groundnut butter and roasted groundnuts	2-4	

Of the above varieties, only 4 were grown in substantial amounts as indicated below. The four main varieties that occurred with the highest frequency were CG7, Red Valencia, ICG-9991 and Homa-bay local and their distribution overall and by division was as shown in Figures 2 and 3.

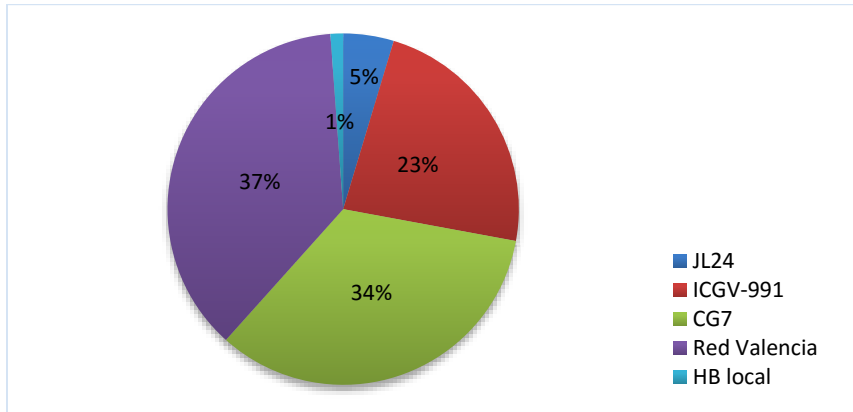


Figure 2: Overall distribution of groundnut varieties cultivated in Homa Bay County

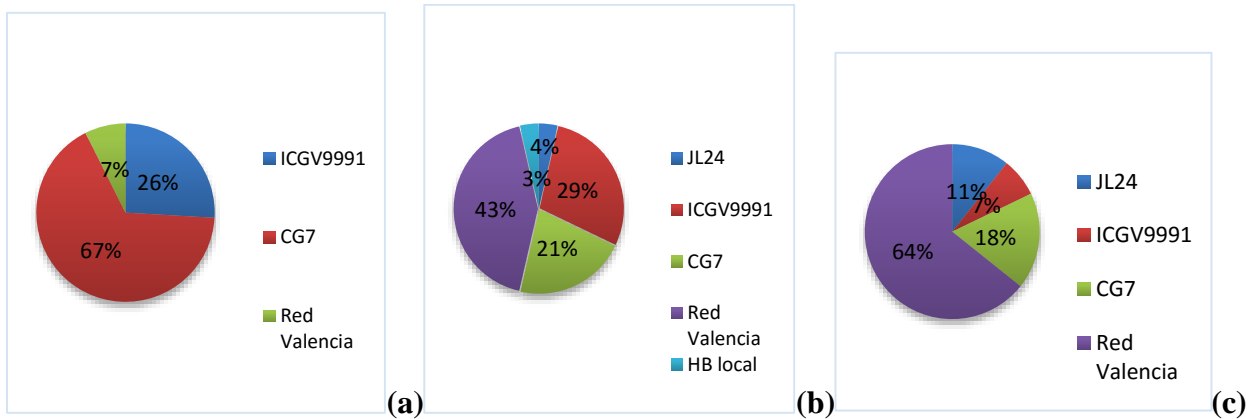


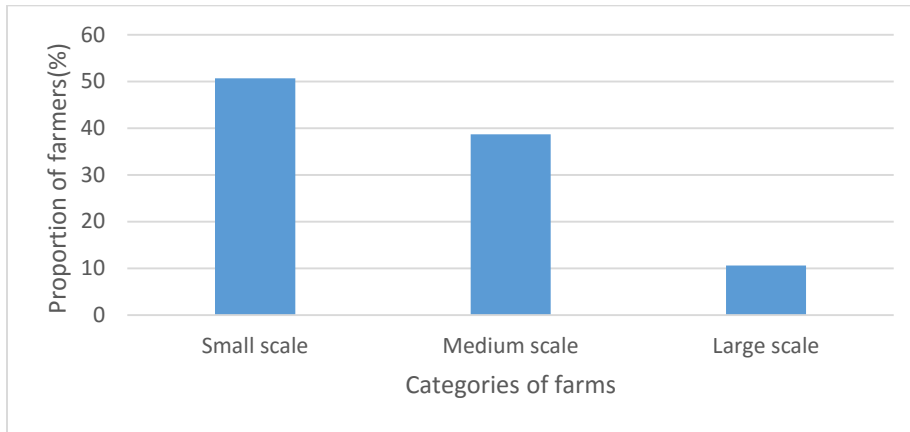
Figure 3: Groundnut varieties planted in Homa Bay County: a) Kobama b) Ndhiwa and c) Nyarongi divisions of Homa Bay County

Seed source and type: a large proportion of farmers (66%) bought the seeds they planted from the local market, 20.1% used their own seeds stored from the previous season, 10.1% bought seeds from the Agro-shop and 3.8% got seeds to plant from neighbors. 63.6% of the farmers used local landrace for planting while 36.4% used improved cultivars.

4.2 Pre-harvest management practices

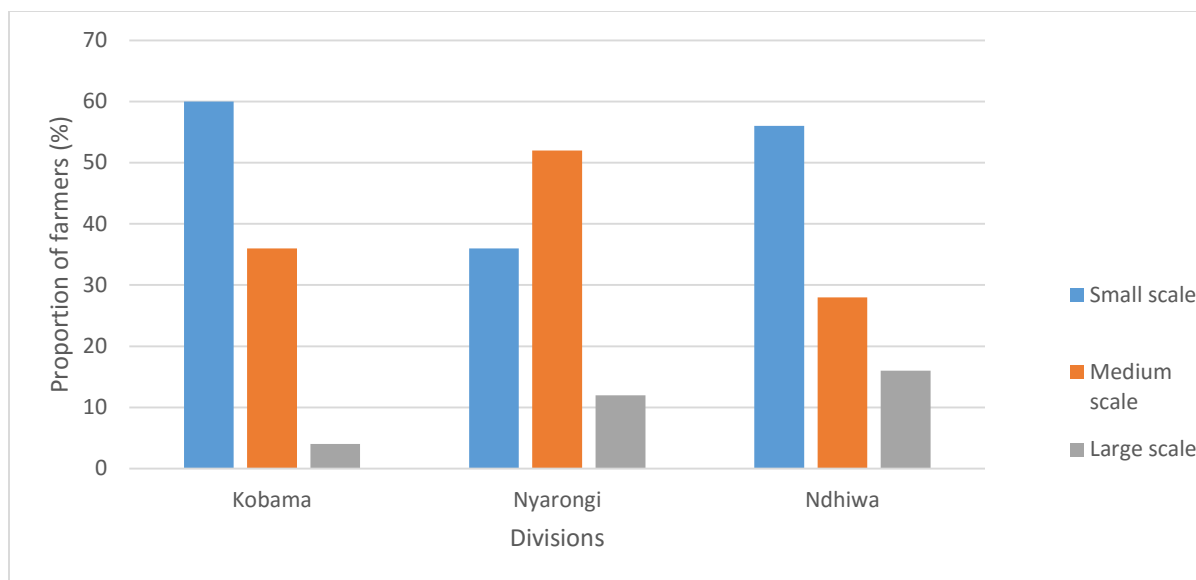
4.2.1 Average farm size and proportion under groundnut production

Fifty one percent of the farmers owned farms of size less than 2 Ha, with 39% and 11% of the farmers with farm sizes ranging between 2-4 and greater than 5 Ha respectively (Figure 4). Out of this, in most of the farms i.e. 74.4% the proportion of under groundnut production was between 0-1.9 Ha, while in the remaining farms, 25.6% the area under groundnut production was between 2.0-3.9 Ha. The average farm size and respective proportion under groundnut production in each of the three divisions was as shown in Figure 5.



*Small scale < 2 ha, medium 2-3.9ha, large > 4 ha

Figure 4: Categories of farm sizes in Homa Bay County



*Small scale < 2 ha, medium 2-3.9ha, large > 4 ha

Figure 5: Categories of farm sizes by Division in Homa Bay County

4.2.2 Cropping systems and soil amendments

A large proportion (80.1%) of the farmers ploughed their farms followed by harrowing before they planted their crops while a 19.9% of the farmers planted their farms immediately after ploughing, (some reasoned that harrowing led to increase in weed populations in the farms, with some claiming lack of resources and others saying they saw no need for doing so since most of the fields had been ploughed in the last season).

Other crops planted on the farm included maize, millet, beans, sweet potatoes, sugarcane and soya beans. Of all the farmers surveyed, 93.2% intercropped their groundnuts with other crops while the remaining 7.7% did not intercrop. All the farmers that practiced intercropping did so with maize (Figure 6, 7 & 8).

Only 19% of the farmers interviewed conducted crop rotation, the remaining 81% did not (Table 3), the cited reasons included small farm sizes and few farms possessed by an individual farmer, and some also claimed ignorance on the same. In cases where crop rotation was done, in the last

season, the farm had been under maize, in most cases intercropped with beans. If maize had been planted in the last season, 76.8% of the farmers left the maize residues to decompose in the farm, 20.8% burnt the residues while 2.4% used the residues as fodder.

Fertilizer use: the majority of the farmers (92.1%) did not use fertilizer while growing groundnuts, of the farmers that did, 85.7% used organic manure which was applied a few weeks before planting, the organic manure used was animal manure. 14.3% of the farmers used commercial fertilizer, which mostly in cases of intercropping, was applied during planting of maize and top-dressed on maize during vegetative growth of groundnuts (Table 3).

Table 3: Farm management practices in Homa Bay County

Division	Cropping systems			
	Mixed cropping (%)	Mono cropping	Crop rotation	Application of amendments
Ndhiwa	100.0	0.0	20.0	4.0
Kobama	92.0	8.0	40.0	12.0
Nyarongi	96.0	4.0	36.0	28.0
Mean	96.0	4.0	32.0	14.7

Percentage proportion of farmers

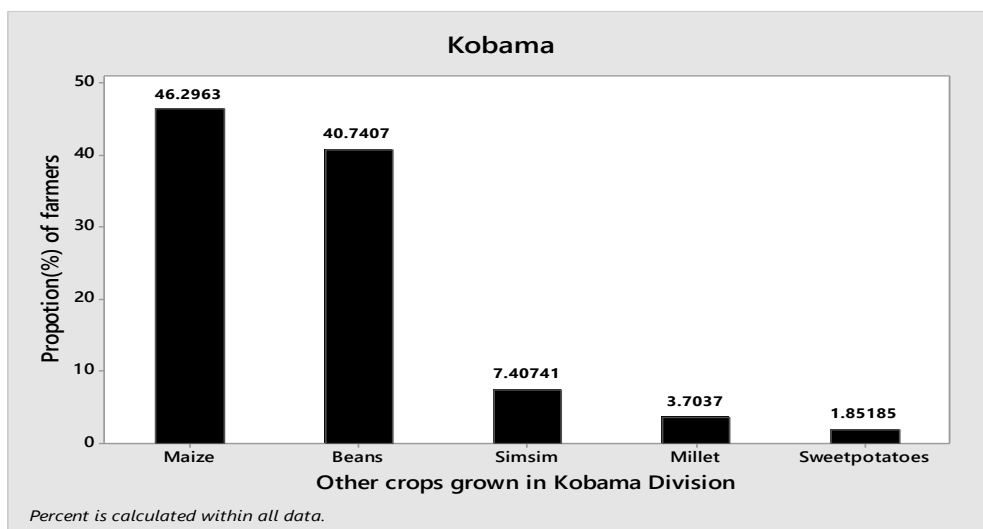


Figure 6: Crops other than groundnuts grown by farmers in Kobama division

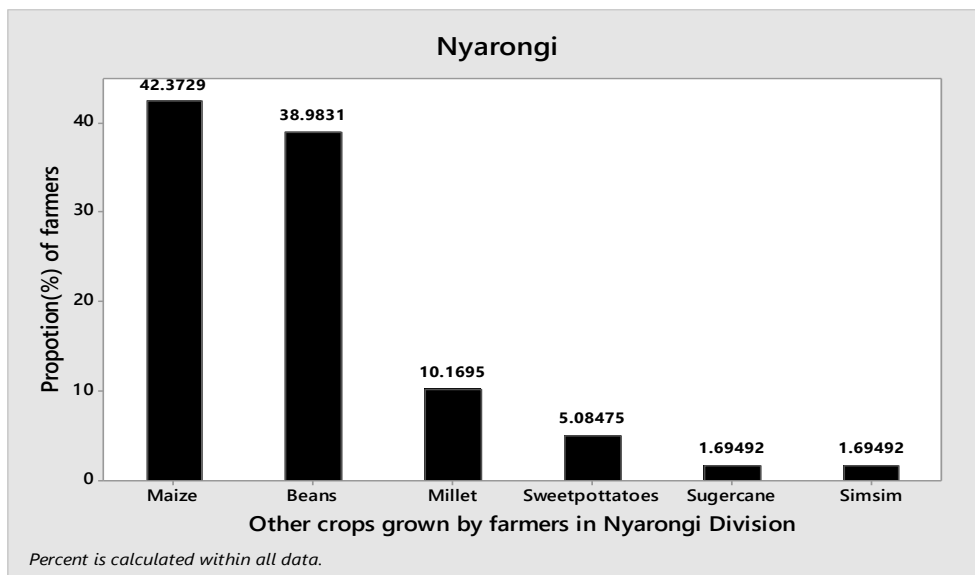


Figure 7: Crops other than groundnuts grown by farmers in Nyarongi Division

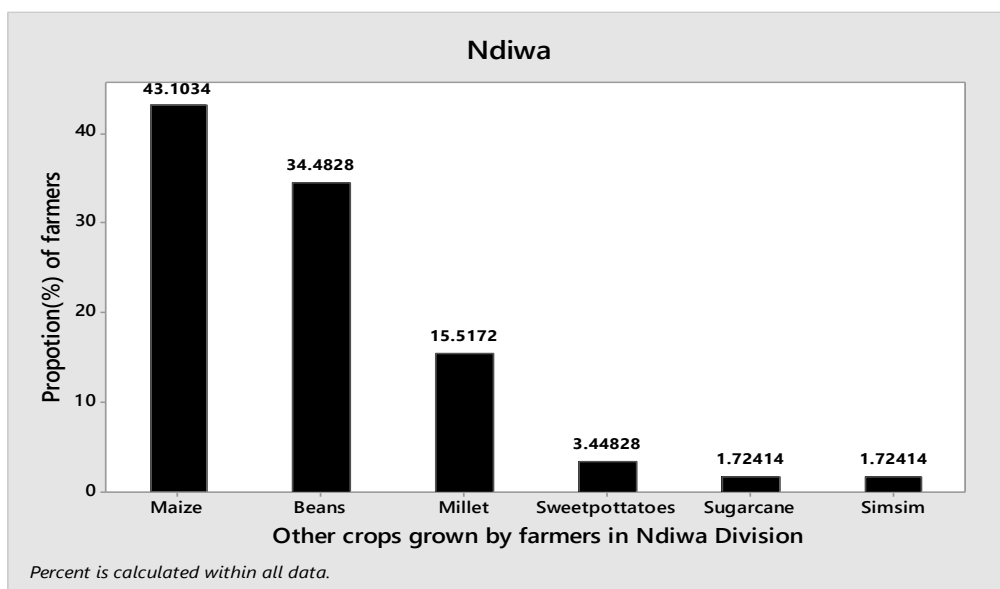


Figure 8: Crops other than groundnuts grown by farmers in Ndiwa Division

Weeding: in the majority of the farms (77.8%), weeding was done twice, 19.85% of the farms, the weeding was done once, while in only a small proportion (2.4%) the weeding was done thrice.

4.2.3 Common pests and diseases

The most common groundnut diseases observed in the area included:

Groundnut Rosette:

It was observed to occur at very high levels and could often produce 100% loss in yield. There were two forms of symptoms seen in the crops: 1. 'chlorotic' (yellow and stunted) (Figure 9) and 2. 'Green' (green and stunted). Chlorotic groundnut rosette was the one mostly observed in the farms. Groundnut rosette was found in majority of the farms (93.9%).



Figure 9: Groundnut chlorotic rosette

Leaf spots:

The two main forms of the leaf spot fungal disease – early and late were both observed by farmers in their farms during the growth period. Early leaf spot which occurred as early as 2 weeks after crop emergence (figure 10). Lesions produced by *Cercospora arachidicola* (Earl spot fungus) were observed to be roughly circular, dark brown on the upper surface with chlorotic (yellow) halos surrounding the darker lesions and a lighter shade of brown on the lower surface of the leaflets. Severe attacks caused heavy defoliation and resulted in a large yield loss. The late leaf spot (caused by *Cercosporidium personatum*) occurred later in the season and was observed to have nearly circular lesions which are darker than those of early leaf spot. Leaf spots were found in about 3.1 % of the farms.



Early leaf spot

Late leaf spot

Figure 10: Groundnut leafspot infection in farms in Homa Bay County

Nematode infections in groundnuts

These were observed as soil-dwelling round worms, less than 1mm long which bore into roots and pods. Their presence in the roots was observed to severely decrease the number of nodules. Symptoms included yellowish foliage and severely reduced production, pod damage was characterized by the appearance of small brown spots which become larger and darker. Nematode infections were found in about 3.0% of the farms observed.

Even with high levels of infections in most of the farms, majority of the farmers (73.9%) did not carry out any form of pest /disease management in their farms, with only 26.1% practicing any form of disease management, out of those who carried out pest/disease management, 97.9% practiced a cultural method of disease management, which involved uprooting the diseased plants and leaving them lying in the farm. 2.1% carried out chemical disease management in their farms.

39% of the farmers did not find drought as a problem to production, with 61% defining drought as a problem (Figure 11).

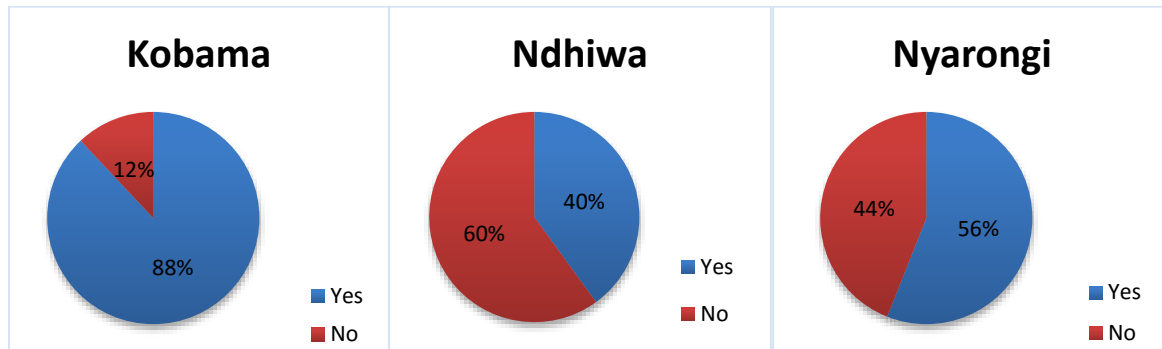


Figure 11: Perception of farmers in Homa Bay County on drought as a production problem

The perception difference may be due to the difference in average rainfall received in the study area.

4.2.4 Harvest and storage of the groundnuts

Majority of the farmers (70.9%) were able to tell that the groundnuts were ready for harvesting by observation of the browning of the leaves, others (25.3%) did random checks by randomly digging up groundnut plants in the farm and checking the nuts had hardened enough, a small proportion (3.8%) used the length of time from planting (averagely 2 and half months) to determine if their groundnuts were ready for harvesting.

Large proportion of the farmers(77.8%) harvested their produce by digging them up using a hand held hoe, other (22.2%) pulled the nuts directly from the ground, the harvest methodology was highly dependent on the soil type and groundnut variety, with pulling from the ground done mainly on Red Valencia grown in loose loamy soils.

4.2.5 Post-harvest agronomic practices

Drying of groundnuts: Majority of the farmers (54.2%) dried their groundnuts on an average of 3-4 days, with the remaining proportion (45.8%) opting for a period of 5-6 days. The period of

drying was heavily dependent on the availability of sunshine, i.e., the drying period could be longer, up to 2 weeks if the weather was cloudy. Some farmers mixed the subsequent harvests with the nuts already drying, this made it difficult to ascertain the exact number of drying days, and also lead to a probable risk of some nuts not properly drying hence vulnerable to post-harvest *Aspergillus* colonization.

Shelling: a large proportion (92.3%) of the farmers shelled their nuts manually, with the remaining 7.7% used a shelling machine. which was manually operated as shown in Figure 12.



Figure 12: Groundnut shelling in Ndhiwa division, Homa Bay County

Storage: all the farmers interviewed stored the groundnuts in unshelled form, shelling was only done prior to sale, consumption or planting, the storage of shelled groundnuts did not go beyond a week in any instance.

A large proportion of the farmers (94.7%) stored their produce in polypropylene bags, only 1.3% of the farmers stored their produce in jute bags, the remaining 3.9% stored their produce in polyethylene (Linear Low Density Polyethylene (LLDPE)) bags on the floor. In all the cases

observed the produce was kept in the house mostly stacked in bags on a corner, the groundnuts meant to be used as seeds for the next planting season were in most cases stored in bags suspended from the roof (Figure 13).



Figure 13: Groundnut storage containers (polypropylene bags) on the floor (Kobama division) and hanged from the roof (Ndhiwa and Nyarongi division)

Table 4: Groundnut storage structures and willingness to upscale groundnut production

Division	Groundnut storage structure		Willingness to upscale production
	Family house	Granary	
Kobama	96.0	4.0	100.0
Ndhiwa	96.0	4.0	100.0
Nyarongi	96.0	4.0	100.0
Mean	96.0	4.0	100.0

Of the farmers interviewed, 83.7% practiced groundnut farming for commercial purposes with only 16.3% growing groundnuts for subsistence purposes. Of the commercial growers, 77.8% took their produce to local market for sale; the remaining 22.3% relied on middlemen for the sale of their produce. The only value addition practice carried on the groundnuts before the sale was shelling. Other value addition practices such as roasting and boiling were done by traders.

4.2.6 General knowledge on aflatoxin contamination of groundnuts.

Majority (92.9%) of the population surveyed had no knowledge of aflatoxin contamination on groundnuts. Of the remaining 7.1% had an idea of aflatoxin contamination (Figure 14), but the knowledge was jumbled up and only two individuals had a clear knowledge of agronomic practices that could be put in place to reduce aflatoxin contamination in groundnuts. These included:

- Use of insect pest attractant plants such as *Desmodium* and *Molato*
- Harvesting groundnuts at maturity
- Proper drying of the produce
- Storage of groundnuts in unshelled state in dry and properly aerated place

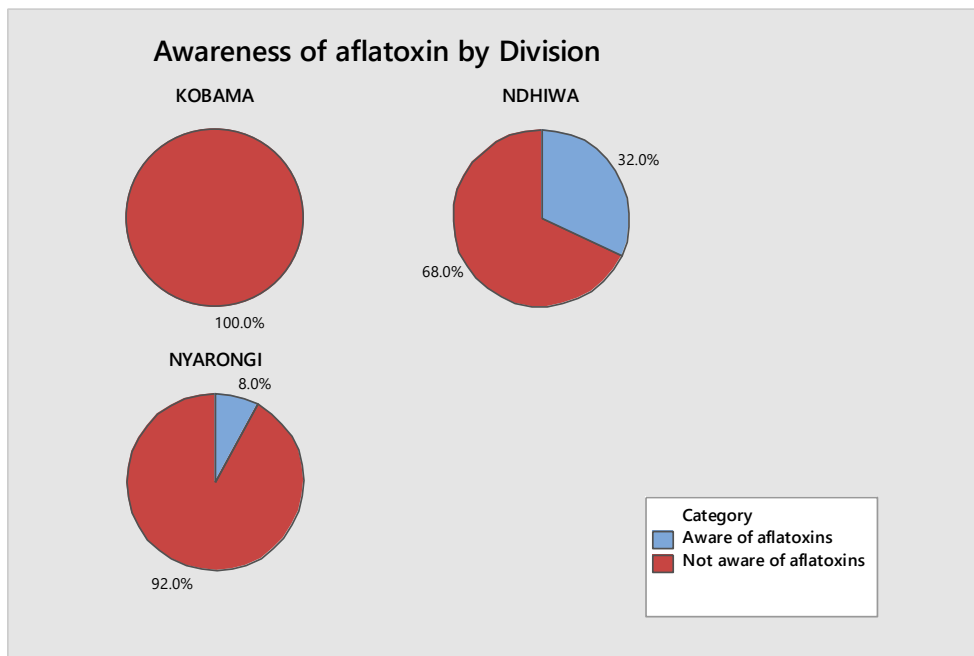


Figure 14: Awareness about aflatoxins among farmers in Kobama, Nyarongi and Ndhiwa divisions

4.2.7 Challenges facing groundnut farmers:

- Intensive labor involved in farming of groundnuts especially during planting, weeding, harvesting and shelling(manually)
- Low returns from the sale of produce due to low market prices especially during harvest period more so if the produce is sold through middlemen
- Unstable market prices. Fluctuating market prices makes planning very difficult
- Soil properties such as soil type and fertility affect quantity and quality of the produce. Groundnuts do well in fertile loamy soils. Farmers with clay soils experience low returns annually
- Lack of quality seeds for planting resulting in low-quality crops prone to diseases and eventually low quality produce unacceptable in international markets. In cases of seed availability, they are usually overpriced
- Lack of proper knowledge on pests, diseases and weed management resulting in low yields
- Lack of agricultural extension services and follow-ups even after the introduction of new seed varieties
- The poor condition of roads, most of the impassable especially during rainy season making it difficult for the produce to reach the market in time
- Unreliable weather patterns making farmer planning difficult and in many cases resulting in huge losses, for example, excessive rainfall during harvest period predisposing the nuts to rotting and fungal colonization

4.3 Population of *Aspergillus* sp. in soil

Various *Aspergillus* sp. were isolated from the soil sampled from Homa Bay County from the soil. The species included *Aspergillus flavus* S-strain, *Aspergillus flavus* L-strain, *Aspergillus niger* and *Aspergillus parasiticus* (figure 15). There was no significant ($p \geq 0.05$) variation in the population of the species in the soil sampled from the different regions in Homa Bay County except for *A. parasiticus*, which was isolated in the highest incidence with a mean population of 2160 CFU/g soil. The highest mean population (1780 CFU/g soil) of *A. flavus* S-strain was in soil sampled from Nyarongi region while the population of *A. flavus* L-strain was highest (240 CFU/g soil) in soil sampled from Ndhiwa division.

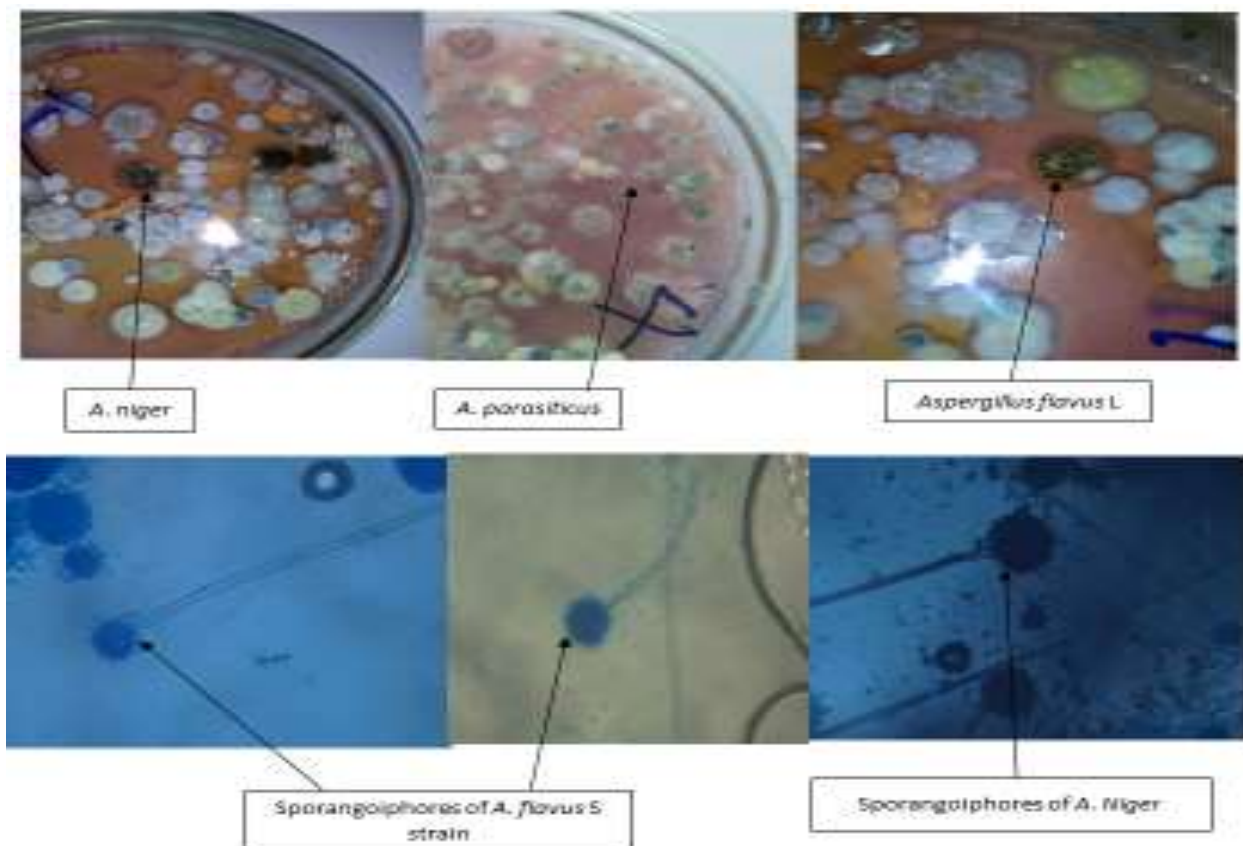


Figure 15: Morphological and cultural characteristics of different *Aspergillus* sp. isolated from soil sampled from various fields in Ndhiwa, Kobama and Nyarongi divisions growing on modified dichloran rose Bengal agar (MDRA)

Table 5: Population of *Aspergillus* sp. in soil and groundnuts sampled from various farms in Ndhiwa area, Homa Bay County at Harvest in 2015

Isolate	Mean ± SE	
	Soil	Nuts
<i>A. flavus</i> L strain	213±54.7	306.6±100.4
<i>A. flavus</i> S strain	1133±338.0	206.6±44.6
<i>Aspergillus parasiticus</i>	920±573.7	1393.3±169.67

Population in CFU/gram of soil

Kruskal-Wallis test to show the relationship in the distribution of the *Aspergillus* species among the three divisions showed that there was no significant difference ($P \leq 0.05$) in the frequency of isolation of the population of the *Aspergillus* isolates from soil from the three divisions. However there was a significant difference in the population of the *Aspergillus flavus* L strain isolated from nuts from the three divisions, but there was no significant difference in the frequency of isolation of the other *Aspergillus* species (Table 6).

Table 6: Frequency of isolation of *Aspergillus* sp isolates from groundnuts and soil from Homa Bay County

<i>Aspergillus</i> isolates from groundnuts			
Isolate	Chi-square values	df	P value
<i>A. flavus</i> L strain	8.6856	2	0.013**
<i>A. flavus</i> S strain	0.0027289	2	0.9986 ns
<i>A. parasiticus</i>	0.010065	2	0.995 ns
<i>Aspergillus</i> isolates from soil			
Isolate	Chi-square values	df	P value
<i>A. flavus</i> L strain	0.23773	2	0.8879 ns
<i>A. flavus</i> S strain	4.594	2	0.1006 ns
<i>A. parasiticus</i>	0.45155	2	0.7979 ns

** Significant difference at $p \leq 0.01$; * significant difference at $p \leq 0.05$; ns- not significant; significant difference at $p \leq 0.05$

Wilcoxon signed rank test with continuity correction with the alternative hypothesis: true location shift is not equal to 0 of *A. flavus* L strain, showed significance difference in the between Kobama and the other two divisions (Table 7), further data exploration shows that no *Aspergillus flavus* L strain was isolated from Kobama division.

Table 7: Frequency of isolation of *A. flavus* L strain in different divisions of Homa Bay County

Variables	V	P
Kobama vs. Ndhiwa	0	0.03603**
Kobama vs. Nyarongi	0	0.0131**
Ndhiwa vs. Nyarongi	35.5	0.858ns

4.3.1 Molecular identification of the toxigenic *Aspergillus* species

All the isolates from both the soil and nut fungi isolates that were positively identified by morphological identification as *A. flavus* and *A. parasiticus* were subjected to molecular identification. DNA extraction followed by PCR, Three primers were used i.e. AFL, Omt and Nor primers.

Twenty two out of 26 isolates were identified to positive for the Afl R gene (transcription activator/pathway regulator gene in aflatoxin biosynthesis pathway) (figure 16). 19 out of 26 isolates were positive for Nor-1 gene (gene coding for reductase enzyme; converts Norsoloriante to (1'S)-Averantin I the aflatoxin biosynthesis pathway) (figure 17). 17 out of 26 isolate were positive for the Omt gene (gene coding for O-methyl transferase A; converts Sterigmatocystin into O-methyl-sterigmatocystin in biosynthesis of aflatoxin B1 and G1, and conversion of Dihydro-sterimatocystin into O-Methyldihydro-sterigmatocystin in the biosynthesis of aflatoxin B2 and G2)(Figure 18). Some isolates overlapped in most of these gene in such a way that 22 out of 26 isolates was positive of at least 1 of the genes leaving four isolates that were identified as nontoxigenic *Aspergillus* species i.e. (Isolates 29, 68, 29B and 29E). (Figure 19). Out of the 22 positive isolates, 3 (28B, 29D, 60 B) were positive for Afla R gene only, the remaining 19 were positive for all the three genes (Afla R, Nor-1 and Omt genes).

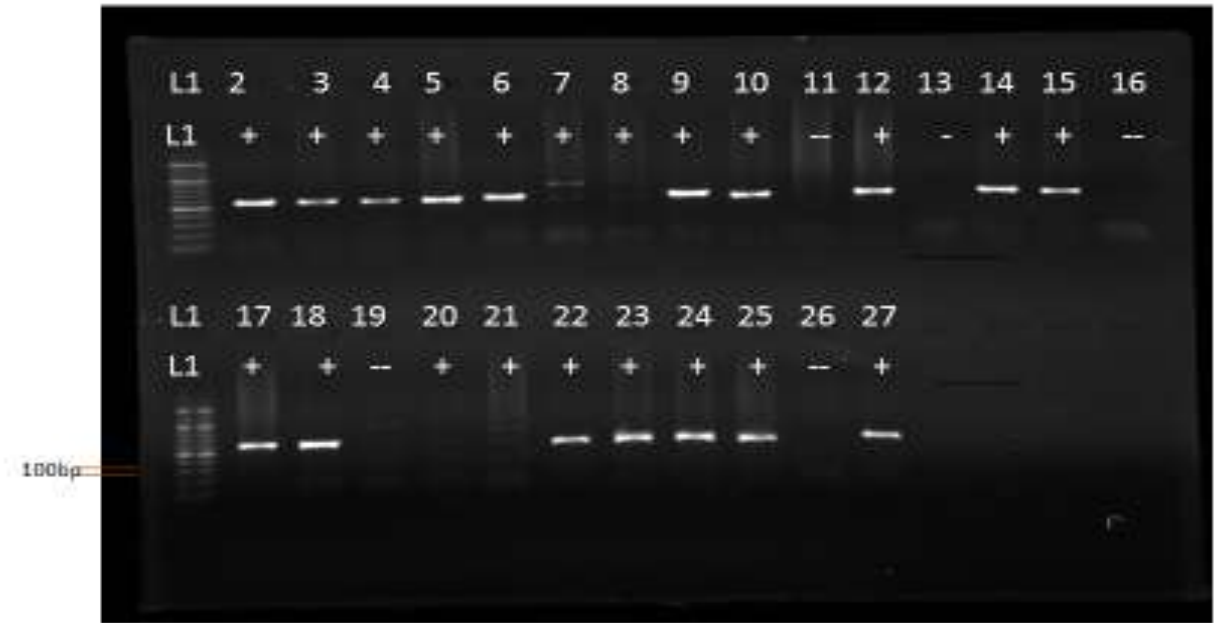


Figure 16: Polymerase Chain Reaction (PCR) bands generated with primers specific for AfIR gene (band size 444 base pairs)

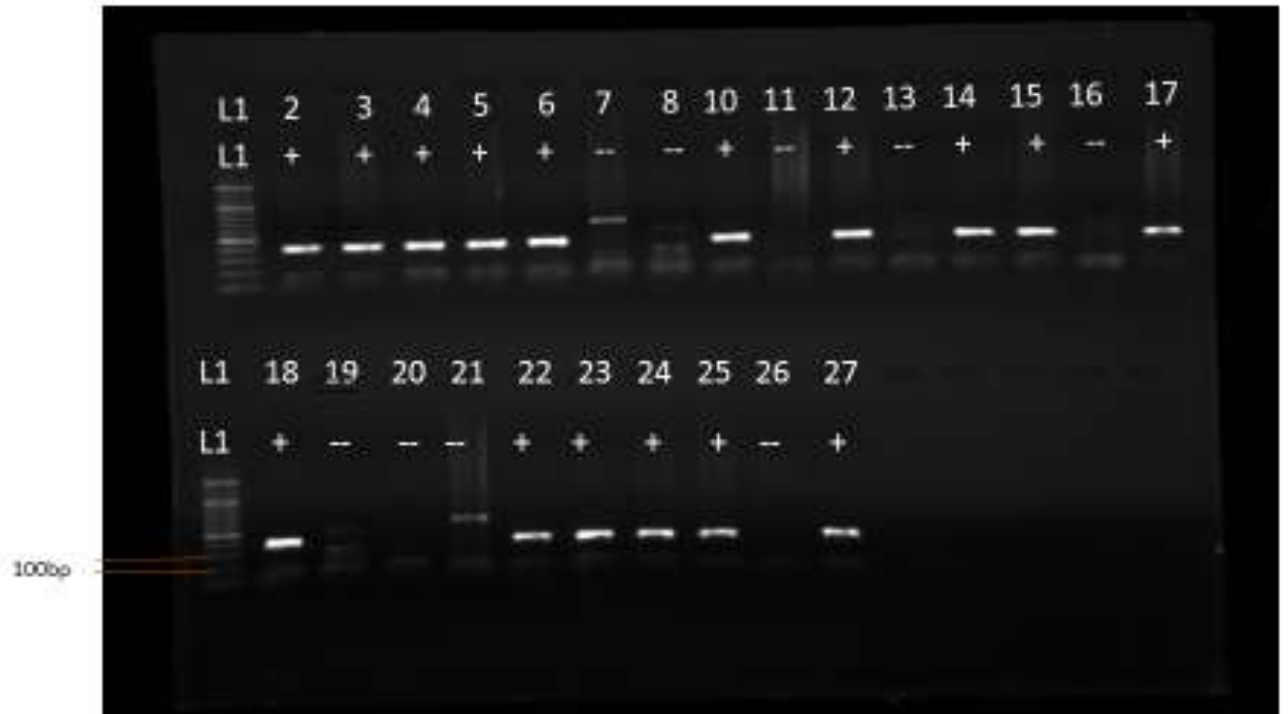


Figure 17: PCR bands generated with primers specific for Nor-1 gene (band size 271base pairs)

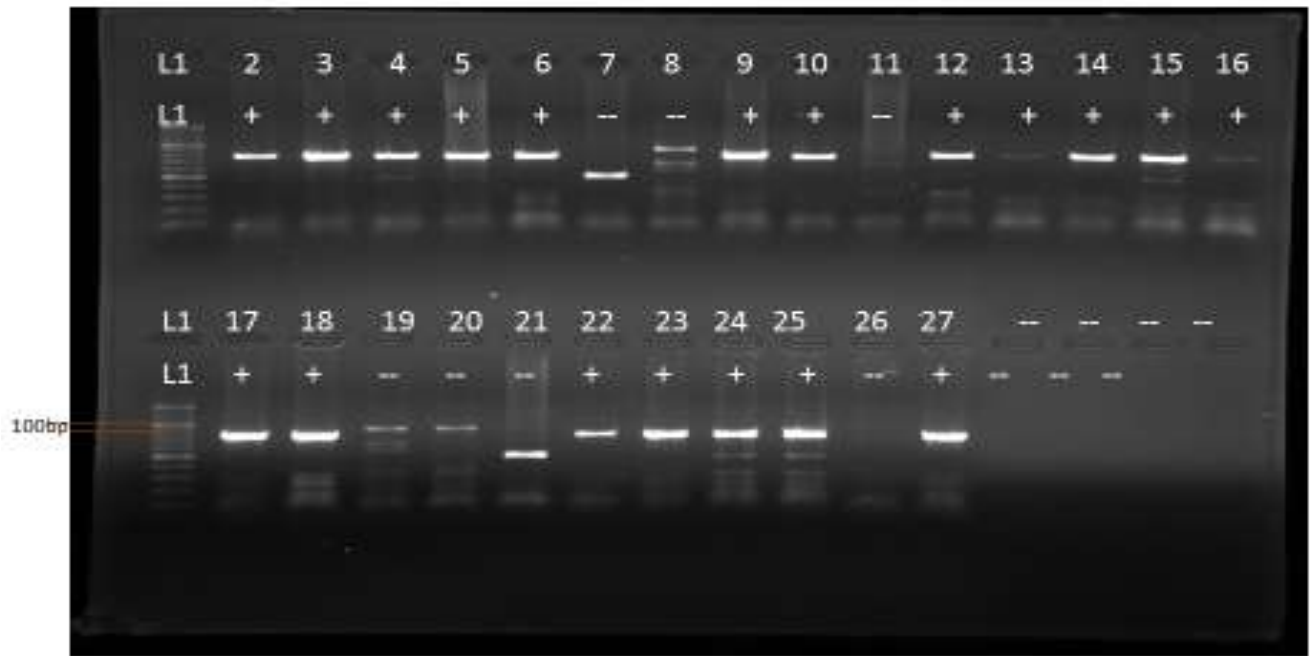


Figure 18: PCR products bands generated with primers specific for Omt gene (Band size 363 base pairs)

Sample id	Plate id	AFLr primers	Omt primers	Nor primers	Sample id	Plate id	AFLr primers	Omt primers	Nor primers
2	39B	+	+	+	16	68	-	-	-
3	73	+	+	+	17	48	+	+	+
4	9	+	+	+	18	42B	+	+	+
5	9B	+	+	+	19	29B	-	-	-
6	42C	+	+	+	20	60B	+	-	-
7	28B	+	-	-	21	28B	+	-	-
8	29D	+	-	-	22	39	+	+	+
9	4	+	+	+	23	73	+	+	+
10	7B	+	+	+	24	7	+	+	+
11	29	-	-	-	25	28B-2	+	+	+
12	39C	+	+	+	26	29E	-	-	-
13	68	-	-	-	27	42B	+	+	+
14	42B	+	+	+					
15	29C	+	+	+					

Figure 19: Molecular identification of various fungal strains based on PCR products of aflatoxin gene primers

4.4 Susceptibility of groundnut varieties to aflatoxin contamination

The levels of aflatoxin ranged from 0- 17.021ppb, 0- 43.23ppb and 0- 25.588 ppb in samples from Kobama, Ndhiwa and Nyarongi respectively. Overall (93.3%) of the groundnut samples had aflatoxin levels below the both the KEBS and EC standards. With 2.7% above EC standards but within KEBS standards and 4% being above both the EC and KEBS standards (Table 8).

Table 8: Proportion of groundnut samples with different aflatoxin content (ppb) in groundnuts from different divisions in Homa Bay County

Division	Proportion of groundnuts containing various concentrations of aflatoxins		
	<= to 4ppb	4- 10ppb	>10ppb
Kobama	92%	4%	4%
Ndhiwa	92%	4%	4%
Nyarongi	96%	0%	4%
Mean	93.3%	2.7%	4%

This shows that majority of the nut produced are within the safe limits for consumption and are acceptable by both the KEBS and EU standards.

Frequency distributions of aflatoxin levels in samples from the three divisions were found not to be significantly different for equal distribution based on Kolmogorov-Smirnoff (K-S) and the Mann-Whitney U two samples test (K-S P = 0.325; Man- Whitney U-test: P = 0.798) for the three divisions (Table 9), the distribution was highly skewed to the left showing that majority of the samples were safe for consumption based on the KEBS and EU regulatory limits.

Table 9: Frequency of distribution of aflatoxin level in Homa Bay County

Divisions	Kobama	Ndhiwa	Nyarongi
Kobama	-		
Ndhiwa	0.3801 ns	-	
Nyarongi	0.9838 ns	0.1969 ns	-

4.4.1 Inter-variety resistance of groundnuts to aflatoxin contamination

Seven varieties of groundnuts were identified based on morphological identification, the groundnut samples were grouped based on varieties and the means of each group compared via ANOVA and the results shows that there is no significant inter-variety difference in terms of aflatoxin accumulation (Table 10).

Table 10: Variety based resistance of different groundnuts to aflatoxin contamination

Variety	Mean concentration
JL 24(Homa Bay local)	1.10 ± 0.13 ^a
ICGV-9991	1.43 ± 0.10 ^a
Red Valencia	1.50 ± 0.09 ^a
CG7	1.44 ± 0.10 ^a
CG2	1.60 ± 0.23 ^a
ICGV-12991	1.92 ± 0.00 ^a
SM 99568	1.24 ± 0.03 ^a
Df	6,61
F	0.581
P-value	0.744

There is no significant difference within the same letter. Different letters are significantly different from each other, i.e. 'a' is significantly different from 'b'

4.3.2 Association between pre- and post-harvest agronomic practices with levels of aflatoxin in groundnuts

A highly significant association ($t = 2.652$; $P = 0.010$) was found between storage state of the groundnuts and aflatoxin levels. The percentage of safe samples according to KEBS standards was higher in samples stored in shelled state (94 %) compared to nuts stored in the unshelled state (84 %). While 14 per cent of samples stored in unshelled state had aflatoxin levels >20 ppb, only 4 % of samples stored in the shelled state were in this category.

A strong association ($t = 2.026$; $P = 0.047$) was also noted between levels of aflatoxin and whether the households considered drought as a problem in production (yes versus no) whereby percentage of safe samples according to KEBS standards was higher in samples form households who

considered drought a problem (96 %) compared to households who did not consider drought as a production problem (90 %). While 2 per cent of samples from households who considered drought as a problem had aflatoxin levels >20 ppb, 7 % of samples from households who did not consider drought a problem were in this category (Table 11).

Table 11: Correlation among different agronomic practices and Aflatoxin levels

Agronomic practices		Std. Error	t value	Pr(> t)	Significance
Pre-Harvest	(Intercept)	9.5947	-0.813	0.4192	ns
	Land under production	0.7156	-0.141	0.888	ns
	Whether or not intercropping was done	3.6565	0.044	0.965	ns
	Crop rotation	1.5866	0.6	0.551	ns
	Plant residue handling	1.3943	0.64	0.5244	ns
	Seed source	0.72	-1.328	0.1892	ns
	Cultivar type	1.4579	1.167	0.2476	ns
	Fertilizer application	2.0739	-1.208	0.2316	ns
	Weeding frequency	1.4216	-1.567	0.1222	ns
	Pest and disease management	1.4643	-0.108	0.914	ns
	Drought problem	1.5118	2.026	0.0472	*
	Harvesting methodology	1.5563	1.601	0.1146	ns
Post-Harvest	Drying duration	1.4246	-0.176	0.8608	ns
	Storage state	2.4478	2.652	0.0102	*

((Dispersion parameter for Gaussian family taken to be 33.22585) Null deviance: 2538.7 on 74 degrees of freedom; Residual deviance: 2026.8 on 61 degrees of freedom

No significant association was detected between levels of aflatoxin and land under production, whether or not the crops were intercropped; seed source; use of fertilizers; weeding frequency; application of crop rotation; disease and pest control; cultivar type; harvest method or drying duration.

4.3.3 Correlation between *Aspergillus* species and aflatoxin concentration

There was a weak negative correlation between *A. flavus* S and L strains, though the correlation was quite insignificant. There was also a weak negative correlation between the fungal populations of the *A. flavus* L and S strains and *A. niger*. There was a positive correlation between the fungal populations of *A. ochraecious* and *A. parasiticus* but the either of the cases the correlation was weak. There was no significant correlation between any of the identified *Aspergillus* species and the concentration of aflatoxins in the groundnuts (Table 12). There was no correlation between the aflatoxin levels found in the groundnuts and the fungal population of any of the fungal species.

Table 12: Correlation among the population of *Aspergillus* species and Aflatoxin levels

	<i>A. flavus</i> L-strain	<i>A. flavus</i> S- strain	<i>A. niger</i>	<i>A. ochraecious</i>	<i>A. parasiticus</i>	Aflatoxin concentration
<i>A. flavus</i> L	1.000					
<i>A. flavus</i> S	-0.0906 ns	1.000				
<i>A. niger</i>	-0.0639 ns	0.0665 ns	1.000			
<i>A. ochraecious</i>	-0.0580 ns	- 0.0448 ns	-0.0766 ns	1.000		
<i>A. parasiticus</i>	0.0206 ns	- 0.1094 ns	- 0.07876 ns	-0.1214 ns	1.000	
Aflatoxin concentration	-0.0525 ns	- 0.1228 ns	-0.0546 ns	0.1606 ns	0.0578 ns	1.000

** Correlation coefficient significant at $p \leq 0.01$; * correlation coefficient significant at $p \leq 0.05$; ns- not significant; correlation coefficient significant at $p \leq 0.05$

CHAPTER FIVE

5: DISCUSSION

The study aimed at determining the varieties of peanuts with resistance to aflatoxin contamination. Also, it sought to analyze the agronomic practices utilized by farmers practicing peanut production in Ndhiwa, Homa Bay County, with significant effects on aflatoxin concentration. Analyzing the gathered information would be helpful in determining the most appropriate production techniques and intervention processes for groundnut production in the area. Moreover, the results will facilitate the recommendation process by providing useful information on the most suitable aflatoxin resistant groundnut varieties for the area. Such will ease and enhance compliance with the standards set forth by the Kenya Bureau of Standards (KEBS) and the European Commission, EC (EC recommends an aflatoxin concentration of 4 ppb while KEBS insists on 10ppb).

5.1 Agronomic practices in peanut production

According to a study conducted by Kumar (2010), farmers with extensive farming lands are inclined to adopt efficient aflatoxin management strategies compared to those with small farming areas. Most farmers at Kobama, Ndhiwa and Nyarongi divisions in Homa Bay County produce groundnuts on small scale basis. These farmers do not use contemporary production techniques in their farming practices but rather capitalize on the traditional crop production systems. They neither use pesticides and insecticides nor practiced any recommended soil amendment methods developed for peanut production. Besides, their lack of knowledge on effective peanut farming procedures like land preparation, crop rotation including disease and pest management among other practices is prominent. Instead, most of these farmers, about 90% of farms, engage in mixed cropping, with maize being the primary crop while groundnuts assuming secondary importance.

Even for farmers who incorporated crop rotation in their activities, peanut production alternates with the maize crops.

According to Wu and Khlangwiset (2010), crops such as peanuts and maize have a high susceptibility to aflatoxigenic fungi like *Aspergillus* sp, explaining the high incidence of the particular species of fungi within the study area. As most farmers use maize in the mixed farming practices and rotation regimes, aflatoxigenic fungi resurgence remains inevitable. Besides, studies conducted in the USA showed a high concentration of *A. flavus* propagules in soils collected from maize fields compared to these from wheat and cotton farms (Abbas *et al.* 2004; Reddy *et al.* 2007). A study conducted by Griffin *et al.* (1981) supports these findings by providing the existence of a greater concentration of *Aspergillus flavus* in soils with continual maize and peanut production within the same fields.

The results of the study also concur with the provisions by Garcia and Cotty (2010). According to these scholars, the previous crop plays a significant role in determining the incidence of both the *A. flavus* L and *A. flavus* S-strains of the fungi. Such findings suggest the possibility of introducing effective crop rotation programs to lower the prevalence of aflatoxin contamination in peanut production. A study conducted by Mutegi *et al.* (2012) in Western Kenya indicated that the percentage of *A. flavus* and *A. parasiticus* isolates responsible for production of aflatoxin B1 and B2 was significantly higher in samples obtained from farmers who did not practice crop rotation compared to those that did. Therefore, as Strosnider *et al.* (2006) contemplate, useful introduction and implementation of rotational programs help to break the life cycle of aflatoxin-producing pathogens.

Despite the fact that most farmers in the study failed to use soil amendments, incorporation of soil amendments in peanut production impacts the level of incidence of fungal pathogens. Application

of lime and farm yard manure as soil amendments have proved effective in reducing *A. flavus* contamination and aflatoxin levels by 50-90% (Waliyar *et al.*, 2008). Lime contains calcium which tends to thicken the cell wall and accelerate pod filling. On the other hand, manure accentuates the growth of saprophytic microorganisms known to suppress pathogenic fungi in soil. Further, as (Reddy *et al.*, 2003) contends, amendments like gypsum provide sufficient calcium to the plant and increase plant tolerance to infections by *A. flavus*. Reported findings of a research by Rajik *et al.* (2011) indicate the potency of soil amendments in efforts to lower the concentration of soil borne pathogens. Besides, Singh and Pathak (2006) also reported the ability of organic compounds to reduce the population of harmful microflora in potato fields. Soil amendments should, therefore, be considered in peanut fields to lower *Aspergillus* contamination and subsequent concentration of aflatoxin in peanut kernels.

Research studies have also revealed a correlation between weather conditions and aflatoxin levels, with the majority focusing on the elements of relative humidity and temperature. Although this study does not focus on these components, it is essential to understand the factors promulgating aflatoxin resurgence and elimination. Farmers based in Ndhiwa rely on rain-fed agriculture in all agricultural practices including peanut production. But, according to Kumar *et al.* (2008), aflatoxin contamination is critical in semi-arid tropical environments with over-reliance on rain for agricultural productivity. On the other hand, irrigation lowers moisture stress, which favors occurrence and prevalence of *Aspergillus* sp, a major fungus in aflatoxin production (Reddy *et al.*, 2003; Kebede *et al.*, 2012).

The results of the study presented a significant variation in the occurrence and spread of the fungi in groundnut family within the regions of study. Peanuts grown in Ndhiwa area showed high susceptibility to foliar diseases, particularly early leaf spot, and late leaf spot. Even with the

occurrence of the aforementioned diseases, the farmers did not use fungicides as management strategies. As postulated by Hell *et al.* (2000), smallholder peanut farming in Western Kenya is typical with disease and pest infestation where meager investments are placed in disease alleviation processes including application of pesticides. According to Mutegi *et al.* (2009), smallholder farmers do not use pesticides or fertilizers in peanut production. Moreover they disregard appropriate agricultural and post-harvest handling practices, which tend to influence aflatoxin concentration. Thus, as in the case of the study, stress surfacing from attack by diseases increased the susceptibility of the crops to aflatoxin contamination.

Further, farmers in the research areas relied on visual observations as a method of determining the appropriate harvest time. During such efforts, they focus on visible changes like the shedding of leaves and color changes. Also, they uproot the plants to check whether pods have matured. These methods can be erroneous as the leaves may remain green long after maturity, especially in a season with high rainfall volumes. However, as Kaaya *et al.* (2006) note, harvest time of peanut influences the quantity of aflatoxin in end product. According to these authors, aflatoxin levels increase up to four times three weeks after the recommended time of harvest. Likewise, the levels upsurge seven times in cases where maize harvest are delayed by four weeks. Regarding early harvests, crops have to be dried effectively to eliminate possibilities of fungal growth. Due to such effects, the harvest technique used by these farmers could explain the existence of pathogenic fungi in the harvested peanuts as it does not provide a precise harvest date.

It is evident that peanut farmers within the study areas had no knowledge of aflatoxin, hence, did not view its existence as a challenge to their production processes. As Kumar (2010) stipulates, lack of awareness contributes to high aflatoxin quantities, as detected in some farms in Kobama,

Ndhiwa and Nyarongi regions. The farmers were unaware of the recommended practices thus had no control measures in place against aflatoxin contamination in peanuts.

5.2 Population of *Aspergillus* species and aflatoxin contamination in peanut kernels

Like the findings by Wagacha *et al.* (2013), the study isolated four strains of *Aspergillus* sp from peanut kernel samples used in the three divisions. These included *A. flavus* L-strain, *A. flavus* S-strain, *A. parasiticus* and *A. niger*. Similar findings were recorded by Mutegi *et al.* (2012), who isolated members of *Aspergillus* section Flavi from peanut kernels in Busia and Homa Bay districts of western Kenya. According to Mutegi *et al.* (2012), the four strains of *Aspergillus* sp are the most predominant species of the fungi in the Western section of the country (>60% incidence rate). A study by Abdela (2009) also reported the prevalence of contamination of peanut samples by different species of the fungus, particularly, *A. niger* and *A. flavus*. In this case, the frequencies for isolation were 29-60% for the *A. niger* strain and 4-52% for *A. flavus*. A different study conducted in Kericho and Eldoret towns in Kenya also recorded high incidence rates of the fungi (Nyirahakizimana *et al.*, 2013). From the findings of the study, 69% of the sampled groundnuts had different strains of *Aspergillus* section including *A. flavus* L strain, *A. flavus* S strain, *A. parasiticus*, *A. tamarii*, *A. caelatus*, *A. alliaceus* and *A. niger*.

Arguably, the high population of *Aspergillus* sp. recorded at the harvest stage may predispose the kernels to high aflatoxin contamination. Increased incidence may have occurred as a result of pest attacks or mechanical damages imposed on the kernels during harvesting. Such occurrences introduce channels for the spread and colonization of aflatoxin-producing fungi (Waliyar *et al.*, 2008). Also, poor storage practices affect the levels of aflatoxin concentration, evidenced by the high population of *A. flavus* S-stain and *A. flavus* L-stains in peanuts accessed from farmer stores. Good peanut storage practices recommend the use of properly ventilated storage material.

However, in the study, 50% of the farmers stored their peanuts in polypropylene bags, most of which had poor ventilations to capacitate free flow of air.

Particular environmental conditions trigger the growth of *A. flavus* in peanuts. As Ribeiro *et al.* (2006) contended, the fungi grows at moisture contents that exceeds 9%, temperature ranges of 25°C and 30° C and, a water activity of between 0.83 aw and 0.99 aw. As further stipulated by these authors, aflatoxin production reaches its optimum at 25°C and a water activity of 0.99 aw with a minimum of 0.87 aw. Therefore, the warm and humid environmental conditions marked across Africa acts a perpetuator of high and widespread aflatoxin rates not only in groundnut production but also other forms of food production practices (Gordon, 2003; Bankole *et al.*, 2006; Wagacha and Muthomi, 2008).

A number of researches have sought to determine and increase the use of good production practices among peanut farmers. Such efforts have mainly focused on improving quality and safety by lowering the rampancy of contamination (Gowda *et al.*, 2002; Turner *et al.*, 2005; Waliyar *et al.*, 2008). Some of the recommended practices include proper drying, grading, and storage. Aside from increasing peanut storage life, efficient drying reduces water concentration, hence, the optimal conditions necessary for aflatoxin resurgence (Sanders, *et al.*, 1982). According to Mestres *et al.* (2004), controlling humidity levels remains a challenge in tropical countries that incapacitates the ability to dry peanuts to appropriate moisture levels. Such a situation increases the susceptibility of the products to aflatoxin.

Based on climatic conditions witnessed in most African countries, the occurrence of *A. flavus* has also been observed in other countries. A study by Eshafie *et al.* (2011) reported the existence of *A. flavus* and aflatoxin in peanut butter in Sudan. As Passone *et al.* (2010) further contend, the major producers of aflatoxin in groundnuts are the *A. flavus* and *A. parasiticus* *Aspergillus* species.

A. flavus is the second leading cause of human aspergillosis and, is known to produce only the B-type aflatoxin (Hedayati *et al.*, 2007; Pasqualotto and Denning, 2008). On the other hand, *A. parasiticus* produces both B- and G-type aflatoxin (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 diversity of the fungal species posing high health risks to peanut consumers extends to other secondary metabolites, and of particular interest are the species of *A. niger*. The *Aspergillus* strain is particularly of great health concern since it produces a wide spectrum of secondary metabolites. According to Frisvad *et al.* (2007) and Weidenbörner (2008), *A. niger* produces ochratoxin A and malformins among other harmful strains.

The population of *A. flavus* S-strain was found to have no correlation with aflatoxin production in Homa Bay County. These findings do not resonate provisions by Wagacha *et al.* (2013). According to Wagacha *et al.* (2013), the incidence and population of *A. flavus* S-strain significantly and positively correlate with the levels of total aflatoxin in peanuts. This may be due to sample size or due to the fact that the identified strains were non-toxigenic. The presence of *A. flavus* S-strain implies a major health problem to consumers as reports have shown its capability to produce great amounts of aflatoxin especially aflatoxin B1 (Mutegi *et al.*, 2012), also classified as class 1 carcinogen (IARC, 1987). In other studies, Garcia and Cotty (2010) reported *A. flavus* S-strain to be the primary cause of aflatoxin contamination events in Africa and North America.

The current study evaluated aflatoxin levels at harvest in the peanut samples used across the three divisions in Ndhiwa area. The findings indicated a 93% compliance with the recommended EU standards of aflatoxin levels (≤ 4 ppb) at harvest. On the other hand, a high proportion of the products (96%) satisfied the threshold provided by the Kenyan regulatory agency (KEBS), ≤ 10 ppb, at harvest. Even though such data indicate high quality and safety levels of the products, the

predisposed storage conditions increase the susceptibility of harvested groundnuts to extensive aflatoxin contamination. Farmers in the area used polypropylene bags and allocated floor sections inside their houses as storage containers.

Based on the effects of aflatoxin contamination, its occurrence in peanuts ought to be of critical concern to the public. High aflatoxin contamination levels (above the 10 ppb limit set by KEBS) have been reported in raw and processed peanuts from different regions in 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), 37% of groundnuts including products like peanut butter and peanut flour sampled from Nairobi, Nyanza and Western Kenya failed to meet the 10 ppb total aflatoxin limit set by the Kenya Bureau of Standards (KEBS, 2007). Also, reports by Diop *et al.* (2000) indicated a mean content of 40 ppb of aflatoxin B1 in over 85% of peanut oil sampled in Senegal. Significant concentrations of the toxigenic compounds were also reported in peanuts sampled from farmers, stockers, traders and processes located Benin. Additionally, studies by Oliveira *et al.* (2009) conducted in Brazil also reported mean total aflatoxin level of 56 ppb in the sampled unprocessed peanuts.

According to Payne (1992) and Atehnkeng *et al.* (2008), Aflatoxin contamination occurs either during crop development or after crop maturation. At the crop development phase, aflatoxin occurrence is propagated by increased subjection of crops to high heat stress, drought or insect damage while after maturation, its incidence occurs as a result of high moisture contents before and after storage. As further postulated by Mutegi *et al.* (2013), the problem escalates when the products are stored in poorly ventilated setups. As such, good storage practices insist on proper ventilations to avoid moisture condensation on the grains that provides an apt environment for the extensive growth of the fungi (Bankole and Adebajo 2003; Hell and Mutegi, 2011).

The high-level aflatoxin contamination recordings in the study samples may have been a result of moderate temperature, rainfall and relative humidity in the Homa Bay County. The area is characterized by the equatorial climate, with an estimated mean annual temperature ranges of between 17.1°C and 34.8°C. Also, it receives annual rainfall amounts of between 250mm and 700mm per annum, providing apt conditions for the occurrence and growth of *Aspergillus* and aflatoxin in groundnuts and other cereals.

CHAPTER SIX

6: CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

Eight varieties were identified in this study as Homa Bay local, ICGV-9991, ICGV-12991, Red Valencia, CG07, CG2, CG3, and SM99568. Over 14 Groundnut varieties are known to grown in western Kenya these include Red Valencia. Minipinta, Bukene, Serere 116, Makulu red, Atika, Asyria Mwitunde,, Texas Groundnut, ICGV-07, ICGV-12988, ICGV-12991, Homabay local, Cianda and SM99508 with other varieties such as ICGV90708 (Valencia type) are grown in other parts of Kenya, particularly in Rift valley region. Most of the varieties identified are in agreement with the types documented to be in the area already apart from CG2, CG3, and SM99568. Not all varieties documented were found in this area most probably because of the sample size and the season. Some varieties are preferred over the others in the first season (March to July).

Agronomic practices carried out in Homa Bay County have predisposed groundnuts to infection by *Aspergillus* species and subsequent contamination by aflatoxins. The farmers lacked awareness on key peanut production practices like proper crop rotation, land preparation methods, common diseases of peanut and their management, did not apply soil amendments or pesticides to control pests and diseases, among others even where crop rotation was practiced, Maize was the main crop used in rotation, the maize stalks from the previous season was in most cases left to rot and ploughed back into the farms this type of practice is very likely to lead to accumulation of *Aspergillus* sp spores in soil over time leading to rise in cases of fungal infection and aflatoxin accumulation.

This study was able to show that as much as there are many groundnut varieties with different attributes, all are equally vulnerable to aflatoxin contamination both pre- and post-harvest, therefore, intervention strategies should be aimed at managing the toxigenic *Aspergillus* species both pre- and post-harvest. The great diversity of aflatoxin producing fungi poses another great risk of aflatoxin contamination in groundnuts. These fungi produce a wide array of other mycotoxins with varied side effects with cases of some mycotoxins being produced by more than one fungi.

Various *Aspergillus* sp. were isolated from the soil and groundnuts sampled from Homa Bay County from the soil. The species included *A. flavus* S-strain, *A. flavus* L-strain, *A. niger* and *A. parasiticus*.

In as much as there was low aflatoxin contamination of groundnuts sampled from Ndhwa area, Homa Bay County with most of the samples being within the safe limits set by the EC and KEBS, with the current production practices in place, there is a great risk of a rapid increase in aflatoxin levels in groundnuts from the area and this will pose a great health concern and may impact negatively on peanut trade locally, regionally and internationally.

6.2 RECOMMENDATIONS

- i. High-quality seeds should be availed to the farmers at affordable rates for planting so as to raise the quality of the crop that is not prone to aflatoxin contamination and eventually produce high quality produce acceptable in international markets.
- ii. More research should be done more-so on different varieties and crossbreeding done to come up with improved varieties with high outputs, visually appealing and resistant to fungal colonization and aflatoxin contamination.

- iii. 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 so as to reduce aflatoxin contamination of groundnuts.
- iv. Farmers should be trained on the use of appropriate drying and storage methods, in cases where possible, mechanical driers should be introduced and marketed to farmers especially those in areas that do not receive adequate rainfall.
- v. Maize crop should be discouraged as the main rotational crop since in most cases the maize itself is an alternative host to *Aspergillus* sp hence their stalks act as reservoirs for the *Aspergillus* sp spores inoculum as they don't decompose fast enough and can be in the soil for up to two years.
- vi. The awareness on the aflatoxin contamination of peanuts should be raised, its implications on human and livestock health and trade and the farmers trained in depth on possible management strategies.
- vii. Surveillance of status of the groundnut quality in terms of aflatoxin contamination should be a continuous activity within the ministry of agriculture and information on types of varieties that do well in particular soil types be made available to all farmers.

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APPENDICES

Appendix 1: QUESTIONNAIRE

Introduction

My name is Boaz Otieno, a Masters in Mycology student at University of Nairobi. I am undertaking my project studies with an objective to assess locally cultivated peanut (*Arachis hypogaea*) varieties for susceptibility to aflatoxin accumulation in Ndhiwa area, in Homa Bay County. The information given here will be confidential and not go beyond my studies. Your honesty and confidentiality in giving information will be highly appreciated.

Section I: Background information:

Farmer ID:.....

Name:.....

Date:../../2015

Age:..... Gender: Male () Female ()

.Division..... Agro-Ecological Zone:.....

Latitude:..... Longitude:..... Elevation (m):.....

Section B: Research questions:

1. Average farm size.....(Acres)

[0.1-1.9=1; 2-3.9=2; 4-5.9=3; 6 and above=4]

2. Area under peanut production.....(Acres)

[0.1-1.9=1; 2-3.9=2; 4-5.9=3; 6 and above=4]

3. What method(s) of land preparation do you practice?.....

[Ploughing=1, Harrowing= 2; ploughing then harrowing =3; others=4]

4. In addition to peanuts, what other crops grown in the farm.....
 [Maize=1, Millet=2, sweet potatoes= 3; Sugarcane= 4; Beans =5, others=6]
5. Do you intercrop peanuts with other crops..... [Yes=1; No=2]
6. If yes, with which crops:.....
 [Maize=1, Millet=2, sweet potatoes= 3; Sugarcane= 4; Beans =5, others=6]
7. Do you practice crop rotation?.....[Yes=1;.No=2]
8. Which crop(s) were planted last season in the area currently under peanuts?
 [Maize=1, Millet=2, sweet potatoes= 3; Sugarcane= 4; Beans =5, others=6]
9. If maize, how did you deal with the residues.....
 [Burnt=1; left to decompose=2; Used as fodder=3; other (specify) _____=4]
10. Where do you get seeds for planting?
 [Own=1; Neighbors=2; Local market=3; Agro-shop=4; others (specify) _____=5]
11. What type of cultivar(s) do you plant.....
 [Improved=1; local landrace=2]
12. Name the peanut varieties planted in this farm (i).....
 (ii)..... (iii)..... (iv).....
13. a) Do you use fertilizer use?_____ [Yes=1; No=2]
 b) If yes what type? _____
 [Commercial fertilizers=1; Organic fertilizers=2]
 c) If yes, when do you apply the fertilizer_____
 [At planting=1; after weeding=2; pegging stage= 3; others (specify) _____=4]
14. How many times do you weed your crops per season? _____
 [Once=1; Twice=2; 3 times or more=3]

15. When do you weed your crops _____
 [Emergence=1; Vegetative growth=2; Flowering=3; Pegging=4; Podding=5; Pod filling=6; Maturity=7]
16. What are the most common peanut pests and diseases in your farm (i).....?
 (ii)..... (iii)..... (iv).....
17. a). Do you practice any pest and disease management in your farm? _____
 [Yes=1; No=2]
 b). if yes what type _____
 [Commercial pesticides=1; Organic pesticides=2; Cultural methods=3]
18. Do you perceive drought as problem to production: _____ [Yes=1; No=2]
19. Apart from drought, what other problems do you encounter in production process?
 (i)..... (ii).....
 (iii).....
 (iv).....
20. How do you tell when your peanuts are ready for harvesting? _____
 [Browning of the leaves=1; random checks by digging=2; length of growth time=3; others (specify) _____=4]
21. How do you harvest your peanuts _____
 [Ox drawn plough=1; digging using hand hoe=2; pulling from the ground=3; others (specify) _____=4]
22. For how long (days) do you dry your peanuts after harvesting? _____
 [1-2=1; 3-4=2; 5-6=3]
23. How do you shell your peanuts? _____

[Manually=1; Use of machine=2; others (specify) _____=3]

24. If you use machine, what kind of machine?.....

25. How do you store your produce? _____

[Jute bag=1; Polypropylene bag=2; Polyethylene bag=3; Bucket=4; Reed basket=5; Tin=6; others (specify) _____=7]

26. Where do you store your produce? _____

[Traditional granary=1; in the house=2; Modern store=3; others (specify) _____=4]

27. On average, how long do you store your peanuts before:

a) Consumption.....

b) (b) Planting.....

c) (c) Selling.....

28. In what state do you store your peanuts after harvest_____

[In shells=1; unshelled=2; powdered form=3; paste=4]

29. Name value addition mechanism done on the peanut before sale/consumption

a).....

b)..... c)..... d).....

30. How do you utilize the peanuts you produce?_____ [Subsistence=1; Commercial=2;

Others(specify)_____ =3]

b). If for commercial use, where do you sell your product _____

[Local markets=1; middlemen=2; others (specify) _____=3]

31. What challenges do you face as a peanut farmer?

(a).....

(b).....

(c).....

(d).....

31. Do you know what aflatoxin contamination in peanuts is? _____[Yes=1; No=2]

a) If yes, list some of the agronomic activities you have put in place in order to reduce aflatoxin contamination in your peanuts

(i).....

(ii).....

(iii).....

(iv).....

Thank you for your time and cooperation