OCCURRENCE OF AFLATOXIN CONTAMINATION ON MAIZE IN THE LOWER EASTERN KENYA AND EVALUATION OF SUPERABSORBENT POLYMERS IN ITS MANAGEMENT

Livine Osiemo Nyakundi

(BSc. Agriculture)

A thesis submitted in partial fulfillment of the requirement for the award of Degree of Masters of Science in Crop Protection

Department Of Plant Science And Crop Protection

Faculty Of Agriculture

University Of Nairobi

2014

DECLARATION

This thesis is my original work and has not been presented for any award of a degree in any other university

Livine Osiemo Nyakundi **Date:**

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

Dr W. M. Muiru **Date:**

Department of Plant Science and Crop Protection

University of Nairobi, Kenya

Dr D. O. Mbuge **Date:**

School of Engineering (Department of Environmental and Biosystems Engineering)

University of Nairobi, Kenya

Dr S. P. Kuate **Date:**

Biochemist, Behavioral and Chemical Ecology Unit, International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya

DEDICATION

To my Late Dad Evans, My mother Mary and my family Lucy, Wayne and Patience

ACKNOWLEDGEMENT

Firstly, I thank the almighty God that I have reached this level of academic ladder, it is all because of His mercy and grace, may His purpose for this favor be fulfilled. I am also grateful to the following people for their support in this work: Dr. William Maina Muiru for his exemplary assistance, guidance, careful examination of my dissertation draft and encouragement that kept me inspired throughout this work; Dr. Duncan Onyango Mbuge my second supervisor from the University of Nairobi Faculty of Engineering Department of Environmental and Biosystems Engineering for his extraordinary patience, understanding and unwavering support has enabled me to complete this study successfully; Dr. Serge Philibert Kuate Biochemist, Behavioral and Chemical Ecology Unit, International Centre of Insect Physiology and Ecology (*icipe*) for his intellectual guidance and encouragement that helped so much in this project.

I also wish to express my profound gratitude to Professor Baldwyn Torto my first supervisor at ICIPE and the Head of the Behavioral and Chemical Ecology Department at the institute for his encouragement, support and suggestions which greatly added value to this work. Further, I am indebted to the staff and fellow research students of Behavioral and Chemical Ecology Department whose encouragement, support and cooperation made it possible to complete my project. In the laboratory, I relied on the help of Onesmus Wanyama, Xavier Cheseto and Kirwa. Their moral support and discussions helped me achieve my research objectives. I am grateful to Ms. Charity Mwangi, the administrative assistant at the Behavioral and Chemical Ecology Department, for her assistance in administrative issues during my training. I recognize the late Mr. F.M. Gathuma, former laboratory technician at the University of

Nairobi Faculty of Agriculture Department of Plant Science and Crop Protection for his assistance in the research work at the Plant Pathology Laboratory in Upper Kabete Campus.

Special recognition goes to the University of Nairobi, Kenya, Institute of Food & Soft Materials, ETH Zurich, Switzerland, International Centre for Insect Physiology (ICIPE), Nairobi, Kenya and International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria whose sponsoring and funding of the project made it possible for me to complete my study.

My heartfelt gratitude goes to all my friends, workmates and family members who have contributed morally and socially for the successful completion of this thesis. Special thanks to my fiancée Lucy for her unrelenting support and endurance during my study. To my family members Paul, Kefa, Carol, Tiberious, Rose, Fred and Lydia whose encouragement has been overwhelming, I sincerely and deeply appreciate you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ACRONYMS AND ABBREVIATIONS

A	Aspergillus
AEZ	Agro ecological zones
ANOVA	Analysis of variance
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin B2
AFs	Aflatoxins
CAST	Council for Agriculture Science and Technology
CDCP	Centre for Disease Control and Prevention
EPZ	Export Processing Zone
ESI	Electron Spray Ionization
EU	European Union
FAO	Food and Agricultural Organization
ICIPE	International Centre of Insect Physiology and Ecology
KEBS	Kenya Bureau of Standards
KEPHIS	Kenya Plant Health Inspectorate Service
LC-MS	Liquid Chromatography-Mass Spectrometry
LM4	Lower Midland Four
LM5	Lower Midland Five
MC	Moisture content
Min	Minutes
MOA	Ministry of Agriculture
PCNB	Pentachloronitrobenzene

ppb	Parts Per Billion
ppm	Parts Per Million
RPM	Revolutions Per Minute
RTP	Room Temperature
SAPs	Super Absorbent Polymers
SPH	Super Porous Hydrogel
Spp	Species
SPSS	Statistical Package for the Social Sciences
USA	United States of America
WHO	World Health Organization

ABSTRACT

Food and feed safety has been a major concern in most countries in the recent times as more knowledge is being generated on the occurrence of natural toxins in foodstuffs and edible plant species. Among the natural toxins, aflatoxins have been given more attention due to their hazardous effects to animals and human beings. A study was conducted on aflatoxin contamination in maize samples collected from lower eastern counties in Kenya. The maize samples were collected through a survey that was conducted in Kitui, Machakos and Makueni counties where aflatoxin contaminations has been a perennial problem. The samples were cultured in czapek dox agar to get the fungal contaminants and the rest analyzed for aflatoxin contamination in the laboratory using liquid chromatography and mass spectrometry (LC-MS). A further study of drying maize using super absorbent polymers (SAPS) was carried out in the laboratory. The desiccants were used in varied ratios of SAP: Maize, whereby the SAP was embedded in a membrane to avoid it mixing up with the maize. The ratios used included 1:1, 1:5, 1:10, 1:20 and control where no SAP was used. The experiment was carried out in three replicates and in different temperatures of 20°C, 30°C and 40°C and frequency change of hydrogels of 24 hours and 48 hours. After 216 hours, the maize samples were ground and aflatoxin concentration analyzed through LC-MS. The results from the questionnaire indicated that post harvest handling of maize predisposes grains to aflatoxin contamination. Among the *Aspergillus spp* isolated, *Aspergillus niger* constituted 50 % and *Aspergillus flavus* at 45% of the total isolates across the counties. In the LC-MS analysis 76% and 65% of samples had total aflatoxin levels below 20 ppb and 10 ppb leaving 24% and 35% of samples having total aflatoxin levels above 20 ppb and 10 ppb respectively. The 20ppb and 10ppb aflatoxin concentration are maximum limits recommended by World Health Organization (WHO)

Kenya Bureau of Standards (KEBS) respectively. Results from maize drying experiment indicated that the ratio 1:1 had no aflatoxin concentration and ratio 1:5 had low concentration of aflatoxin of less than 10 ppb in all treatments. The control had high concentration of aflatoxin above 10 ppb in all treatments and 1:10 and 1:20 had aflatoxin levels dependent on temperature and frequency change of hydrogels. There was a significant drop of aflatoxin concentration in ratio 1:10 and 1:20 in both frequency change of hydrogel compared to where hydrogel was not changed. It was noted that temperature, moisture content, amount of (Super absorbent polymers) SAP used and time taken to dry maize influenced the drying rate and overall aflatoxin contamination. The study revealed that use of 1:1 ratio can dry maize and avoid aflatoxin contamination, other ratios can also be used but with regular change of hydrogels in 24 or 48 hours to achieve lower contamination of aflatoxin of less than 10 ppb.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Superabsorbent polymers (SAP's) are classes of polymers that are known to absorb large amounts of water of up to 4,000 times their weight than traditional absorbent material (Esposito *et al*., 1996). They consist of a network of polymer chains that are cross-linked to avoid dissolution. The ionic functional group along the polymer chains encourages diffusion of water within the network (Raju *et al*., 2003).

There are a number of applications of the superabsorbent polymers which include; disposable personal hygiene products, agriculture, horticulture, waste management, electronics and construction (Barbucci *et al*., 2000). In agriculture, Superabsorbent polymers have been used as soil additives to increase the water retention of soils which can replace peat, the traditional moisture retention aid for soil (Barbucci *et al*., 2000). Miller (1979) suggested that the performance of superabsorbent polymers as a water retaining additive is greater in soils that are well draining such as sand. In addition, they have also been used for efficient utilization of water and fertilizers in plants.

Aflatoxins(AFs) belong to a closely related group of secondary fungal metabolites. These mycotoxins are highly toxic metabolites produced mainly by *Aspergillus flavus* and *A. parasticus.* Aflatoxin contamination can occur when a crop is in the field or during harvest, drying, and storage. However, most contamination occurs during the post-harvest stage especially when the produce is not handled properly to suppress growth of the toxigenic fungi (Wilson *et al*., 1992). Kenya has experienced several aflatoxicosis outbreaks during the last

twenty five years most of which have occurred in Makueni and Kitui districts in Eastern Province (CDCP, 2004). Both districts are prone to food shortage because of poor and unreliable rainfall and very high temperatures.

The Food and Agriculture Organization/World Health Organization (FAO /WHO) Joint Experts Committee on Food Additives has established guidelines for maximum food aflatoxin levels to reduce the amount of contaminated food that reaches consumers and animals (Ezzeddine, 1995). Although Kenya has adopted the WHO aflatoxin limit of 10 parts per billion (ppb) for humans (FDCS Act,. 1978), enforcing this limit is difficult, especially for homegrown maize that is consumed primarily by the subsistence farm household with perhaps a portion sold to local markets. Maize grown on small-scale farms does not appear typically in national commercial markets where aflatoxin testing is performed routinely. Instead, the grain is either used within the homestead or sold to local small-scale distributors and millers (Lewis *et al.,* 2005).

Severe aflatoxin outbreaks were reported in Eastern Province of Kenya during April through June of 2004 and 2005. In the year 2004 AFs contamination in maize resulted in 317 human cases of severe aflatoxin poisoning, with 125 deaths (CDCP, 2004; Julia, 2005). In 2005, another aflatoxicosis outbreak in Makueni and Kitui districts affected 75 people, resulting in 32 deaths (Lewis *et al*., 2005). Outbreak investigations and follow-up studies suggested that locally produced maize from subsistence farming was the likely source of contaminated grains responsible for the two outbreaks (CDCP, 2004).

Harvesting in these regions is mostly done during the wet season when farmers need to plant new crop and concurrently harvest and stock their produce which by then is not fully dry for

future use. Due to lack of proper drying facilities, the produce may get infected by *Aspergillus* spp. and lead to aflatoxin contamination. Therefore, use of superabsorbent polymers to dry the produce may be an alternative technology to reduce such cases.

1.2 Problem statement and Justification

In the last few years major grain losses have been reported as a result of field and postharvest aflatoxin contamination of maize, and more importantly loss of lives from consumption of contaminated maize in Kenya. Post-harvest losses are a serious concern since the lost grains represent loss of investment such as seed, fertilizer, herbicide, pesticide, irrigation water, labor, machine time and energy and are also a central health issue in society.

The main causes of post-harvest aflatoxin contamination are high temperature, relative humidity of the surrounding air and moisture content of the grain. In technology based storage structures, proper control of temperature, relative humidity and moisture content is easily achieved using electric or fossil fuel powered heaters, dehumidifiers, air conditioners and air circulation fans. Peasant farmers have no access to in-storage conditioning of maize facilities neither do they have sufficient knowledge on post harvest handling of their maize produce and therefore aflatoxin contamination normally results when maize is harvested wet or during the rainy season.

Given the substantial impact of the outbreaks of aflatoxicosis in the past years, this study's goal is to identify the farmers' post harvest practices that may influence their produce to Aspergillus growth leading to aflatoxin contamination and same time develop simple and efficient technology that can be used by resource challenged farmers to dry their maize produce after harvest thus reduce cases of aflatoxicosis.

1.3 Main objective

To reduce the losses associated with mycotoxin contamination of maize by use of superabsorbent polymers (SAPs)

1.3.1 Specific objectives

1. To identify the fungal contaminants and types of aflatoxins produced in the maize samples from lower eastern region of Kenya
2. To determine the effectiveness of super absorbent polymers (SAPs) / hydrogels in the control of *Aspergillus* spp and subsequent aflatoxin contamination in maize

1.4 Research hypothesis

1. Farmers' post harvest handling practices of maize in the lower eastern region of Kenya contributes to Aspergillus growths that lead to high levels of aflatoxin contamination in their maize
2. Super absorbent polymers (SAPs) can dry maize fast enough to make conditions for Aspergillus growth unfavorable and therefore reduce aflatoxin contaminations

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Mycotoxins

Mycotoxins are natural toxic secondary metabolites produced by certain fungi in agricultural products that are susceptible to mould infestations (Bennett and Klich, 2003; Wagacha and Muthomi, 2008; Morenoa *et al*. 2009). The most common fungal mould that produces these mycotoxins are Aspergillus, Fusarium and Penicillium.

The most important mycotoxins are aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisin, and T-2 toxins. Among these mycotoxins, aflatoxins are the most widely studied due to their effects both to human and animals (Okoth and Kola, 2012). Aflatoxins are produced by a group of *Aspergillus spp* namely *Aspergillus flavus* and *Aspergillus parasiticus* (Nassir and Jolley, 2002). There are more than 13 different aflatoxins produced in nature but the most common ones are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) of which AFB1 is most common and most toxic (Okoth and Kola, 2012). *Aspergillus spp* contaminate maize and other agricultural products during production, harvest, transportation, storage and food processing (CAST, 2003; Murphy *et al*., 2006).

2.2 Identification of *Aspergillus* genus

The genus is large consisting of more than 180 accepted anamorphic species with teleomorphs described in nine different genera (Pitt and Samson, 2000). The genus is divided to seven sub genera which in turn are further divided into sections (Klich, 2002). *Aspergillus* mold species are found throughout the world and are the most common type of fungi in the environment

(Suhaib *et al.,* 2012). Sixteen species of this genus are dangerous to humans, animals and plants as they cause diseases and infections.

The color of mould's surface differs from species to species and can be used for identification of this genus. The rate of growth of the colony can also be used for identification as some *Aspergillus spp* grow quickly than others for example seven days of growth at 25 °C most *Aspergillus* colony will be between 1 to 9 cm in diameter but some like *Aspergillus nidulans* and *Aspergillus glaucus* grow more slowly and will be between 0.5 and 1 cm within the same time. The genus is easily identified by its characteristic conidiophores but species identification and differentiation is complex for it is traditionally based on range of morphological features (Suhaib *et al*., 2012).

Macromorphological features which are considered include conidia and mycelia color, colony diameter, colony reverse color, presence or absence of sclerotia and cleistothecia (Klich, 2002). Table 2.1 gives guidelines on how identification is done in various fungal species cultured in czapek dox agar for 7 days in terms of colony colors. Micromorphological features mainly are dependent on seriation, shape and size of vesicles, conidia and stipe morphology, presence or absence of hulle cells, morphology of cleistothecia and ascospores (Klich, 2002). There are several taxonomic identification keys and guides such as Klich's key and Rapper and Fennell keys are available and can be used for ease of identification (Klich, 2002; Rapper and Fenell, 1965).

Rapid and accurate methods of identification have been developed like the molecular method but are expensive and are not as commonly used compared to microscopy and cultural methods (ASM, 2004).

Table 2.1: Cultural features used for identification of *Aspergillus species* (Suhaib *et al*., 2012)

SPECIES	SURFACE	REVERSE
A. clavatus	Blue-green	White, brownish with age
A. flavus	Yellow-green	Goldish to red brown
A. fumigatus	Blue-green to gray	White to tan
A. glaucus	Green with yellow areas	Yellowish to brown
A. nidulans	Green, buff to yellow	Purplish red to olive
A. niger	Black	White to yellow
A. terreus	Cinnamon to brown	White to brown
A. versicolor	White, then yellow, tan, pale green or pink	White to yellow or purplish red
A.parasiticus	Grey – green	Brownish

2.3 Effects of aflatoxin contamination

Maize is the most important staple food for the majority of the Kenyan population (EPZA, 2005; Kimanya *et al*., 2008) which is consumed at an average intake of 400g per person per day (Muriuki and Siboe, 1995; Shephard, 2008). However, the grain is vulnerable to degradation by mycotoxigenic fungi which include *Aspergillus*, *Fusarium* and *Penicillium*. Maize contamination by fungi does not only reduce its quality through discolouration and reduction of nutritional value but also lead to mycotoxin production (Okoth and Kola, 2012).

Maize contamination with aflatoxin is a global concern as maize is one crop that is widely cultivated by many countries across the globe. In Kenya alone more than 40% of diets in both

rural and urban communities are composed of maize and maize products (Mwihia *et al*., 2008).

Mycotoxins have caused significant economic losses associated with their impact on human health, animal productivity and trade (Wagacha and Muthomi, 2008). Acute aflatoxin poisoning in humans causes hepatitis, jaundice and gastrointestinal injury with high morbidity and mortality (CDCP, 2004). This means that ingestion of high doses of aflatoxin results to acute aflatoxicosis and in severe cases leads to liver failure and death which has been the case in most aflatoxicosis outbreaks in Kenya (Okoth and Kola, 2012). The prolonged exposure by ingesting low doses of aflatoxin contaminated products over a long period of time increases the risk of liver failure to humans. In case of children, aflatoxin contaminated food retards growth and their development which can lead to nutritional challenges (CDCP, 2004).

In livestock, aflatoxin contamination reduces food utilization efficiency and reproductivity of animals and in rare cases may lead to death. It can also suppress the immune system of animals leading to frequent occurrence of infectious diseases (Gray *et al*., 2009). When animals take aflatoxin in contaminated feed, this raises human health concerns as the aflatoxin can appear in animal products such as meat, milk and eggs in case of poultry which in turn can affect whoever consumes these by products (Gray *et al*., 2009).

The Food and Agricultural Organization (FAO) estimates that between 25% and 50% of agricultural crops worldwide are contaminated by mycotoxins (Fandohan *et al*., 2003; Lewis *et al*., 2005; Wagacha and Muthomi, 2008).

The estimated value of maize lost to aflatoxin is $225 million per year, out of the $932 million due to all the mycotoxins in the United States (Betran and Isakeit, 2003). It is reported

that in 2011 the government of Kenya banned sale of 2.3 million bags of maize from small growers due to the contamination of aflatoxin with levels being higher than the accepted limit (Mathias and Macharia, 2013). This resulted to serious loss of income and other inputs to these farmers.

2.4 Occurrence of mycotoxin poisoning in Kenya

Mycotoxin poisoning in humans and animals occurs through ingestion, inhalation and absorption through the skin (Park and Liang, 1995; Wagacha and Muthomi, 2008). High-level exposure may cause instant death while long-term chronic effects include cancer, mutagenicity, hepatotoxicity and nervous disorders (KEPHIS, 2006; Fang and Clark, 2004). At the farm level, the real problem is that contaminated maize may appear just like the normal grain without any outward physical signs of fungal infection. Destruction of aflatoxins by conventional food processing is difficult because they are typically resistant to heat and detection is complicated due to limitations in analytical capacity (Muthomi *et al*, 2009).

Aflatoxins B1, B2, G1, and G2 are produced by some strains of *A. flavus*, *A. parasiticus* and *A. nomius* (Cotty, 1997) with aflatoxin B1 being the most common. Aflatoxin poisoning has continued to cause disease and death of many people in rural areas of Eastern and Central provinces of Kenya. The first outbreak of aflatoxicosis in Kenya was reported in 1978 and again in 1984-1985, when a large numbers of dogs and poultry died due to aflatoxin poisoning (Manwiller, 1987). In 1978, aflatoxin contamination in dog meal exceeded 150ng/g with the highest being 3000ng/g (Manwiller, 1987).

Other outbreaks occurred in 1981, 2001, 2004, 2005, 2006, 2007 and 2008 resulting in sickness, death and destruction of contaminated maize (Bennett and Klich., 2003; Lewis *et al*.,

2005; KEPHIS, 2006; KEPHIS, 2006, MOA, 2008; Probst *et al*., 2007; Reddy and Raghavender, 2007; Shephard, 2008).

The largest outbreak reported in the world during the last 20 years was in 2004 when 317 cases were reported with 125 deaths in Makueni, Kitui and neighboring districts (Lewis *et al*., 2005). Maize from the affected area contained as much as 4,400ng/g aflatoxin B1, which is 440 times greater than the 10ng/g tolerance level set by the Kenya Bureau of Standards. Most of the aflatoxin poisoning outbreaks occurred in remote villages and, therefore, the number of people affected could have been higher than reported (Lewis *et al*., 2005).

Investigations after the outbreak and follow up studies suggested that locally produced maize from subsistence farming was the likely source of contaminated grains (CDCP, 2004). Despite Kenya adopting the aflatoxin limit of 10 parts per billion (ppb), enforcing this is a challenge especially for homegrown maize mainly consumed by subsistence farm households with little or none being sold to local markets. According to Lewis *et al*. (2005), maize grown by subsistence farmers don't appear in the national commercial markets where aflatoxin testing is done as a routine but they are either used locally within the homestead or at times sold to local small scale distributors and millers who later sell it within the local markets. This has posed a big challenge especially to the concerned government departments and other stakeholders who enforce the law.

2.5 Causes of aflatoxin contamination in maize

Aflatoxin in maize is caused by a group of fungi mainly from Aspergillus genus. The species involved according to Muthomi *et al*. (2009) are *Aspergillus flavus* and *Aspergillus parasiticus*. These species produce aflatoxin B1, B2, G1 and G2 as their metabolites once they

infect the maize and maize products or other agricultural products. The Aspergillus group of fungi is widely spread in nature and can be found in air, soils and other agricultural products (Okoth and Kola, 2012).

The aflatoxin contamination in maize has been associated with drought combined with high temperature as well as insect injury (Betran and Isakeit, 2003). Favorable temperatures for Aspergillus growth and sporulation ranges between 30°C and 40°C on which the fungi can multiply and spread to contaminate most agricultural products (Mutungi *et al*., 2008). According to Saleem *et al*. (2012), injuries either from insect or physical causes can expose the maize to Aspergillus contamination both at pre or post harvest stage of the product. It is worthwhile to note that genotype, soil types, and insect activity are important in determining the likelihood of pre-harvest contamination (Cole *et al*., 1995).

Poor harvesting practices, improper storage and less than optimal conditions during transport and marketing can also contribute to fungal growth and multiplication of mycotoxins (Bhat and Vasanthi, 2003; Wagacha and Muthomi, 2008). It is reported that cold room storage reduced aflatoxin contamination in maize compared to warehouse and bin storage at room temperature (Saleem *et al*., 2012). This implies that under low temperatures the activity of *Aspergillus* species is hindered and therefore is unable to grow and sporulate to cause aflatoxin contamination. Other poor post harvest handling practices like using plastic containers for storage of under dried maize have been reported to cause more aflatoxin contamination unlike well aerated bags and stores (Mwihia *et al*., 2008). As reported by Wagacha and Muthomi (2008), transporting in poorly aerated containers, drying maize on ground contact with soil can expose the maize to *Aspergillus* contamination which may lead to aflatoxin in the maize.

The moisture content of the maize or other agricultural products during and after storage is an important factor that can determine the extent to which the product can be contaminated with aflatoxin. According to Mutungi *et al*. (2008), the appropriate moisture content of maize ready for storage is below 14%. When moisture content is above this level it is considered as wet and if bulked in a poorly aerated place, this can generate heat and create favorable microenvironment for *Aspergillus* multiplication and may lead to aflatoxin contamination (Mwihia *et al*., 2008). Rewetting of already dry maize increases the moisture content of maize and as noted by Hughs (1970), the rate of *Aspergillus* growth and subsequent aflatoxin contamination in these samples was higher than freshly harvested maize under similar conditions.

2.6 Prevention of aflatoxin contamination in maize

The contamination of aflatoxin in maize has been widely studied at different levels and it has been concluded that the contamination can occur either in the field (pre - harvest) or during and after the harvest has been done (Gray *et al*., 2009). Several strategies have been used to prevent aflatoxin contaminations as follows:

2.6.1 Breeding for resistance

Several screening tools have been developed and used to facilitate corn breeding for developing germplasm resistant to fungal growth and/or aflatoxin contamination (Brown *et al*., 2003). Sources of resistance to *Aspergillus* infection and aflatoxin contamination in corn have been identified, but commercial hybrids have not been developed. This is largely due to the difficulty in finding elite lines that maintain high yields and maintain resistance within multiple environments (Clements and White, 2004). Many new strategies that enhance host

plant resistance against aflatoxin involving biotechnologies are being explored and are reviewed by Brown *et al*. (2003). These approaches involve the design and production of maize plants that reduce the incidence of fungal infection, restrict the growth of toxigenic fungi or prevent toxin accumulation.

2.6.2 Biological control

Another potential means for toxin control is the bio-control of fungal growth in the field. Numerous organisms have been tested for biological control of aflatoxin contamination including bacteria, yeasts, and nontoxigenic (atoxigenic) strains of the causal organisms (Yan et al., 2008) of which only atoxigenic strains have reached the commercial stage. Biological control of aflatoxin production in crops in the US has been approved by Environmental Protection Agency and a commercial product based on a toxigenic *Aspergillus flavus* strains is being marketed (Afla-Guard®). In Africa, two isolates of *A. flavus* have been identified as atoxigenic strains to competitively exclude toxigenic fungi in the maize fields. These strains have been shown to reduce aflatoxin concentrations in both laboratory and field trials, reducing toxin contamination by 70 to 99% (Atehnkeng *et al*., 2008). A mixture of four atoxigenic strains of *A. flavus* of Nigerian origin has gained provisional registration (AflaSafe) to determine efficacy in on-farm tests and candidate strains have been selected for Kenya and Senegal.

2.6.3 Control of environmental factors

Environmental factors that favor *A. flavus* infection in the field include high soil and/or air temperature, high relative humidity, high rates of evapo-transpiration, reduced water availability, drought stress, nitrogen stress, crowding of plants and conditions that aid the

dispersal of conidia during silking (CAST, 2003). Significant correlations exist between Agro ecological zones (AEZ) and aflatoxin levels, with wet and humid climates and drier regions after longer storage periods increasing aflatoxin risk (Hell *et al*., 2000).

Agro ecological zones are geographic areas that share similar biophysical characteristics for crop production, such as soil, landscape, and climate. Modeling of interactions between host plant and environment during the season can enable quantification of pre-harvest aflatoxin risk and its potential management (Boken *et al*., 2008). Predictive growth models for fungal and mycotoxin developments are available and have been reviewed by Garcia *et al*. (2009).

Factors that influence the incidence of fungal infection and subsequent toxin development include invertebrate vectors, grain damage, oxygen and carbon dioxide levels, inoculum load, substrate composition, fungal infection levels, prevalence of toxigenic strains and microbiological interactions. High incidence of the insect borer *Mussidia nigrivenella* Ragonot, was positively correlated with aflatoxin contamination of maize in Benin (Setamou *et al*., 1998). Storage pests, in particular *Cathartus quadricollis* Guerin and *Sitophilus zeamais* Motschulsky, play an important role in the contamination of foods with fungi, especially those that produce toxins (Hell *et al*., 2003; Lamboni and Hell, 2009).

2.6.4 Crop management

Management practices that reduce the incidence of mycotoxin contamination in the field include timely planting, optimal plant densities, proper plant nutrition, avoiding drought stress, controlling other plant pathogens, weeds and insect pests and proper harvesting (Bruns, 2003). Tillage practices, crop rotation, fertilizer application, weed control, late season rainfall,

irrigation, wind and pest vectors all affects the source and level of fungal inoculum, maintaining the disease cycle in maize (Diener *et al*., 1987).

Extended field drying of maize could result in serious grain losses during storage (Borgemeister *et al*., 1998; Kaaya *et al*., 2006), and as such harvesting immediately after physiological maturity is recommended to combat aflatoxin problems. Kaaya *et al*. (2006) observed that aflatoxin levels increased by about 4 times by the third week and more than 7 times when maize harvest was delayed for four weeks. However, after early harvesting products have to be dried to safe levels to stop fungal growth.

Moisture and temperature influence the growth of toxigenic fungi in stored commodities. Aflatoxin contamination can increase 10 fold in a three day period when field harvested maize is stored with high moisture content (Hell *et al*., 2008). It is recommended that harvested commodities should be dried as quickly as possible to safe moisture levels of 10 – 13% for cereals. Achieving this through simple sun-drying under the high humidity conditions is difficult. Even, when drying is done in the dry season, it is not completed before loading grains into stores like observed by Mestre *et al*. (2004) and products can be easily contaminated with aflatoxins.

There are several technologies that increase the efficacy of grain drying and reduce the risk of toxin contamination even under low-input conditions which include the use of drying platforms, drying outside the field, drying on mats among others (Hell *et al*., 2008). Other technological solutions like use of dryers, could aid in reducing grain moisture rapidly (Lutfy *et al*., 2008). However, these dryers are not used by most farmers because large capital

investments are needed to acquire them. Gummert *et al*. (2009) observed that use of dryers had a positive effect on maintaining rice quality and reducing mycotoxin risk.

2.6.5 Post harvest management

Aflatoxin contamination of foods increases with storage period (Kaaya and Kyamuhangire, 2006). It is compounded in by excessive heat, high humidity, lack of aeration in the stores, and insect and rodent damage resulting in the proliferation and spread of fungal spores.

Traditional storage methods are divided into two types, namely temporary storage that is mainly used to dry the crop and permanent storage that takes place in the field or on the farm. It is difficult to promote new storage technologies, such as the use of metal or cement bins, to small-scale farmers due to their high cost. Many farmers store their grains in bags, especially polypropylene which are not airtight, but there is evidence that this method facilitates fungal contamination and aflatoxin development (Hell *et al*., 2000; Udoh *et al*., 2000). Presently there are efforts to market improved hermetic storage bags in Africa, based on triple bagging developed for cowpea (Murdock *et al*., 1997) which has been or is being tested for other commodities (Ben *et al*., 2009).

Smoking is an efficient method of reducing moisture content and protecting maize against infestation by fungi. The efficacy of smoking in protecting against insect infestation was found to be high. About 4 to 12% of farmers in the various ecological zones in Nigeria used smoke to preserve their grains, and this practice was found to be correlated with lower aflatoxin levels in farmers' stores (Udoh *et al*., 2000). Other compounds used for seed fumigation like ethylene oxide and methyl bromide were found to significantly reduce the incidence of fungi including toxigenic species on stored groundnuts and melon seeds (Bankole

et al., 1996). Use of pesticides to control mycotoxins and their efficacy, have been reviewed by D'Mello *et al*. (1998), but their use by farmers is not always well practiced and deaths due to pesticide use have been reported.

Aflatoxin is unevenly distributed in a seed lot and may be concentrated in a very small percentage of the product (Whitaker, 2003). Sorting out of physically damaged and infected grains (known from colorations, odd shapes and size) from the intact commodity can result in 40-80% reduction in aflatoxins levels (Park, 2002; Fandohan *et al*., 2005). The advantage of this method is that it reduces toxin concentrations to safe levels without the production of toxin degradation products or any reduction in the nutritional value of the food and can done manually or by using electronic sorters.

Clearing the remains of previous harvests and destroying infested crop residues are basic sanitary measures that are also effective against storage deterioration. Cleaning of stores before loading in the new harvests was correlated to reduced aflatoxin levels (Hell *et al*., 2008). Separating heavily damaged ears i.e. those having greater than 10% ear damage also reduces aflatoxin levels in maize (Setamou *et al*., 1998). Wild hosts, which constitute a major source of infestation for storage pests, should be removed from the vicinity of stores (Hell *et al*., 2008).

Mycotoxins in contaminated commodities can also be reduced through food processing procedures that involve processes such as sorting, washing, wet and dry milling, grain cleaning, dehulling, roasting, baking, frying, nixtamalization and extrusion cooking. These methods and their impact on mycotoxin reduction have been reviewed by Fandohan *et al*. (2008). Dehulling maize grain can reduce aflatoxin contamination by 92% (Siwela *et al*.,

2005). The other strategies to reduce the risk of aflatoxin ingestion are dietary change which significantly reduces risk of liver cancer (Strosnider *et al*., 2006).

2.7 General description of superabsorbent polymers

According to the International Union of Pure and Applied Chemistry (IUPAC) Recommendations (2004), a superabsorbent polymer is a material that can absorb and retain extremely large amounts of a liquid relative to its own mass. The liquid absorbed can be water or an organic liquid.

Mass production of SAP hydrogels started at the end of the last century. In the beginning superabsorbent polymers were made from chemically modified starch and cellulose, and from other polymers like PVA (polivynyl alcohol) or PEO (polyethylene oxide). Nowadays they are made from partially neutralized, lightly cross-linked polyacrylic acid. SAPs are water swelling, but not water soluble (Elliot, 2004). They are characterized by an ability to absorb big amounts of water. Depending on the quality and chemical composition, SAP can retain up to 4000 times more pure water than its dry mass but water containing urine or metal ions the absorption capacity reduces by up to ten times (Glados and Maciejewski, 1998).

Water absorbing polymers are classified as hydrogels. When cross-linked they absorb aqueous solutions through hydrogen bonding with water molecules. A SAP's ability to absorb water is a factor of the ionic concentration of the aqueous solution (Raju *et al*., 2003). The total absorbency and swelling capacity are controlled by the type and degree of cross-linkers used to make the gel. Low density cross-linked SAPs generally have a higher absorbent capacity and swell to a larger degree. These types of SAPs also have a softer and stickier gel formation.

High cross-link density polymers exhibit lower absorbent capacity and swell, but the gel strength is firmer and can maintain particle shape even under modest pressure.

2.8 Desiccants used for drying maize

Desiccants have generally been used in the past for drying seed rather than grain. They are used to minimize reduction in seed longevity as a result of moisture accumulation and high temperatures associated with hot air drying. Different desiccants have been used. The use of silica gel for drying seed maize has been studied and concluded that using a silica gel:seed ratio of between 1:1 and 1:2.5 in sealed containers with the maize and gel in contact maximized the storage life extension of seed longevity (Daniel *et al*., 2009).

Aluminium silicates also called drying beads or "zeolites" were reported to have a higher affinity for moisture than silica gel and were found to swell by 20-25% of their original weight. Desiccant:seed ratios between 3:2 and 1:4 were used. It was shown that saturated drying beads may be dried and reused (Bradford *et al*., 2011).

Bentonite, a montmorillonite and type of common clay based desiccant, whose chemical composition is hydrated aluminium silicate with sodium and calcium as the common exchangeable cations has also been studied. A 1:1 corn to bentonite ratio was used and achieved drying to about 18% moisture content in less than 24 hours. Sturton *et al*., (1983) reported that bentonite swelled up to 20 times its original volume in water, developed drying equations for the swelling of the desiccant and showed that the bentonite could be blown off the grain, leaving an ash content of 1.7%.

The possibility of using charcoal and maize meal and previously dried seed to dry wet seed has been proven (KEW, 2010). A combination of desiccants bentonite and calcium chloride in

addition to solar drying reduced moisture content from 38% to 15% within 24 hours for 90 kg of maize (Thoruwa *et al*., 2007).

2.9 General applications of SAPs

The largest use of SAP is found in personal disposable hygiene products, such as baby diapers, protective underwear and sanitary napkins (Deyu, 2003). SAP is also used for blocking water penetration in underground power or communications cables and control of spill and waste aqueous fluid in waste management. In agriculture, Superabsorbent polymers have been used as soil additives to increase the water retention of soils which can replace peat, the traditional moisture retention aid for soil (Barbucci *et al*., 2000; Orzeszyna et al 2005). Miller (1979) suggested that the performance of superabsorbent polymers as a water retaining additive is greater in soils that are well drained such as sand. On top of that they have also been used for efficient utilization of water and fertilizers in plants (Miller, 1979).

CHAPTER THREE

3.0 Fungal contaminants and types of aflatoxins produced in the maize samples from Lower Eastern Region in Kenya

3.1 Abstract

Aflatoxin poisoning outbreaks have been reported in the lower eastern region of Kenya since 1978. These outbreaks have caused a lot of concern as some have led to loss of lives. This study was conducted to assess the fungal contaminants on maize in the region and more importantly the exposure to aflatoxicosis due to *Aspergillus spp* contamination. Seventy two (72) maize samples were obtained from farmers interviewed in the survey that was conducted in October 2012. The samples were obtained from different counties as follows; Kitui (24), Machakos (24) and Makueni (24). The samples were first assessed for general fungal contaminants and then for *Aspergillus* species contaminants. *Aspergillus* species were isolated using czapek Dox agar medium. The aflatoxin quantification was carried out using LC-MS analysis. *Fusarium*, *Aspergillus* and *Penicillium* were the predominant fungal species that were isolated and among the *Aspergillus* species, *A. niger* and *A. flavus* were the most predominant. Aflatoxin B1 (AFB1) was most predominant and was recorded to levels of 128.8ppb followed by aflatoxin B2 (AFB2) at 21.3ppb and aflatoxin G1 (AFG1) at 3.3ppb all being highest levels recorded in Makueni county. Aflatoxin G2 (AFG2) was never detected in all maize samples. Most samples (□70%) were safe for consumption as they had aflatoxin levels less than 10ng/g. this can be attributed to the >75% of farmers being aware of the mycotoxin problem and >60% know how to mitigate the problem. However, to some few farmers <20%, poor post harvest handling practices might be the main source of Aspergillus contamination which led to high level of aflatoxin in their maize samples as revealed in this study. Practices such as drying maize directly on the ground, using

plastic bags to store maize and not having simple and efficient technology to detect the maize dryness are some of the practices that create favorable conditions for Aspergillus contamination.

From the study it is clear that the government and other stakeholders have succeeded in awareness campaign which is far above 75%. Empowering of farmers with simple technologies to monitor the level of moisture content, dry their produce fast enough and store in proper way can be recommended as this will significantly contribute to eradication of this perennial problem in the region.

3.2 Introduction

The safety of food and feed has been a major concern in most countries in the last years as more knowledge is being generated on the occurrence of natural toxins in foodstuffs and edible plant species. Among these natural toxins are mycotoxins which are toxic metabolites of fungi contaminating food and feed, phytotoxins produced by algae contaminating fishery products and the plant toxins in edible plant species (Okoth and Kola, 2012). Among the three natural toxins named above, mycotoxins have been given more attention due to their hazardous effect both to animals as well as human beings (Miller, 1991).

Mycotoxins are toxic secondary metabolites produced by certain fungi in agricultural products that are susceptible to mould infestations (Bennett and Klich, 2003; Wagacha and Muthomi, 2008; Morenoa *et al*., 2009). The most important mycotoxins are aflatoxins, ochratoxins, deoxynivalenol, zearalenone, fumonisin and T-2 toxin. There are more than 300 known mycotoxins produced naturally of which aflatoxins is the most widely studied (Okoth and Kola, 2012).

Aflatoxins are natural metabolites produced by *Aspergillus* species of fungi primarily *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus* fungi are found in air, soil, seeds and plant debris and can contaminate maize, peanuts, peanut meal, cotton seed, cotton seed meal and beans. According to Nassir and Jolley (2002), aflatoxins are primarily associated with maize and maize products than any other crop.

There are more than 13 known different types of aflatoxins of which the most common ones produced in maize are AFB1, AFB2, AFG1 and AFG2. It is appreciated that aflatoxin contamination only becomes dangerous for human consumption after certain levels are

exceeded. These levels are normally specified for aflatoxin contamination of type B1 (AFB1) and total aflatoxin contamination. The most conservative levels are specified by the European Union as 2ng/g and 4ng/g for AFB1 and total aflatoxin respectively (Rahmani, *et al*., 2010). In Kenya, the acceptable level of AFB1 was initially 20 ng/g (Onsongo, *et al*., 2004) but has now been revised to 10ng/g for total aflatoxin (Muthomi, *et al*., 2012).

Aflatoxin poisoning outbreaks in Kenya have been reported over the years but the most severe outbreak reported in the history was in 2004 when 317 cases were reported with 125 deaths in Makueni, Kitui and neighboring districts (Lewis *et al*., 2005). Maize from the affected areas contained as much as 4,400ng/g aflatoxin B1, which is 440 times greater than the 10 ng/g tolerance level set by the Kenya Bureau of Standards (KEBS). It is believed that most of the aflatoxin poisoning outbreaks occurred in remote villages and, therefore, the number of people affected could have been higher than reported (Lewis *et al*., 2005).

The aflatoxin contamination in maize has been associated with drought combined with high temperature as well as insect injury (Betran and Isakeit, 2003). Genotype, soil types, drought and insect activity are important in determining the likelihood of pre-harvest contamination (Cole *et al*., 1995). Poor harvesting practices, improper storage and less than optimal conditions during transport and marketing can also contribute to fungal growth and production of mycotoxins (Bhat and Vasanthi, 2003; Wagacha and Muthomi, 2008).

The major factors that have been reported to contribute to aflatoxin production in maize include moisture content (Manoch *et al*., 1988), relative humidity and temperature in storage (Moreno and Kang, 1999) storage period (Liu and Yu, 2006) and storage types (Roy and Chourasia, 2001).

The objectives of this study were to assess the farmers' practices during post harvest handling that may lead to aflatoxin contaminations in the lower eastern region of Kenya. The study was also undertaken to determine the distribution of fungal contaminants with the main focus being *Aspergillus spp.* and associated aflatoxin contamination in the region.

3.3 Materials and methods

3.3.1 Evaluating farmers' post harvest practices that may lead to aflatoxin contamination

A survey was carried out using the open and closed questionnaire in lower eastern regions in Kenya where cases of aflatoxin contaminations on maize have been frequently reported. In open questionnaires farmers were asked questions and given chance to respond on their own words unlike in closed questionnaire where farmers were asked questions with already a set of answers provided to choose from. The region that was targeted for the survey included Machakos, Kitui and Makueni counties.

From each county two agro ecological zones were identified these being LM4 and LM5 whereby twelve farmers were randomly identified and interviewed by help of area agricultural officers with the assumption that all answers given by the farmer were correct and true to the best of his/her knowledge.

From each farmer one kilo of maize from the August / September harvest was purchased and packed in a paper bag then delivered to the laboratory at University of Nairobi in Upper Kabete Campus. Isolation of *Aspergillus* s*pp* was done from ten kernels from each sample in

three replicates and the remainder stored in 4 °C for further analysis. A total of 72 maize samples were collected twenty four from each county.

3.3.2 Isolation and identification of fungi

Aspergillus species were isolated from whole maize samples collected from farmers in the lower eastern region of Kenya. The grains were surface sterilized using 3% sodium hypochlorite then 30 kernels from each sample were plated on the growth medium. The medium was prepared using Czapek Dox medium (agar 20 g, sucrose 30 g, $NaNO_3$ 2 g, KCl 0.5 g, $MgSO_4.7H_2O$ 0.5 g, $FeSO_47H_2O_2$ 0.01 g, K_2HPO_4 1 g and distilled water 1000 ml) amended with 20 ppm of tetracycline, streptomycin, penicillin and pentachloronitrobenzene (PCNB).

The plates were incubated at room temperature (RTP) for up to fourteen days whereby the kernels showing the growth of various fungi were counted then those showing *Aspergillus* spp were separated from the rest for further identification. *Aspergillus* colonies were further sub-cultured in Czapek Dox agar medium for up to 14 days and identified to species level based on both cultural and morphological characteristics.

3.3.3 Analysis of aflatoxin in maize samples

One kilo maize from each sample was crushed to very fine powder/flour using a Retsch rotor mill (model SK 1, Germany). Ten gram of maize flour was weighed into 100mls falcon tubes. Forty milliliter of acetonitrile: water (84:16) was added and vortexed for 5min. Forty micro-liters of internal standard griesofulvin 5mg/ml was added to the mixture and vortexed for thirty minutes and left to settle for another thirty minutes. Six milliliters of the supernatant was drawn and filtered through multistep 228 aflapat column. Four milliliters of the filtrate was

evaporated to dryness in the hood. After evaporation, it was re-constituted in 400 micro-liters of methanol: water (20:80), vortexed for 5min, centrifuged at 10,000 rpm for 3 min and supernatant analyzed using LC-MS.

The LC-MS consisted of a quaternary LC pump (Model 1200) coupled to Agilent MSD 6120-Single quadruple MS with electrospray sourced from Palo Alto, CA. The system was controlled using ChemStation® software (Hewlett-Packard). Reversed-phase liquid chromatography was performed on an Agilent technology 1200 infinite series, Zorbax SB C_{18} column, 2.1 x 50 mm, 1.8 μm (Phenomenex, Torrance, CA).

The sample were dissolved in 100% B (MeOH) (LC-MS grade, Sigma, St. Louis, MO), vortexed and centrifuged at 10,000 rpm to remove insoluble material before analysis by LC-MS. The mobile phase used a gradient program initially 80:20 (A:B), [(A 5% formic acid in LC-MS grade ultra pure H_2O, Sigma, St. Louis, MO] to 0:100 at 10 min and maintained at this solvent proportion for 15min , 80:20 at 26 min to 30 min which was the run time. The flow rate was 0.7 ml min^{-1}. Injection volume was 10 μl and data was acquired in a full-scan positive-ion mode using a 100 to 800 *m/z* scan range. The dwell time for each ion was 50 ms. Other parameters of the mass spectrometer were as follows: capillary voltage, 3.0 kV; cone voltage, 70 V; extract voltage, 5 V; RF voltage, 0.5 V; source temperature, 110ºC; nitrogen gas temperature for desolvation, 380ºC; and nitrogen gas flow for desolvation, 400 L/h. Injection volume was 10 μL.

Authentic standards for B1, B2, G1 and G2 each of concentration 3ng/μl and an internal standard griseofulvin were purchased from Sigma-Aldrich (California, USA) and used to

construct calibration curves (Appendix 2.1 to 2.10). The aflatoxin content in the samples was determined by interpolating from the curves.

3.4 Data collection and Analysis

Data collection for the questionnaire and laboratory cultures was done as per appendix 1, 5 and 6 and analysis done for post harvest handling practices and fungal contaminants isolated from the maize using SPSS version 20 and Microsoft excel. Data collection LC-MS analysis was as per Appendix 2.14 to 2.19, interpretation as per Appendix 3 and Microsoft excel software was used. The mean aflatoxin levels were analysed using R statistical software at 95 degrees of confidence level.

3.5 Results

3.5.1 Farmers' maize handling practices

All the samples were homegrown but with mixed varieties as by the time of the study most farmers had just harvested their crop. Most respondents were owners of the farms and from the data analysis of the questionnaire it was revealed that most respondents were subsistence farmers (Table 3.1) and 33% practice both cash and subsistence farming. This explains the spread of aflatoxin within the local markets.

A number of farmers (40%) dry their maize directly on the ground (Table 3.2) a practice that may result to contamination with Aspergillus. Furthermore, it takes most farmers up to two weeks to dry their maize to 13% moisture content level required before storage (Table 3.3). Farmers use change of color, sound and others bite the maize kernels with their teeth to determine whether the maize is dry and ready for storage (Table 3.4).

The study also revealed that most farmers (50%) store their maize in plastic bags (Table 3.5). Plastic bags can retain moisture and accumulate heat which may promote Aspergillus growth and sporulation. Some farmer's (20%) store their maize in granaries and sisal bags which are well aerated and can minimize moisture and heat accumulation resulting to reduced aflatoxin contamination.

In terms of awareness, most farmers are already aware of the problems associated with aflatoxicosis (Table 3.6) and at same time they can rate the level or extent of the problem in their respective areas (Table 3.7). This is as a result of government and other stakeholders promoting aflatoxin awareness campaigns in the entire region. The farmers are also aware of the practice they can carry out to mitigate against the aflatoxin problem (Table 3.8). It is worthwhile to note that most farmers are not aware of the tolerant varieties and those claiming to be aware are not certain and specific on maize varieties that are tolerant or resistant to aflatoxin contamination (Table 3.9). The results show that some post harvest handling practices by some farmers expose them to aflatoxicosis although it is quite clear that most farmers from this region have done what it takes to avoid the aflotoxicosis problems.

Table 3.1: Purposes for which maize production is done in the lower eastern counties in Kenya

County	Cash crop	Subsistence	Both cash and subsistence	Total
Kitui	12.5	54.2	33.3	100
Machakos	8.3	55.2	36.5	100
Makueni	16.7	50	33.3	100

Table 3.2: Frequency of methods used by farmers to dry maize after harvest in the lower eastern counties of Kenya

County	Direct on the ground(A)	Use tarpaulins/sisal(B)	Both A and B	Total
Kitui	37.5	54.2	8.3	100
Machakos	41.7	41.7	16.6	100
Makueni	50.0	12.5	37.5	100

Table 3.3: Duration taken by farmers to dry maize after harvest in the lower eastern counties of Kenya

County	One week	Two weeks	Three weeks	> 3 weeks	Total
Kitui	45.8	41.7	8.3	4.2	100
Machakos	20.8	41.7	29.2	8.3	100
Makueni	16.6	55.3	19.8	8.3	100

Table 3.4: Frequency of methods used by farmers to determine the dryness of maize in the lower eastern counties of Kenya

County	Change of the sound (A)	Use teeth (B)	Change of color (C)	Both A and C	Total
Kitui	20.8	4.2	33.3	41.7	100
Machakos	12.5	8.3	50	29.2	100
Makueni	16.7	4.2	45.8	33.3	100

Table 3.5: Storage methods used by the farmers to store their maize after harvest in the lower eastern counties of Kenya

County	Granaries (A)	Polystyrene bags (B)	Sisal bags (C)	Plastic containers (D)	Both B, C & D	Totals
Kitui	16.7	8.3	12.5	12.5	50	100
Machakos	25.3	4.2	29.2	8.3	33	100
Makueni	29.2	4.2	12.5	8.3	45.8	100

Table 3.6: Awareness of contamination of maize by aflatoxin in the lower eastern counties in Kenya

County	Aware	Not aware	Totals
Kitui	79.2	20.8	100
Machakos	75	25	100
Makueni	100	0	100

Table 3.7: Seriousness of aflatoxin as perceived by farmers on the aflatoxin problems in the lower eastern counties in Kenya

County	Not serious	serious	Very serious	Not aware	Totals
Kitui	20.8	8.3	54.2	16.7	100
Machakos	33.3	16.7	37.5	12.5	100
Makueni	12.5	25	54.2	8.3	100

Table 3.8: Mitigation methods used by farmers in the lower eastern counties of Kenya to address aflatoxin problems.

County	Proper drying	Sorting	Proper storage	Totals
Kitui	75	8.3	16.7	100
Machakos	62.5	12.5	25	100
Makueni	58.3	16.7	25	100

Table 3.9: Farmers awareness on the existence of aflatoxin tolerant maize varieties in lower eastern counties in Kenya

County	Aware	Not aware	Totals
Kitui	16.7	83.3	100
Machakos	4.2	95.8	100
Makueni	8.3	91.7	100

3.5.2 Isolation of fungal contaminants from the maize samples

Figure 3.1 shows the types and frequency of occurrence of fungi found in the maize samples purchased from farmers. The fungal isolates identified were Fusarium as the most predominant followed by Aspergillus, Penicillium and Rhizopus in that order. A small number (□10%) of samples were clean without any fungal contaminants.

A. niger was found to be the predominant isolates in the Aspergillus species followed by *A. flavus, A. fumigatus and A. versicolor* in that order (Figure 3.2). From the results *A. niger* had an average of 50% and *A. flavus* 45% across the three counties. This was so despite the fact that the two counties Kitui and Makueni have more cases of afltoxin contamination than Machakos. This also shows that not all of samples with *A. flavus* results to aflatoxin contamination.

Plate 1 shows sample of kernels in petri dish during fungal culturing in the laboratory and plate 2 shows a sample of pure culture of *A. flavus* cultured in the laboratory while plate 3 shows the pure culture of *A. niger*.

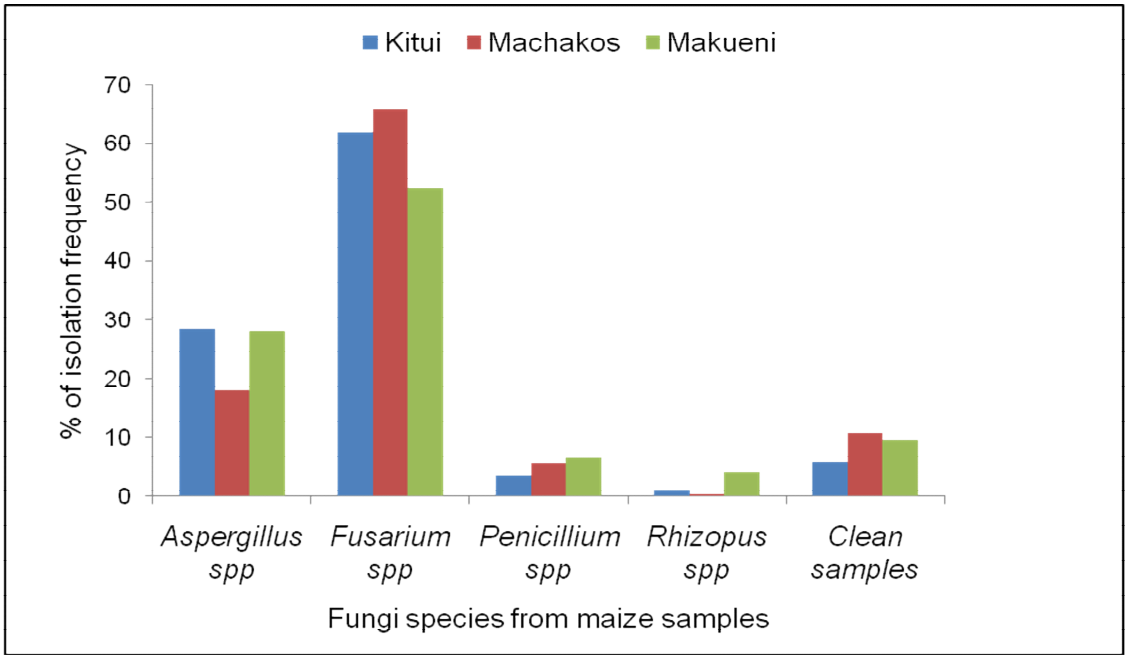


Figure 3.1: Percentage frequency of various fungi isolated from the maize samples collected from lower eastern counties in Kenya.

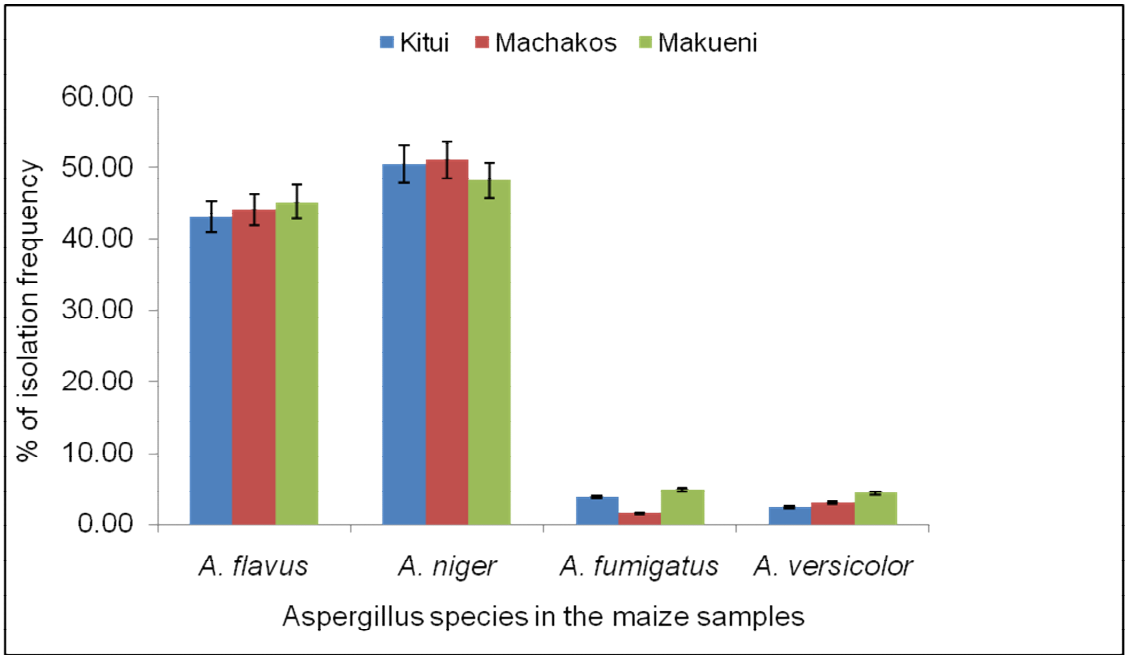


Figure 3.2: Percentage frequency of *Aspergillus species* isolated from maize samples from lower eastern counties in Kenya.

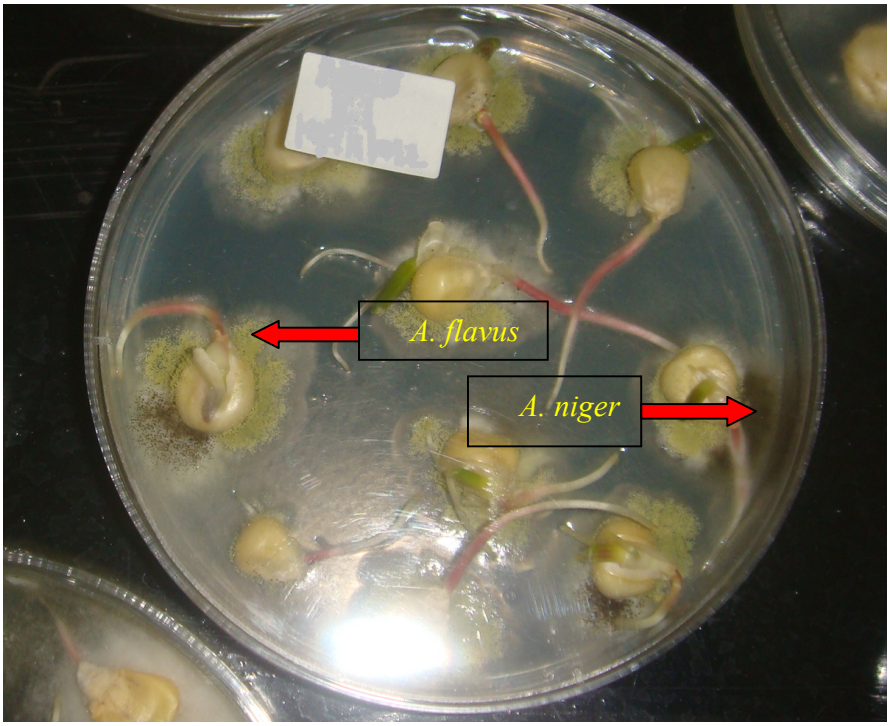


Plate 1: Growth of *A. flavus and A. niger* colonies from maize sample.

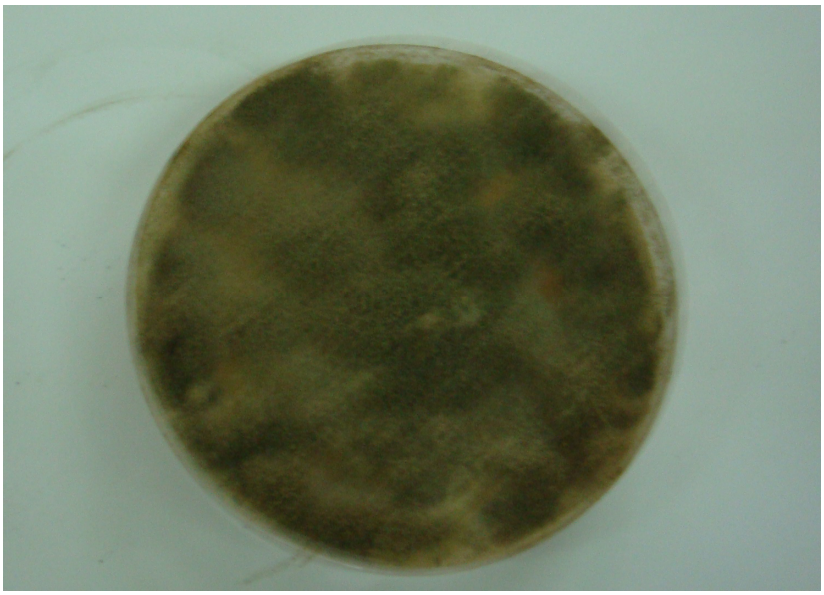


Plate 2: An isolation plate showing pure culture of *A. flavus*

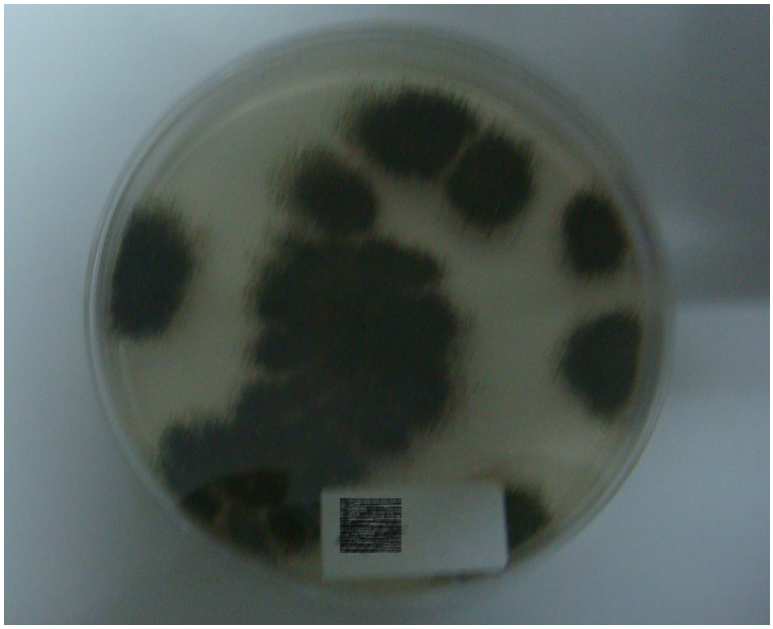


Plate 3: An isolation plate showing pure culture of *A. niger*

3.5.3 Aflatoxin content in the maize samples

Results from the analysis done revealed that high level of aflatoxin across the three counties were above the world health organization (WHO) recommended maximum limit of 20ppb and Kenya bureau of standard (KEBS) maximum limit of 10ppb (Figure 3.3 – 3.6). The aflatoxin levels ranged from 0 – 123ng/g in Kitui, 0 – 87ng/g in Machakos and 0 – 128ng/g in Makueni (Figure 3.6).

The result from Kitui shows that 29.2% (7 samples) had aflatoxin levels above 20ppb and 41.6% (10 samples) had aflatoxin levels above 10ppb as per WHO and KEBS recommendations. This leaves only 70.8% samples safe as per WHO and 58% samples safe for human consumption as per KEBS recommendations (Figure 3.6).

In Machakos, samples that had levels above 20 ppb were 12.5% (3 samples) and those above 10ppb were 25% (6 samples). This shows that 87.5% and 75% samples were safe for consumption as per WHO and KEBS recommendations respectively (Figure 3.6).

From Makueni, it shows that 29.2% (7 samples) were above 20 ppb and 37.5% (9 samples) were above 10 ppb. This indicates that 70.8% and 62.5% of samples were safe for human consumption according to WHO and KEBS recommendations respectively (Figure 3.6). The result from the three counties also indicates that AFB1 was predominantly high in all samples followed by AFB2 and AFG1. The analysis did not detect any levels of AFG1.

Table 3.10 to 3.12 shows the means of aflatoxin levels in all the three counties. It reveals that aflatoxin is highly variable at 95% confidence interval in the counties. The results from the tables further indicate that aflatoxin levels within and across the two agro ecological zones (LM4 and LM5) were highly significant. The variability may be due to different maize handling procedures that farmers within and across the agro ecological zones use during pre and post harvesting stages of their produce.

In summary the results from the three counties (Figure 3.8) indicates that total aflatoxin above 20 ppb was 23.6% and those above 10 ppb was 35% of total samples collected. This reveals that 76.6% and 65% of the total samples were safe for human consumption according to recommendations of maximum limits by WHO and KEBS respectively.

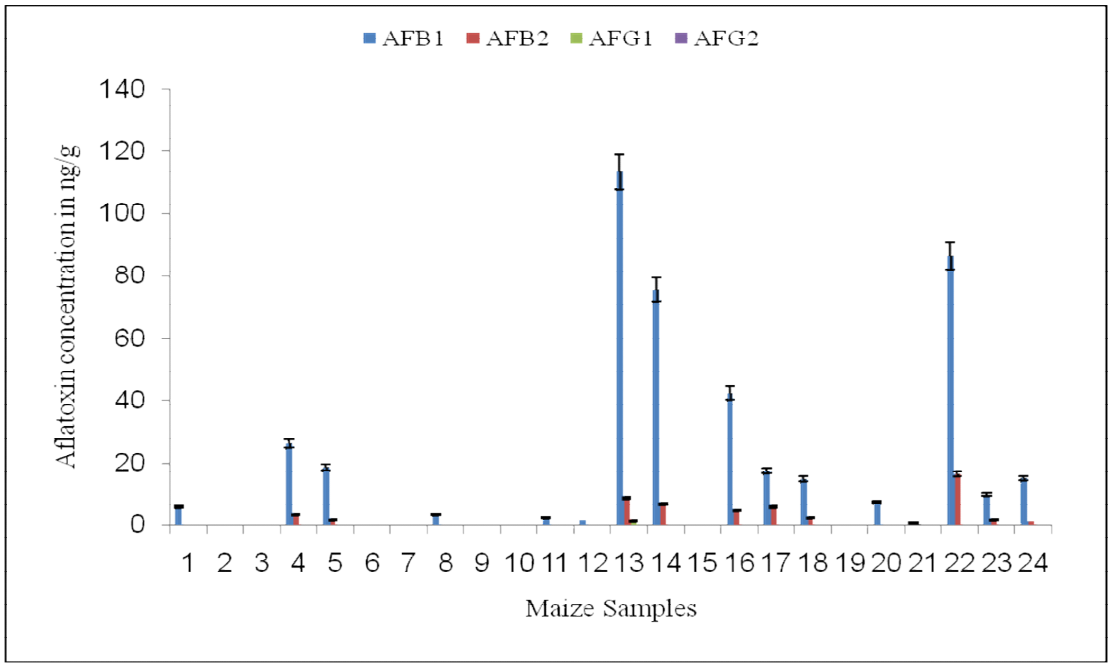


Figure 3.3: Concentration of aflatoxin in ng/g in maize samples collected from Kitui county of lower estern in Kenya.

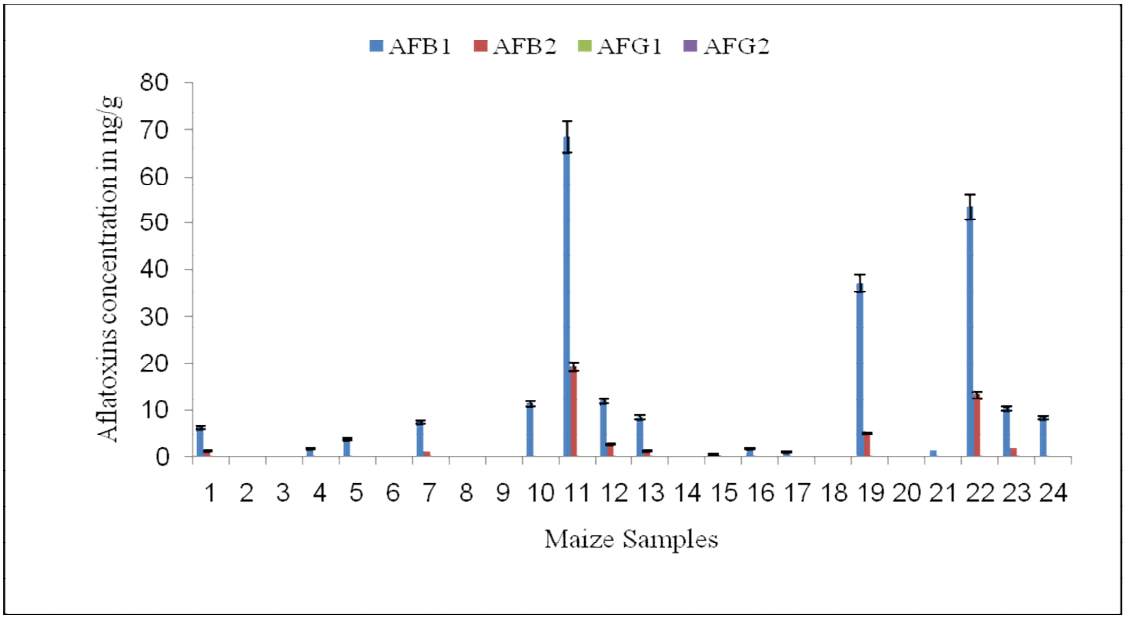


Figure 3.4: Concentration of aflatoxin in ng/g in maize samples collected from Machakos county of lower eastern in Kenya.

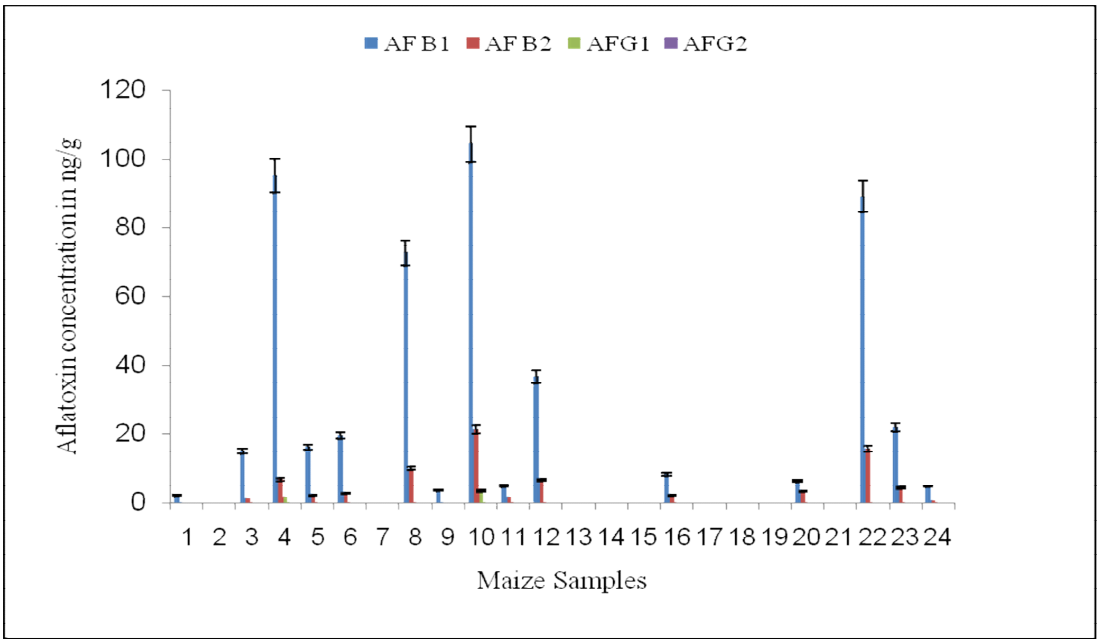


Figure 3.5: Concentration of various aflatoxins in ng/g in maize samples collected from Makueni county of lower eastern in Kenya.

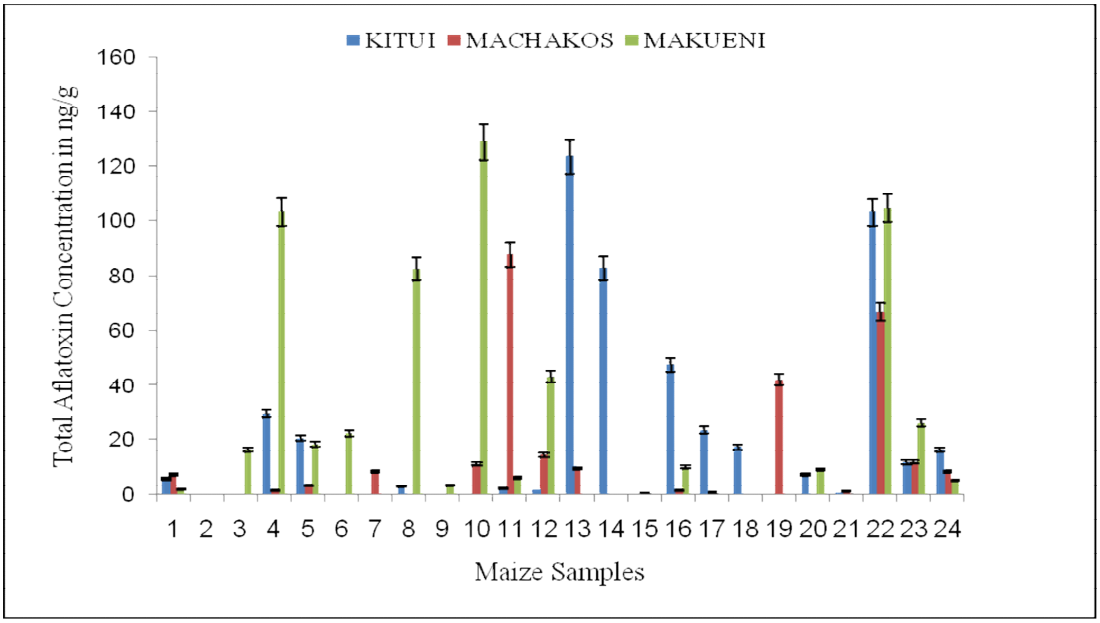


Figure 3.6: Total aflatoxin concentration in maize samples collected from lower eastern counties in Kenya.

Table 3.10: Mean aflatoxin levels in maize samples obtained from various farmers in two Agro ecological zones (LM4 and LM5) in Kitui County

Maize sample	Mean aflatoxin ± Se
Lower midland 4 (LM4)	
1	5.80±0.45 ej
2	0.00±0.00 a
3	0.00±0.00 a
4	29.72±0.38 b
5	20.60±1.16 cd
6	0.00±0.00 a
7	0.00±0.00 a
8	3.23±0.55 ae
9	0.00±0.00 a
10	0.00±0.00 a
11	2.32±0.28 ae
12	1.82±0.38 ae
Lower midland 5 (LM5)	
13	123.54±1.36 f
14	82.61±1.65 g
15	0.00±0.00 a
16	47.35±1.36 h
17	23.48±0.69 c
18	17.33±0.63 di
19	0.00±0.00 a
20	7.51±0.42 j
21	0.73±0.12 a
22	103.12±1.74 k
23	11.83±0.51 l
24	16.38±0.45 i

Means accompanied by similar letters are not significantly different (F_{23} = 2168, P<0.001, n=72)

Table 3.11: Mean aflatoxin levels in maize samples obtained from various farmers in two Agro ecological zones (LM4 and LM5) in Machakos County

Maize sample	Mean aflatoxin ± Se
Lower midland 4 (LM4)	
1	7.35±0.35 c
2	0.00±0.00 a
3	0.00±0.00 a
4	1.67±0.08 ab
5	3.55±0.31 b
6	0.00±0.00 a
7	8.34±0.23 cd
8	0.00±0.00 a
9	0.00±0.00 a
10	11.32±0.37 ef
11	87.65±0.37 g
12	14.56±0.43 h
Lower midland 5 (LM5)	
13	9.63±0.38 de
14	0.00±0.00 a
15	0.46±0.24 a
16	1.56±0.25 ab
17	0.89±0.12 a
18	0.00±0.00 a
19	42.07±0.80 i
20	0.00±0.00 a
21	1.25±0.09 ab
22	66.73±0.38 j
23	12.04±0.36 f
24	8.34±0.20 cd

Means accompanied by similar letters are not significantly different (F_{23} = 3298, P<0.001, n=72).

Table 3.12: Mean aflatoxin levels in maize samples obtained from various farmers in two Agro ecological zones (LM4 and LM5) in Makueni County.

Maize sample	Mean aflatoxin ± Se
Lower midland 4 (LM4)	
1	1.98±0.08 ag
2	0.00±0.00 a
3	16.67±0.56 b
4	103.23±3.24 c
5	18.18±0.55 b
6	22.22±0.66 d
7	0.00±0.00 a
8	82.44±0.34 e
9	3.45±0.19 afg
10	128.83±0.20 h
11	6.24±0.17 fij
12	43.50±0.47 k
Lower midland 5 (LM5)	
13	0.00±0.00 a
14	0.00±0.00 a
15	0.00±0.00 a
16	10.07±0.13 i
17	0.00±0.00 a
18	0.00±0.00 a
19	0.00±0.00 a
20	9.23±0.25 ij
21	0.00±0.00 a
22	104.79±0.76 c
23	26.05±0.40 d
24	5.26±0.29 gj

Means accompanied by similar letters are not significantly different (F_{23} = 2168, P<0.001, n=72).

3.6 Discussion

The study revealed that poor post harvest handling practices may be contributing to production of aflatoxin for most farmers. The methods used for drying, detecting whether the maize is dry, duration taken to dry the maize and storage are the practices that expose the maize to Aspergillus contamination. According to Wagacha and Muthomi (2008), there is high frequency of *A. flavus* isolation in soils from different agro ecological zones from the eastern region of Kenya. This indicates that farmers should avoid contact of their maize with soil during harvesting and drying to avoid contaminations.

Use of plastic bags for maize storage should also be avoided. Mwihia *et al*., (2008) noted that plastic bags accumulate moisture and heat that can promote aflatoxin contamination. Plastic bags are poorly aerated but cheaper and more readily available than the preferred sisal bags or improved granaries (Turner *et al*., 2005).

One of the optimum conditions for growth and subsequent production of *A. flavus* is moisture content of above 14% (Mutungi *et al*., 2008). Farmers in these regions have simple methods of determining the moisture content of their maize during and after storage (Muhia *et al*., 2008). They mainly use traditional methods of sound, change of color and use of teeth. This may result to storing of wet maize as maize cannot dry uniformly which may results to some pockets in the bags or some individual kernels creating favorable conditions for aflatoxin contamination.

The duration of storage has a significant effect on the levels of aflatoxin in the maize (Sumbali, 2001). Aflatoxin production depends upon storage conditions like; moisture content (Manoch *et al*., 1988), relative humidity and temperature (Moreno and Kang, 1999), storage period (Liu and Yu, 2006) and storage types (Roy and Chourasia, 2001). Rewetting of the stored maize

should be avoided as Hugh *et al* (1970) noted that rewetted maize had more contamination of *A. flavus* and other Aspergillus than freshly harvested maize under similar conditions. In this regard, farmers should make sure their storage facilities are water proof with no leakages from roof tops for those who store their maize in granaries and those who store them in their houses to put their maize on wooden raised pallets and not in contact with the ground to avoid rewetting as much as possible.

Most farmers were aware of aflatoxin problems in their respective areas. This can be attributed to the various public awareness campaigns run by Food and Agricultural Organization and the Ministry of Agriculture after the 2004 aflatoxin outbreak (Strosnider *et al*., 2006). Despite this, the levels of aflatoxin is still high and therefore empowering of farmers with simple technologies to monitor the level of moisture content, dry their produce fast enough and store in proper way can significantly contribute to eradication of this perennial problem in the region.

The maize samples were purely from home grown maize but as per the data (Table 1) quite a substantial number of farmers (□33%) grow maize for both cash and subsistence. This reveals how the aflatoxin spreads through the local markets. According to Okoth *et al* (2012), markets are the major source of chronic aflatoxin exposure to the larger population in Kenya.

The fungi that were isolated included Fusarium, Aspergillus, Penicillium and Rhizopus predominant in that order. Aspergillus species isolated were *A. niger, A. flavus, A. fumigatus and A. versicolor. A. niger* was more frequently isolated from the three counties than the rest of the Aspergillus species. No presence of *A. parasiticus* was isolated despite being closely related to *A. flavus*.

Aspergillus flavus and *Aspergillus parasiticus* are common and widespread moulds in nature and can grow on various substrates such as soil, agricultural products like maize, peanuts, beans, cotton seed, cotton seed meal among others, decaying vegetations including crop residues, hay and can also be found in air (Njapau *et al*., 1998 and WHO, 2002). The presence of *A. flavus* in some samples across the three counties is a concern as aflatoxins are primarily associated with maize infested with this species of Aspergillus.

Aflatoxin contaminations can occur when the crop is in the field or during harvest, drying and storage. Most contamination occurs during post harvest stage in case the produce is not handled properly to reduce chances of *Aspergillus* contamination (Yadgiri *et al*., 1970 and Wilson *et al*., 1992). From this study it is assumed that most of the contaminations by *A. flavus* might have occurred during the maize handling practices from harvest to storage as such practices like drying maize on the direct ground, storing on plastic bags, maize taking long to dry are just but among the practices that might have created conducive environment for Aspergillus contamination.

Aflatoxin B1, B2 and G1 were detected in maize samples from Kitui and Makueni but only aflatoxin B1 and B2 were detected in maize samples from Machakos County. The presence of aflatoxin B1 in some samples above 10ng/g is of concern as it is considered to be the most toxic to human and even livestock. Cotty and Cardwell (1999) reported presence of aflatoxin B1 and B2 in the presence of *A. flavus* but Saleem *et al*. (2012) reported aflatoxins B1, B2, G1, and G2 in the presence of *A. flavus*. Although *A. parasticus* was not reported, aflatoxin G1 was detected. Aflatoxin G1 and G2 is the general indicators of *A. parasiticus* (Egel *et al*., 1994). Detection of G1 proves the association of these aflatoxins with *A. flavus*.

Probst *et al*. (2010), determined aflatoxin content in maize from eastern province of Kenya and found 41% of samples were below 20ng/g. Results from this study indicates change of trend as overall in the region, 76.6% of the samples had aflatoxin below 20ng/g while 65% had aflatoxin levels below 10ng/g. These are as per recommendations by WHO and KEBS, respectively. Machakos was least affected with 87.5 % and 75 %, followed by Makueni 70.8% and 62.5% then lastly Kitui with 70.8% and 58% aflatoxin levels below 20ng/g and 10ng/g, respectively. This drop in terms of exposure can be attributed to the various campaigns that have been carried out in the region and this has created awareness to farmers about the problem and mitigation measures.

3.7 Conclusions

Despite the public awareness in the region, aflatoxin contamination still remains a threat to maize consumers from the region which is evident by high levels of aflatoxin in their maize above the maximum limits put by both WHO and KEBS of 20ng/g and 10ng/g respectively.

The study reveals that most of the aflatoxin contaminations are as a result of poor handling practices of maize during and after harvest. Most population in the country is peasant farmers and entirely dependent on nature as they cannot afford modern facilities for drying their maize and even detecting when the maize is dry.

Aflatoxin B1 was predominantly high among the maize samples and exposure to these especially on small amounts over a long period can lead to chronic aflatoxicosis. This is true in cases where contaminated samples from individual farmers end up in the market where they are mixed with others and sold to the larger population.

It is also worthwhile to note that most of the aflatoxin exposure is from homegrown maize from individual farmers within the counties. This is true as maize from individual farmers analyzed in this study had a higher total aflatoxin content of up to 128 ppb and because most farmers offload the excess produce to the market when they get bumper harvest. Most of the maize that comes from outside the counties especially in form of food aid is usually tested before it gets to the target population.

3.8 Recommendations

Post harvest handling practices that will discourage fungal growth and subsequent mycotoxin contamination should be encouraged in the region. This will include post harvest practices that will improve the drying process and storage conditions.

New simple and farmer friendly technologies should be developed to help farmers dry their maize, determine when the maize is dry and store their produce without exposing them to fungal contaminations.

New varieties tolerant or resistant to *Aspergillus flavus* contamination should be developed for farmers in this region to curb this perennial aflatoxicosis problem.

CHAPTER FOUR

4.0 Effectiveness of super absorbent polymers in the control of maize contamination with aflatoxin

4.1 Abstract

Aflatoxin contamination of maize occurs in the field or during harvesting, drying and storage. Most of the contamination occurs during the post harvest handling unlike during pre harvest periods. Aflatoxin contamination is caused by *Aspergillus species* mainly *Aspergillus flavus and Aspergillus parasticus*. The favorable conditions that favor the growth, sporulation and spread of these fungi are high temperature, moisture content of maize above 14%, duration of maize storage and storage types of the maize. The aim of the study was to develop simple and efficient technology which can be used by farmers to dry their maize fast enough to the recommended moisture content of 13% before storage and maintain the same thereafter. Various super absorbent polymers were selected on basis of their drying capacity, drying rate, form of the SAP, whether it is food grade, strength before and after adsorption of water, dewatering characteristics and number of wetting and drying cycles before loss of absorbency. On basis of the above poly acrylic acid (pa) which is sodium salt granules was selected and tested in this study. The effectiveness of the use of superabsorbent polymers (SAP) as desiccants in drying maize and control of aflatoxin contamination was studied by mixing different ratios of SAP: maize in sealed containers in which the moisture content (MC) and the aflatoxin levels were monitored. The experiments were carried out at different temperatures and frequency of change of hydrogel. The levels of different types of aflatoxin and total aflatoxin for each treatment were determined by LC-MS analysis. It was found that between the ratios of SAP: maize of 1:1 up to 1:5, there was little or no aflatoxin contamination after

drying to 13% moisture content (MC). However, for the ratios 1:10 and 1:20, the aflatoxin contamination depended on temperature and duration taken to reach the final moisture content. It was noted that ambient temperature, frequency of change of SAP, duration of drying and the ratio of SAP: maize influenced the drying rate and the aflatoxin contamination. From this study, it is clear that super absorbent polymers can be used to dry maize fast enough to avoid aflatoxin contamination in maize. It was revealed that the ratio of SAP used to dry the maize to that of maize has a significant effect both to rate of maize drying and overall contamination of maize with aflatoxin. Therefore use of SAPs can be recommended as a viable alternative method for drying maize to avoid aflatoxin contamination.

4.2 Introduction

Superabsorbent polymers (SAPs), also called hydrogels and super porous hydrogels (SPH) are known for their ability to absorb large amounts of water. One gram of hydrogel may absorb more than 4000 g of water in 200 minutes, with half of this water being absorbed within the first 12 minutes (Karuwita, 2008). SAPs were first used in agriculture for drainage but have now found use in health products such as baby diapers and sanitary pads (Delgado *et al*., 2009). Due to their moisture absorbing capacity, it was hypothesized that they may serve as a possible solution to aflatoxin contamination, operating as drying agents for maize during the post-harvest (storage and drying) stage.

Such qualities as high water absorption capacity qualify SAPs as desiccants that can be used in grain drying although they have never been tried before. According to Deyu (2003), there would be distinct advantages in using SAPs to dry both seed (for planting) and grain (for human consumption) after harvest.

The swelling rate and swelling capacity of the SAPs have value in aflatoxin control in the sense that slow or restricted absorption is likely to result in *Aspergillus* growth and sporulation and eventually aflatoxin contamination in wet maize. Hence the presence of aflatoxin contamination would indicate ineffective drying rate of the maize or slow and poor absorption of moisture by the polymer (Deyu, 2003; Saleem, 2012).

One other important property that applies to grain for consumption is whether the SAP is food grade or not. The SAP selected for grain drying would have to comply with food safety requirements if it is to be allowed for use with commodities for human consumption. There are many materials from which SAPs are made of. However, the most commonly available

SAP has been identified as sodium polyacrylate. It is currently used in the manufacture of SAPs which are listed as food grade such as the BASF products Luquafleece® and Luquasorb® that are applied in fish and meat packaging containers (Elliot, 2004).

SAPs are fairly easy to manufacture from local materials and may also be made from natural products even in developing countries. For instance, superabsorbent hydrogel has been processed from native cassava starch-Poly [sodium acrylate-co-acrylamide], by alkaline hydrolysis of starch/PAN physical mixture. This is because graft copolymerization of vinyl monomers onto natural polymers is an efficient approach to achieve biopolymer-based super absorbing hydrogels. SAPs have exceptional properties such as biocompatibility, biodegradability, renewability, and non-toxicity which are desirable properties for maize drying (Ekebafel *et al*., 2011).

The findings of different researchers show that the use of desiccant for drying both seed and grain is highly promising and needs to be studied further and perfected (Glados *et al*., 1998). Compared with the other desiccants, SAPs have a higher absorption capacity rate, can be re-used many times as well as other properties that make them suitable for use as desiccants for drying maize (Ekebafel *et al*., 2011). It is for this reason that SAPs were studied in this project. However, the project investigated not just the drying but also sought to establish the drying regimes that would minimize aflatoxin contamination.

4.3 Materials and methods

4.3.1 Description of SAP used in drying experiments

The super absorbent polymer (SAP) used in the drying and Aflatoxin tests was poly acrylic acid (PA) which is mainly sodium salt lightly cross-linked. It was a powder of particle size 99% < 1,000 μm purchased from Sigma Aldrich, product code: 43,636-4. Since the SAP forms a sticky gel and adheres to the maize when it absorbs moisture, the SAP was enclosed in a porous tea bag membrane for all the experiments.

4.3.2 Determination of the progression of *Aspergillus* contamination and the effect of SAPs in its control

A fresh sample of maize at a moisture content of 32.5% (approximate moisture content of maize at harvest) that had been confirmed not to be contaminated with *Aspergillus* was shelled manually and weighed into 100g and put into a hermetic glass bottle. The grain was mixed with SAP in tea bags following SAP to Maize ratios: 1:1, 1:5, 1:10 and 1:20. There were three replicates for each ratio. In each sample, the quantity of maize was set at 100g while the quantities of SAP used were 100, 20, 10, and 5g to match the required SAP to Maize ratios. The SAP was placed in 5g tea bags and the maize spread evenly around. The mixtures of SAP and maize were placed in ovens at 20°C, 30°C and 40°C until the experiments were stopped after 216 hours. Other than 1:1 ratio, the effect of SAP on drying and aflatoxin contamination was further tested by varying the intervals at which the SAP was changed as follows; no change of SAP throughout the experimental period, change of SAP after every 48 hours and change of SAP after every 24 hours.

The maize samples were ground using a Retsch rotor mill (model SK 1, Germany). Samples were then drawn at the end of the experiment for quantification of *Aspergillus* metabolites. Liquid Chromatography Mass Spectrometry (LC-MS) was used for quantification of *Aspegillus* metabolites.

4.3.3 Extraction, purification and LC–MS Analysis

The aflatoxin extraction, purification and LC- MS analysis of maize samples was done as explained in section 3.3.3.

4.3.4 Quantification of aflatoxins

Authentic standards for B1, B2, G1, and G2 each of concentration 3ng/µl were purchased from Sigma-Aldrich (California, USA). Various concentrations of aflatoxin (3ng/µl, 30ng/µl, 60ng/µl and 90ng/µl) were prepared and used to generate the standard curves (Appendix 2.6 to 2.9) which allowed for external quantification of each targeted aflatoxin. An example of a calibration curve is given in Appendices 2.1 to 2.4.

An internal standard griseofulvin was used to calculate the response factor. An internal standard is a known amount of compound different from the analyte that is added to the unknown. The signal from the analyte is compared with signal from the internal standard to find out how much analyte is present. The internal standard is desirable when sample losses occur during sample preparation steps prior to analysis thus it is used to correct errors. A known quantity of internal standard was added to the unknown prior to analysis, the ratio of the standard analyte remains constant as a similar fraction of each is lost in any operation. The internal standard calibration

curve was developed by preparing various concentrations: 20, 40, 60, 80 and 100 ng/µL each concentration analyzed in three replicates (Appendices 2.5 and 2.10).

4.4 Data collection and analysis

ChemStation® software (Hewlett-Packard) was used for LC-MS analysis and data acquisition (Appendix 4.8). Data was generated in three dimensional, one as total ion current (TIC) chromatogram a plot which displays on the y-axis summed signal intensity and the x-axis retention time in minutes across the entire range of masses being detected at every point in the analysis. In addition to signal strength, it generates mass spectral data that can provide valuable information about the molecular weight, structure, identity, quantity, and purity of a sample. Mass spectral data adds specificity that increases confidence in the results of both qualitative and quantitative analyses. The aflatoxin in the maize samples were extracted, identified and quantified based on their eluting time and mass fragmentation pattern related to the authentic standards analyzed.

Peak areas of the excitation curves such as those in (Appendices 2.11 to 2.13) were used for quantification. Using the calibration curves the area obtained was converted to the concentration of aflatoxin contamination in ng/µl and later converted to ng/g (Appendix 3). The results were compared with the standard recommended levels of aflatoxin in maize for human and livestock consumption. The mean aflatoxin levels were analysed using R statistical software at 95 degrees confidence level.

4.4 Results

The results showed that aflatoxin was detected in all maize samples except where the ratio of maize to super absorbent polymers used was high and that is 1:1 ratio across all experiments

(Table 4.1, 4.2 and 4.3). In this particular ratio it took less than 48 hours to dry maize from 32.5% moisture content to less than 13% moisture content which is recommended before the maize can be stored for future use. From the results as shown on tables (Table 4.1-4.3) it is clear that aflatoxin levels were high in all the control experiments than all other treatments. The total aflatoxin content of 229.72ng/g was recorded in the control experiment compared to 170.83ppb which was second highest. Maize samples in the control never dried but the moisture content rose slightly to 33% and maintained that level until the experiment was terminated after 216 hours. This result of the control indicated that no drying took place and it yielded the most aflatoxin contamination for all the temperature levels after the 216 hours duration of the experiment.

In all treatments where the SAPs were not changed during the drying process, all samples had a higher aflatoxin levels and although some drying took place, no samples dried to the recommended moisture content of 13% after 216 hours. This trend changed when the super absorbent polymers were changed after both 24 and 48 hours.

Table 4.4 to 4.6 shows analysis of aflatoxin within and across each experiment. It shows that there were high significant differences on the mean level of aflatoxin content in all experiments where no change of superabsorbent polymers as compared where the polymers were changed after every 24 and 48 hours.

Table 4.7 shows comparison of the mean aflatoxin levels across all experiments where SAP to Maize ratio of 1:10 and 1:20 was used. The results shows that there was high variability across all ratios where there was no change of hydrogel and where frequency of change of hydrogel was 24 and 48 hours there were no much variability. This means that ratio 1:10 and 1:20 can be used alternatively.

Table 4.1: Aflatoxin levels, final moisture content and time taken to dry the maize samples in the oven at a temperature of 40°C at various ratios of maize to super absorbent polymers

SAP: Maize	AFB1(ng/g)	AFB2 (ng/g)	AFG1 (ng/g)	AFG2 (ng/g)	Total AF(ng/g)	Final MC(%)	Time to final MC(Hrs)
Control	162.28	39.19	21.96	6.29	229.72	33	216
No change of super absorbent polymers							
Ratio 1:1	0	0	0	0	0	12.37	48
Ratio 1:5	12.59	14.11	5.93	0.54	33.17	17.97	216
Ratio1:10	109.96	35.2	10.93	0.87	156.96	22.23	216
Ratio1:20	120.68	37.07	12.19	0.89	170.83	25.50	216
Change of super absorbent polymers every 48 hours							
Ratio 1:5	0.92	0	1.22	0	2.14	12.8	144
Ratio 1:10	7.97	7.83	2.91	0	18.71	12.63	168
Ratio 1:20	11.27	11.84	3.47	0	26.58	13	192
Change of super absorbent polymers every 24 hours							
Ratio 1:5	0	0	0	0	0	12.7	120
Ratio 1:10	6.43	6.57	1.48	0	14.48	12.2	144
Ration1:20	8.7	7.6	3.05	0	19.35	12.67	192

Table 4.2: Aflatoxin levels, final moisture content and time taken to dry the maize samples in the oven at a temperature of 30°C at various ratios of maize to super absorbent polymers

SAP: Maize	AFB1(ng/g)	AFB2 (ng/g)	AFG1 (ng/g)	AFG2 (ng/g)	Total AF(ng/g)	Final MC(%)	Time to final MC(Hrs)
Control	134.58	45.19	18.7	11.46	209.93	33	216
No change of super absorbent polymers							
Ratio1:1	0	0	0	0	0	12.93	48
Ratio1:5	6.53	5.52	6.16	0	18.21	19.10	216
Ratio1:10	97.96	11.08	12.74	0.47	122.25	23.40	216
Ratio1:20	118.16	16.37	14.28	0.65	149.46	26.07	216
Change of super absorbent polymers every 48 hours							
Ratio 1:5	0.68	0	1.2	0	1.88	12.83	120
Ratio1:10	6.88	0	2.05	0	8.93	13.00	168
Ratio1:20	7.13	0	3.13	0	10.26	13.17	192
Change of super absorbent polymers every 24 hours							
Ratio 1:5	0	0	0	0	0	12.67	120
Ratio1:10	4.09	4.35	2.13	0	10.57	13.03	180
Ratio1:20	4.13	4.09	2.89	0	11.11	13.13	192

Table 4.3: Aflatoxin levels, final moisture content and time taken to dry the maize samples in the oven at a temperature of 20°C at various ratios of maize to super absorbent polymers

SAP: Maize	AFB1(ng/g)	AFB2 (ng/g)	AFG1 (ng/g)	AFG2 (ng/g)	Total AF(ng/g)	Final MC(%)	Time to final MC(Hrs)
Control	61.66	22.29	14.99	0	98.94	33	216
No change of super absorbent polymers							
Ratio1:1	0	0	0	0	0.00	12.75	72
Ratio1:5	5.92	0	0	0	5.92	18.33	216
Ratio1:10	46.94	6.48	7.01	0	60.43	22.40	216
Ratio1:20	52.99	9.39	8.94	0	71.32	26.07	216
Change of super absorbent polymers every 48 hours							
Ratio 1:5	0.14	0	0	0	0.14	12.83	120
Ratio1:10	2.59	0	0.66	0	3.25	12.83	192
Ratio1:20	3.65	0	1.21	0	4.86	12.97	216
Change of super absorbent polymers every 24 hours							
Ratio 1:5	0	0	0	0	0.00	12.90	120
Ratio1:10	0.71	0	1.45	0	2.16	12.97	192
Ratio1:20	1.57	0	1.70	0	3.30	12.97	216

Table 4.4: Mean aflatoxin levels in maize samples dried at 20°C using various ratios of super absorbent polymers to maize.

SAP: Maize Ratio	Mean aflatoxin ± Se
No change of super absorbent polymers	
1:1	0.00±0.00 c
1:5	5.92±0.54 f
1:10	60.43±1.02 a
1:20	71.32±1.09 e
control	98.94±0.57 g
Change of super absorbent polymers every 24 hours	
1:5	0.00±0.00 c
1:10	2.16±0.16 bc
1:20	3.30±0.20 bf
Change of super absorbent polymers every 48 hours	
1:5	0.14±0.01 c
1:10	0.20±0.20 bd
1:20	4.86±0.24 df

Means accompanied by similar letters are not significantly different (F_{10} = 4715, P<0.001, n=33).

Table 4.5: Mean aflatoxin levels in maize samples dried at 30°C using various ratios of super absorbent polymers to maize.

SAP: Maize Ratio	Mean aflatoxin ± Se
No change of super absorbent polymers	
1:1	0.00±0.00 e
1:5	18.21±0.18 b
1:10	122.25±2.64 a
1:20	149.46±1.57 c
control	209.93±5.55 d
Change of super absorbent polymers every 24 hours	
1:5	12.67±0.76 b
1:10	13.03±0.65 b
1:20	13.13±0.69 b
Change of super absorbent polymers every 48 hours	
1:5	12.83±0.53 b
1:10	13.00±0.54 b
1:20	13.17±0.40 b

Means accompanied by similar letters are not significantly different (F_{10} = 1367, P<0.001, n=33)

Table 4.6: Mean aflatoxin levels in maize samples dried at 40°C using various ratios of super absorbent polymers to maize.

SAP: Maize Ratio	Mean aflatoxin ± Se
No change of super absorbent polymers	
1:1	0.00±0.00 f
1:5	33.17±0.97 e
1:10	156.96±2.04 a
1:20	170.83±0.64 c
control	229.72±2.74 g
Change of super absorbent polymers every 24 hours	
1:5	0.00±0.00 f
1:10	14.48±0.43 b
1:20	19.35±0.40 b
Change of super absorbent polymers every 48 hours	
1:5	2.14±0.10 f
1:10	18.71±0.44 b
1:20	26.58±0.31 d

Means accompanied by similar letters are not significantly different (F_{10} = 5499, P<0.001, n=33)

Table 4.7: Comparison of the performance of super absorbent polymers ratios 1:10 and 1:20 in aflatoxin contamination at different temperatures.

Temperature	SAP:Maize Ratio	Mean aflatoxin ± Se
No change of Super absorbent polymers		
40°C	1:10	156.96 ± 2.04 j
40°C	1:20	170.83 ± 0.64 f
30°C	1:10	122.25±2.64 c
30°C	1:20	149.46±1.57 h
20°C	1:10	60.43±1.77 d
20°C	1:20	71.32±1.09 i
Change of super absorbent polymers every 24 hours		
40°C	1:10	14.48±0.43 ab
40°C	1:20	18.96±0.43 a
30°C	1:10	12.27±0.65 b
30°C	1:20	13.13±0.69 b
20°C	1:10	2.16±0.16 e
20°C	1:20	3.30±0.20 e
Change of super absorbent polymers every 48 hours		
40°C	1:10	18.71±0.43 a
40°C	1:20	26.58±0.31 g
30°C	1:10	13.0.31±0.31 b
30°C	1:20	13.17±0.40 b
20°C	1:10	3.25±0.20 e
20°C	1:20	4.86±0.20 e

Means accompanied by similar letters are not significantly different ($F18 = 3,214$, $P<0.001$, $n=54$)

When a ratio of 1:5 of SAP: Maize was used, very little or no aflatoxin contamination was detected in all cases, except for the case when the SAP was not changed through the full duration of the experiment and a value of 17.97ng/g was detected for total aflatoxin concentration. This was within the world health organization (WHO) standard of 20ng/g but above the Kenyan standard (10ng/g) and the European Union (EU) standard (4ng/g), respectively. However, when the SAP was changed every 24 or 48 hours, the value was less than 4ng/g. In all the cases, when the SAP was changed every 24 hours, no aflatoxin was recorded at the ratio 1:5. Both ratios of 1:10 and 1:20 (SAP: Maize) recorded high levels of aflatoxin contamination. However, these ratios met the Kenyan standard at 20°C and when SAP was changed every 48 and 24 hours.

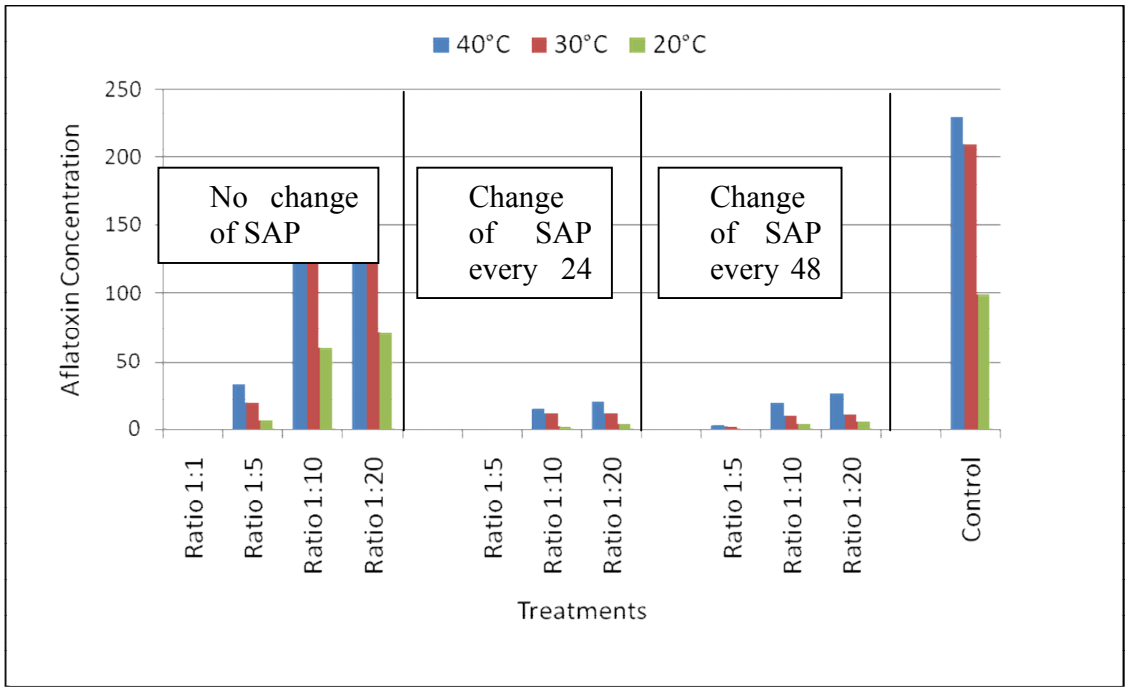


Figure 4.1: Total aflatoxin content in all maize samples dried using different ratios of super absorbent polymers to Maize at diffent temperatures.

From the above graph (Figure 4.1), the study reveals that aflatoxin content was higher at 40°C and 30°C compared to 20°C across all treatments. Control had higher aflatoxin content than all other treatments followed by 40°C, 30°C and 20°C in that order. In cases where super absorbent polymers were changed both in 24 and 48 hours respectively there was a significant drop in aflatoxin concetration implying that other than using higher ratio of 1:1 to dry the maize, lower ratios of up to 1:20 can be used and attain same results of drying maize with little aflatoxin concentration. From the graphs (Figure 4.2, 4.3 and 4.4) it was revealed that all types of aflatoxin were detected in all temperature regimes. Aflatoxin B1 was the highest followed by B2, G1 and G2 in that order.

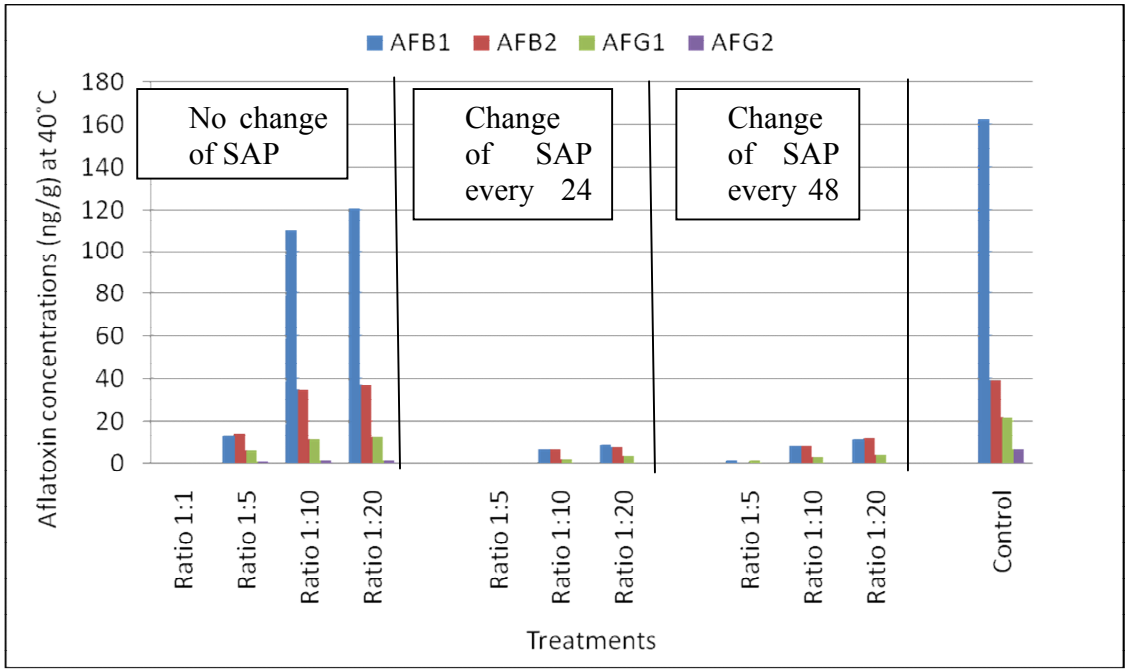


Figure 4.2: Aflatoxin content in maize samples dried using different ratios of super absorbent polymers to Maize at 40°C

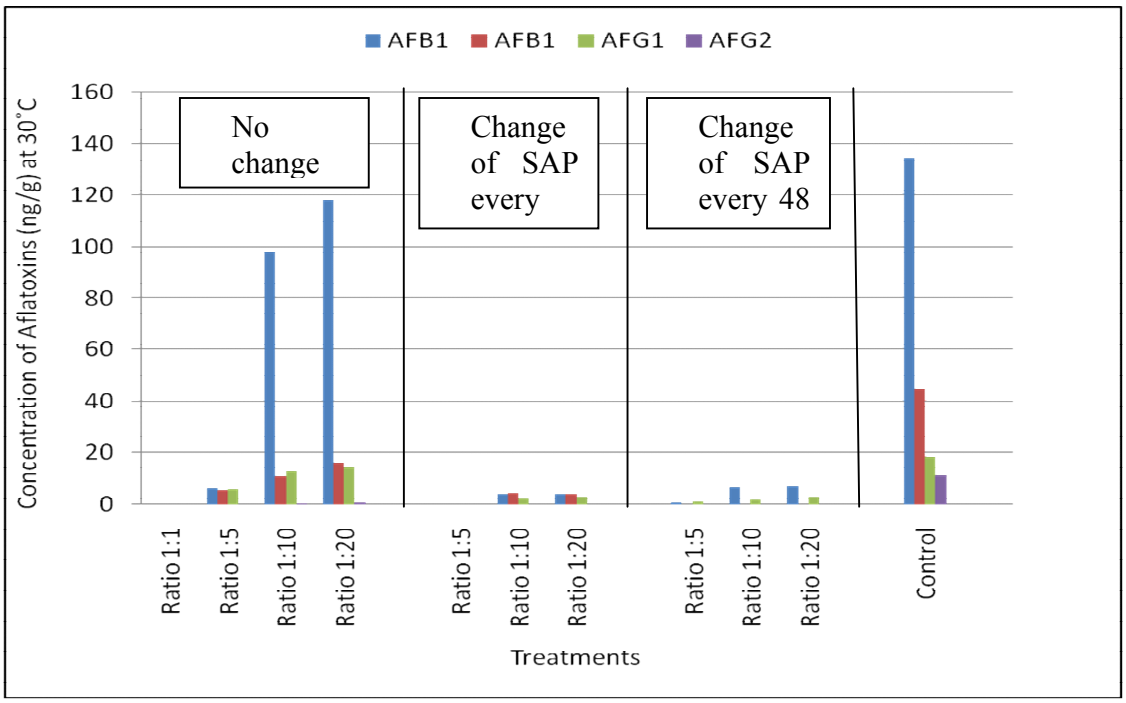


Figure 4.3: Aflatoxin content in maize samples dried using different ratios of super absorbent polymers to Maize at 30°C

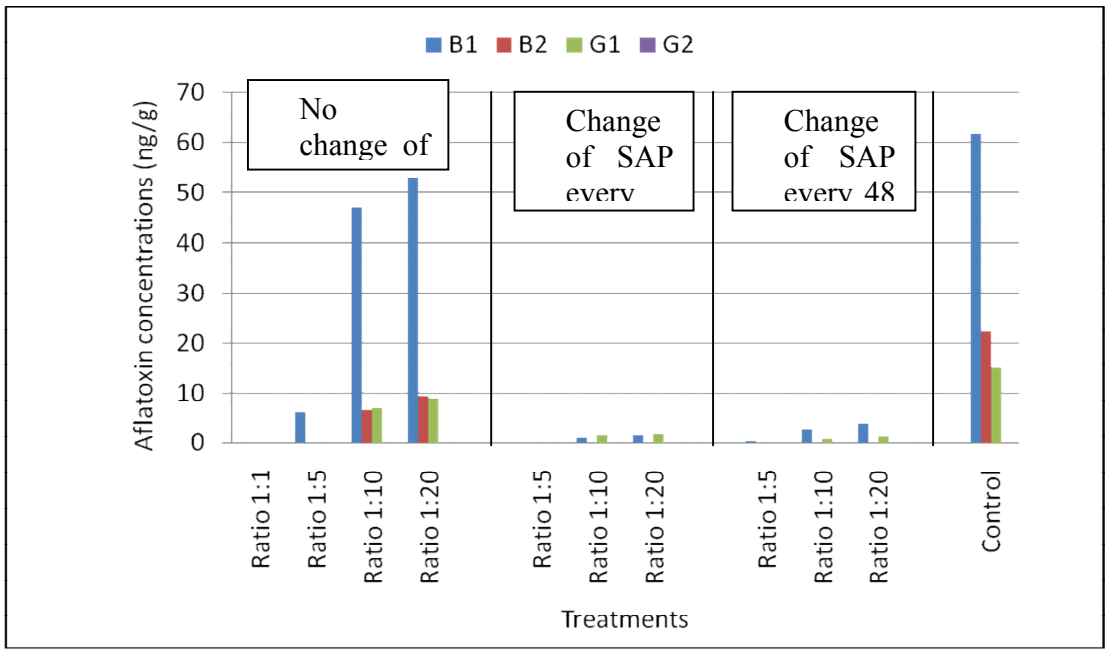


Figure 4.4: Aflatoxin content in maize samples dried using different ratios of super absorbent polymers to Maize at 20°C

The EU and Kenyan standard for aflatoxin contamination were superimposed on the curve to determine how much drying time would be sufficient to prevent aflatoxin contamination. It was determined that the increase in total aflatoxin contamination is exponential over time as evidenced by the equations for each curve and the corresponding values of R^2 that were relatively high. From Figure 4.5, it can be deduced that drying should be accomplished within 160, 170 and 220 hours at 40°C, 30°C and 20°C, respectively to prevent aflatoxin contamination beyond the prescribed EU standard. To meet the Kenyan standard, drying needed to be completed within 180 hrs for 30°C and 40°C and 240 hours at 20°C (obtained by extrapolation).

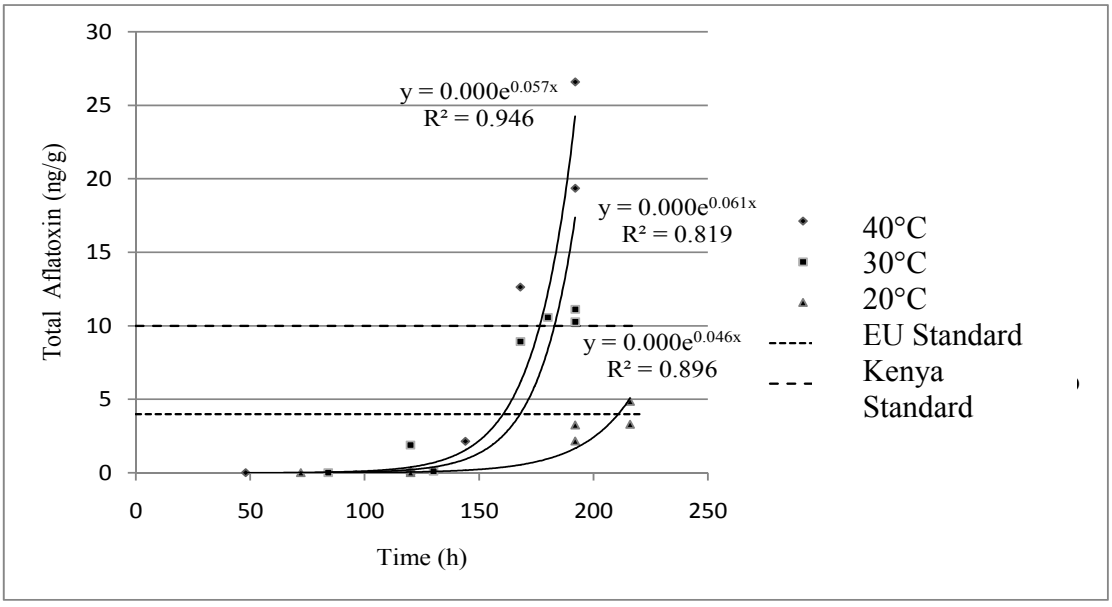


Figure 4.5. Relationship between time to drying and total aflatoxin concentration

A relationship was established between time taken to dry a sample to 13% MC and the level of aflatoxin contamination by considering only samples that actually dried to 13% by the time the experiments were terminated at 216 hrs. All the data for samples that didn't dry to 13% MC was left out in this particular analysis. The data was plotted for 40°C, 30°C and 20°C and is presented in Figure 4.6 below.

The data shows that aflatoxin content can be associated with high moisture content when under favorable conditions. The results show European standards and Kenyan standards the moisture content of maize has to be below 14% moisture content to avoid aflatoxin contamination but depending on various conditions.

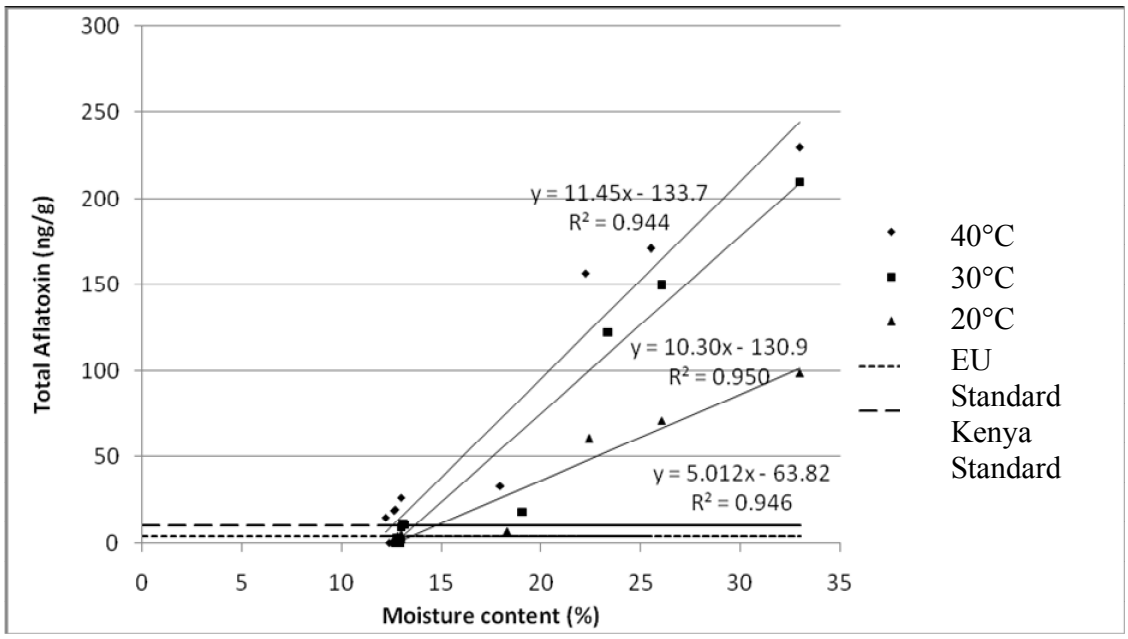


Figure 4.6: Relationship between final moisture content and total aflatoxin contamination

4.5 Discussion

The duration of drying for the purposes of this experiment was the time required to reduce the grain to the safe moisture content (MC) of 13% below which contamination by mycotoxins is unlikely. The duration of drying was observed to be closely related to if not dependent on both the SAP to Maize ratio as well as on the frequency of change of the SAP as seen from Tables 4.1 through 4.3. With no change of SAP, all the SAP: Maize ratios apart from the SAP to Maize ratio of 1:1 did not dry to the required 13% MC. In all the cases where drying did not reach 13% MC, the aflatoxins were observed to be much higher than when the drying to 13% MC was achieved before the 216 hours when the experiment was terminated as the wet samples showed signs of rotting.

In general, it was found that drying by SAP reduced the aflatoxin contamination and that the more SAP is used the less the incidence of aflatoxin contamination. To be absolutely sure that there will be no aflatoxin contamination, a ratio of 1:5 (SAP:Maize) or less is recommended. Higher ratios (such as 1:10 and 1:20) may prevent aflatoxin contamination below 20 °C and with changes of SAP every 24 hours.

However, in terms of desiccant drying using SAP, it is a significant improvement from past studies which gave ratios of 1:1, 1:2.5 and 1:4 for bentonite, silica gel and aluminum silicates, respectively as effective in drying (Bradford *et al*., 2011; Daniel *et al*., 2009; Sturton *et al.*, 1983). According to Daniel *et al*. (2009) the desiccants used were not food grade and were not drying maize but as per the results from this study, the dessicants used are food grade and have proved to dry the maize that can be used for human and livestock consumption and at the same time reduce the aflatoxin contamination in maize.

A separate analysis was done to determine the effect of the final moisture content on aflatoxin contamination. For all the experiments, the final moisture was recorded and plotted against the corresponding aflatoxin contamination (Figure 4.2). The results for each temperature level were plotted separately. It was determined that the relationship between the final moisture content was linear and that the higher the moisture content, the greater the aflatoxin contamination. The result from Figure 4.2 shows the short range of moisture contents in which maize have to be maintained to prevent aflatoxin contamination. To meet the EU standard, the final MC should be less than 13% and to meet the Kenyan standard the MC should be less than 15% for the range of temperatures considered. Given that maize is hygroscopic and absorbs moisture from the atmosphere, this presents challenges of storage in that the maize has to be dried to below 13% MC and stored under hermatic conditions where it may not absorb moisture. Mutungi *et al*. (2008) established that aflatoxin contamination is likely when the moisture content is 14%. This confirms that maize has to be dried to a moisture content of below 14% for storage for future use to take place. Keeping maize under moisture content of 13% will be safe as the maize will not generate any heat within to create favorable conditions for Aspergillus growth and subsequent aflatoxin contaminations.

The *Aspergillus flavus* and *Aspergillus paraisticus* that invade and contaminate under dried maize and other agricultural products to cause aflatoxin are dependent on temperature. According to Mutungi *et al*. (2008) and Hugh *et al*. (1970) the temperatures above 30°C are most favourable for growth and sporulation of *Aspergillus spp*. This might be the reason why most samples above 30°C had higher aflatoxin content since the temperature is more favorable for Aspergillus contamination.

The low temperature has an effect on Aspergillus growth which results to aflatoxin contamination. According to Saleem *et al*. (2012), maize stored in cold room (7 ± 2°C) for 21 days had very low aflatoxin contamination compared to those in ware house and metal bins at room temperature. The study showed that at 20°C despite the maize taking long hours to dry the aflatoxin contamination were far much lower as when compared with 30°C and 40°C with same ratios of maize to super absorbent polymers used to dry the maize. In this case under low temperatures, maize drying might take long but once the temperature is not very favorable for Aspergillus growth and sporulation, the aflatoxin levels are likely to be lower. The study shows that maize under 20°C can be stored at 15% moisture content without being contaminated with aflatoxin unlike those under 30°C and 40°C which have to be stored below a moisture content of 13% moisture content. From this study it is clear that the rate at which the maize dries has a bearing to the contamination of aflatoxin in the maize samples. Under favorable temperature and moisture content, maize samples in temperatures ranges above 30°C which took more than 150 hours to dry all had a higher aflatoxin contamination compared to samples that dried within 72 hours. The drying in 1:1 ratios took the shortest time (less than 48 hours) and no aflatoxin was recorded in all samples unlike the rest. This can be applied in times when the maize is ready for harvest and the drying is slow especially when farmers are harvesting and it is raining and the farmers have inadequate drying space and materials. In such cases aflatoxin contamination are likely to be more especially in the tropics and sub tropics where temperatures are relatively high (Saleem *et al*., 2012).

The study showed that LC-MS Analysis was able to detect all aflatoxin as being present in the samples that had contamination. AFB1 was most predominant and is known to be most toxic (Okoth *et al.,* 2012). The levels of AFB1 were very high in most samples above both the

KEBS and EU recommendations. The presence of AFB1 and AFB2 indicates that *Aspergillus flavus* was the most predominant species which contributed to this kind of contamination. According to Saleem *et al*. (2012) aflatoxin B1,B2,G1 and G2 were detected from maize samples contaminated entirely with *Aspergillus flavus*. The same was recorded by Chulze (2010) and Medina-Martinez (2000).

4.6 Conclusions

It can be concluded that the duration of drying and temperature has a significant effect on aflatoxin contamination in maize. From the study AFB1, AFB2, AFG1 and AFG2 were all detected but varied in different temperature regimes. More contaminations were detected at higher temperatures of 30°C and 40°C than low temperature of 20°C meaning that Aspergillus growth and activity was best supported by high temperatures and the activity slowed down at lower temperatures (20°C). In the tropics and sub tropics high temperatures are experienced and this is the reason why afloxicosis is commonly reported in these regions.

The rate of drying had an effect on aflatoxin contamination as indeed maize samples that dried within 48 hours none was contaminated but those which took a longer time to dry had a significant level of contamination above the WHO, KEBS and EU recommended standards. This shows that the faster the drying the less the contamination but the longer the drying takes place under favorable conditions the higher the aflatoxin contamination in the maize.

From the study above, it is clear that the rate of drying maize, temperature, and moisture content are main factors that contributed to high aflatoxin levels in some maize samples. Therefore to make an intervention, one has to manipulate these factors in order to reduce or minimize the exposure to aflatoxin contamination.

The study indicates that super absorbent polymers can be used to dry maize. The ratio of SAP to Maize has an effect across all temperature regimes. The higher the ratio the better as no aflatoxin contamination can be realized but when resources are limited, the SAPs can be changed at different time intervals dried and re used to dry the maize. Therefore drying with SAP is a viable alternative to other drying operations. This is supported by the fact that the SAP can be dried and reused many times.

4.7 Recommendations

The study revealed that drying of maize with SAP reduces aflatoxin contamination but the reduction depends on the ratio of SAP to Maize with ratios below 1:5 being most effective but higher ratios such as 1:10 and 1:20 being applicable depending on temperature. Therefore use of SAPs for drying maize is recommended whereby ratios below 1:5 are suitable for fast drying in all temperatures but ratios of 1:10 and 1:20 are recommended where the SAP is regularly changed within 24 or 48 hours at lower temperatures like of 20°C.

The study was carried out in a controlled environment and it is recommended that further studies be carried out in the open and in farmers' environment to support the findings.

Further work on the economics of drying maize by use of super absorbent polymers needs to be done to ascertain the viability and affordability by the peasant farmers who are most affected by aflatoxin contamination in maize.

CHAPTER FIVE

5.0 General discussion

The whole study revealed that post harvest aflatoxin contamination is influenced by handling practices that various farmers use. According to Kaaya *et al*. (2006) harvest after physiological maturity combats aflatoxin problems as he noted that the levels of aflatoxin increased by four times by the third week and more than seven times in the fourth week after physiological maturity.

From the study, most farmers delay their harvest due to financial and other environmental factors beyond their control, a reason that might be influencing the presence of aflatoxin in their maize produce. The maize that was used in this study for drying using super absorbent polymers was harvested after physiological maturity and from the experiments it was revealed that the maize that dried faster especially where the ratio of SAP : Maize was 1:1 no aflatoxin contamination was noted compared to others where there was some delay in drying.

The duration of drying has an effect on aflatoxin contamination as where the drying took less than 48 hours under high temperature of above 30°C no trace of aflatoxin contamination was recorded compared to where the drying was delayed above 48 hours. However, no aflatoxin contamination was recorded after 72 hours of drying at 20°C which can be attributed to unfavorable conditions for *Aspergillus* growth due to low temperatures. According to Diener *et al*. (1987), the optimal conditions for *Aspergillus* development is 36 to 38°C, with a high humidity above 85% which is a similar conditions that experiments for drying maize at 30 and 40°C experienced, same to under dried maize that is stored in the plastic bags by some farmers.

Temperature and moisture influence the growth of the toxigenic fungi in stored commodities. Hell *et al*. (2008) observed that aflatoxin contamination can increase up to 10 fold in a three day period when harvested maize is stored with high moisture content. Harvested commodities should be dried as fast as possible to a safe moisture content of 10 – 13% for all cereals (Hell *et al*., 2008 and Mutungi *et al*., 2008).

The same scenario was replicated to all control experiments where no hydrogels were used to dry the maize recording the highest aflatoxin contamination across all the experiments in all the temperatures followed by where the polymers were not changed. This created a favorable microclimate for Aspergillus growth and sporulation which resulted to aflatoxin contamination.

The farmers from the area of study have simple methods of determining the moisture content of their maize by using traditional methods such as sound of the maize, color and bite by their teeth to determine the dryness of their maize (Muhia *et al*., 2008). This method is not adequate as may result to storage of wet maize as their may exist some pockets or individual kernels that are still wet which while in the store can create favorable condition for aflatoxin contamination. Given that the aflatoxin awareness is far above 79% and still experiencing the same problem of aflatoxin contamination by some farmers, simple and effective technology for detecting moisture content should be availed to the farmers to curb this perennial problem.

From the LC-MS analysis it was evident that AFB1, AFB2 and AFG1 were detected but AFB1 was most predominant and was detected in higher levels of up to 128 ng/g in Makueni County and 229ng/g at 40°C in the control experiment. The predominance of AFB1 in both experiments is a great concern as it is linked to hepatocellular cancer (Strosnider *et al*., 2006).

There is a very high risk of Hepatitis B and Hepatitis C carriers to develop liver cancer when they are exposed to aflatoxin AFB1 (Williams et al., 2003). According to Saleem *et al*. (2012), the presence of AFB1, AFB2, AFG1 and AFG2 indicates the presence of *A*. *flavus* as most likely aspergillus spp that contaminated the produce. The same was confirmed by Chulze (2010) and Medina-Martinez (2000). This is true as samples from farmers in the lower eastern counties that recorded high AFB1 were the ones that had high presence of *A*. *flavus*. Exposure to AFB1 in small amounts over a long time may lead to chronic aflatoxicosis (Strosnider *et al*. 2006) this might be the true in the scenario where contaminated maize from individual farmers end up in the market where they are mixed with others and sold to a larger population.

The scenario from the study reveals drop in terms of exposure to aflatoxin in the lower eastern regions which is highly attributed to public awareness campaigns by FAO and MOA after the major outbreak in 2004 (Strosnider *et al*. 2006). Despite this drop there are still cases of aflatoxin contamination in the region which needs to be addressed by availing simple and easy to use technology for farmers and in this study use of super absorbent polymers in drying of maize can be an alternative means to reduce the exposure to aflatoxin contamination.

5.1 General conclusion

From the study it was revealed that aflatoxin contamination is common among the farmers from lower eastern region of Kenya despite the awareness being above 79%. The exposure is attributed to post harvest handling practices by the affected farmers more so lacking the simple and modern technology for drying and handling their maize after harvest.

Temperature, moisture content and humidity influence the aflatoxin contamination in maize as revealed in the study. Interfering with one or two of the factors will significantly affect the

aflatoxin contamination as in this case observed in 20°C compared to both 30 and 40°C. It was further revealed that use of super absorbent polymers can significantly affect the level of aflatoxin contamination based on the ratio used and frequency of change of SAPs.

From the study it is revealed that use of super absorbent polymers technology can be an alternative means to reduce the aflatoxin contamination in maize. The polymers are cheap and can be used several times to dry maize as long as it is dried before re-use. At high temperature use of SAPs up to 1:10 can dry maize fast enough to reduce aflatoxin contamination and at low temperature of 20°C the ratio of 1:20 can be used and achieve the same results.

5.2 General recommendation

The level of public awareness is very high and therefore stake holders should engage in developing simple and farmer friendly technologies that farmers can use to dry their maize fast enough and be able to measure their moisture levels in order to store their maize at appropriate moisture content and avoid contamination by *Aspergillus flavus.*

It is evident that super absorbent polymers can be used to dry maize at any given condition depending on the ratios used and frequency of their change. This reveals that other than using high ratios, lower ratio like 1:10 can be used and achieve the same result as 1:1. With the fact that SAPs can be re-used several times, it makes economical sense to dry the maize using SAPs to achieve low aflatoxin contamination. This can be a viable alternative among other drying technologies especially to peasant farmers.

References

American Society for Microbiology (ASM). (2004). Washington, DC. clinical microbiology workforce issues; http://www.asm.org. Retrieved 2/10/2013.

Atehnkeng, J., Ojiambo, P.S., Ikotun, T., Sikora, R.A., Cotty, P.J., Bandyopadhyay, R. (2008). Evaluation of atoxigenic isolates of *Aspergillus flavus* as potential biocontrol agents for aflatoxin in maize, Food Additives and contaminants: Part A 25, 1264-1271.

Bankole, S.A., Eseigbe, D.A., Enikuomehin, O.A. (1996). Mycoflora and aflatoxin production in pigeon pea stored in jute sacks and iron bins. Mycopathologia 132, 155–160.

Barbucci, R., Magnani, A. and Consumi, M. (2000). Swelling behavior of arboxymethylcellulose hydrogels in relation to cross-linking, pH, and charge density. Macromolecules, 33: 7475-7480.

Ben, D., Liem, P., Dao, N., Gummert, M., Rickman, J. (2009). Effect of hermetic storage in the super bag on seed quality and milled rice quality of different varieties in Bac Lieu, Vietnam. International Rice Research Notes, 31(2). Retrieved February 12, 2014, from http://www.philjol.info/index.php/IRRN/article/view/1138/1035

Bennet, J. and Klich, M. (2003). Mycotoxins. Clinical Microbiology Reviews (3), 497-516.

Betran, F. and Isakeit, T. (2003). Aflatoxin accumulation in maize hybrids of different maturities. Agronomy Journal 96, 565-570.

Bhat, R. and Vasanthi, S. (2003). Food safety and food security and food trade –mycotxin food safety risk in developing countries. International Food Policy Research Institute. Retrieved in August 10, 2013, from http://www.ifpri.org

Boken, V.K., Hoogenboom, G., Williams, J.H., Diarra, B., Dione, S., Easson, G.L. (2008). Monitoring peanut contamination in Mali (Africa) using the AVHRR satellite data and a crop simulation model. International Journal of Remote Sensing 29, 117-129.

Borgemeister, C., Adda, C., Sétamou, M., Hell, K., Djamamou, B., Markham, R.H., Cardwell, K.F. (1998). Timing of harvest in maize: Effects on post harvest losses due to insects and fungi in central Benin, with particular references to *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). Agriculture, Ecosystem and Environment 69, 233-242.

Bradford, K. J., Dahal, J., Bajracharya, B., Mishra, K., Kunusoth, A. N. G., Ranga and J. Van Asbrouck, HortCRSP and USAID. (2011). New technology for postharvest drying and storage of horticultural seeds: zeolite desiccant beads, http://hortcrsp.ucdavis.edu/main/4Seeds.html. Retrieved 10/8/2013.

Brown, R.L., Chen, Z.-C., Menkir, A., Cleveland, T.E. (2003). Using biotechnology to enhance host resistance to aflatoxin contamination of corn. African Journal of Biotechnology 2, 557-562.

Bruns, H.A. (2003). Controlling aflatoxin and fumonisin in maize by crop management. Journal of Toxicology, Toxin Reviews 22, 153–173.

Centers for Disease Control and Prevention. Outbreak of aflatoxin poisoning-Eastern and Central provinces, Kenya. MMWR. 2004; 53: 790-793.

Chulze, S. N., (2010). Strategies to reduce mycotoxins levels in maize during storage: A review. Food additives contaminants, 27: 651-657

Clements, M.J., White, D.G. (2004). Identifying sources of resistance to aflatoxin and fumonisin contamination in corn grain. Journal of Toxicology, Toxins Reviews 23, 381-396.

Cole, R., Domer, J. and Holbrook C. (1995). Advances in mycotoxin elimination and resistance. American Peanut Research and Education Society, Stillwater OK, 456-74.

Cotty, P. J., (1997). Aflatoxin producing potential of communities of Asperillus section flavi fro cotton producing areas in the United States. Mycological Research 101 : 699 – 704

Council for Agricultural Science and Technology (CAST). (2003). Mycotoxins – Risks in plants, animals and human systems. Task Force Report, No. 139, 1-191. Ames, Iowa, USA.

Daniel, I. O., Oyekale, K. O., Ajala, M. O., Sanni, L. O. and Okelana, M. O. (2009). Physiological quality of hybrid maize seeds during containerized-dry storage with silica gel. African Journal of Biotechnology Vol. 8 (2), Pp. 181-186.

Delgado, J.M., Vázquez da Silva, P.Q., Nasser, R.O., Gonçalves M.P. and Andrade, C.T. (2009). Water Sorption Isotherms and Textural Properties of Biodegradable Starch-based Super-absorbent Polymers, Defect and Diffusion Forum Vols. 283-286 pp 565-570, Trans Tech Publications, Switzerland.

Deyu, G. (2003). Superabsorbent polymer composite (SAPC) materials and their industrial and high-tech applications. Ph.D. Thesis, Technische Universität, Bergakademie, Freiberg, Germany.

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S., Klich, M.A. (1987). Epidemiology of Aflatoxin, in formation by *Aspergillus flavus*. Annual Review of Phytopathology 25, 240-270.

D'Mello, J.P.F., Macdonald, A.M.C., Postel, D., Dijksma, W.T.P., Dujardin, A., Placinta, C.M. (1998). Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. European Journal of Plant Pathology 104, 741-751

Egel, D. S., Cotty, P. J., and Elias, K. S. (1994). Relationship among isolates of Aspergillus section flavi that vary in aflatoxin production. Phytopathol., 84: 906- 912

Ekebafel, L. O., Ogbeifun, D. E. and Okieimen, F. E. (2011). Effect of native cassava starch-poly (sodium acrylate-co-acrylamide) hydrogel on the growth performance of maize (*zea mays*). Seedlings. American Journal of Polymer Science 1(1): 6-11.

Elliot, M. (2004). Superabsorbent Polymers, BASF AG, Ludwigshafen, Germany.

Esposito, F., Del Nobile, A., Mensitieri, G. and Nicholais, L. (1996). Water sorption in cellulose-based hydrogels. Journal of Application of Polymer Science., 60:2403-2407.

Export Processing Zones Authority (2005). Grain production in Kenya. Export Processing Zones Authority, Athi River, Kenya. http//:www.epza.com.Retreived 2/7/2013.

Fandohan, P., Hell, K., Marasa, W. and Wingfield, M. (2003). Infection of maize by fusarium species and contamination with fumonisins in Africa. African Journal of Biotechnology 2 (12), 570 – 579.

Fandohan, P., Hell, K., Marasas, W.F. (2008). Food processing to reduce mycotoxins in Africa. Pre and postharvest management of aflatoxin in maize. In Leslie, J.F., Bandyopadhyay, R., Visconti, A., (Eds) Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CABI Publishing, Wallingford, UK. pp. 302-309.

Fandohan, P., Zoumenou, D., Hounhouigan, D.J., Marasas, W.F., Wingfield, M.J., Hell, K. (2005). Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. International Journal of Food Microbiology 98, 249-59.

Food, drugs and chemical substances Act, CAP. 254, Laws of Kenya, Regulation. (1978).Fung F. and Clark R. (2004). Health effects of mycotoxins: a toxicological overview. Journal of Toxicology 2004; 42:217–234.

Garcia, D., Ramos, A.J., Sanchis, V., Marín, S. (2009). Predicting Mycotoxins in Foods: A Review. Food Microbiology 26, 757- 769.

Glados, S. and Maciejewski, M. (1998). Hydrogels synthesis and applications. Wiadomooeci Chemiczne, 52, 101-124.

Gray, M., Charlse,H., Julie, M., Dan, L., and Alison, R. (2009). Aflatoxin in corn. Iowa State University Extension PM 1800.

Gummert, M., Balingbing, C.B., Barry, G., Estevez, L.A. (2009). Management options, technologies and strategies for minimised mycotoxin contamination of rice. World Mycotoxin Journal 2, 151-159.

Hell, K., Cardwell, K., and Poehling, H. (2003). Relationship between management practices, fungal infection and aflatoxin for stored maize in Benin. Journal of Phytopathology 151, 690-698.

Hell, K., Cardwell, K.F., Setamou, M., Poehling, H.M. (2000). The influence of storage practices on aflatoxin contamination in maize in four agro-ecological zones of Benin, West Africa. Journal of Stored Products Research 36, 365–382.

Hell, K., Fandohan, P., Bandyopadhyay, R., Cardwell, K., Kiewnick, S., Sikora, R., Cotty, P. (2008). Pre- and postharvest management of aflatoxin in maize. In Leslie, J.F., Bandyopadhyay, R., Visconti, A., (Eds) Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade. CABI Publishing, Wallingford, UK. pp. 210-219.

Hughs, L. T. and Paul, A. H. (1970). Effects of moisture content and temperature on aflatoxin production in corn. Journal of applied microbiology Iowa state university 1970; 19: 781-784.

International Union of Pure and Applied Chemistry, (2004). Definitions of terms relating to reactions of polymers and to functional polymeric materials (IUPAC) recommendations (2003). Pure Applied Chemistry, 76, 4, 889-906.

Julia, R. (2005). Liver cancer and aflatoxin: New information from the Kenyan outbreak. Environ. Health Perspect. 2005; 113: A837-A838.

Kaaya, A.N., Kyamuhangire, W. (2006). The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. International Journal of Food Microbiology 110, 217–223.

Kaaya, A.N., Kyamuhangire, W., Kyamanywa, S. (2006). Factors affecting aflatoxin contamination of harvested maize in the three agro-ecological zones of Uganda. Journal of Applied Sciences 6, 2401–2407.

Kenya Plant Health Inspectorate Service (KEPHIS) (2006). Mycotoxins and Food Safety. KEPHIS Headquarters, Nairobi, Kenya.

KEW. (2010). Improving the identification, handling and storage of "difficult" seeds

Kimanya, M., Meulenaer, B., Tiisekwa, B., Ndomondo-Sigonda, M., Devlieghere, F., van Camp, J. and Kolsteren P. (2008). Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages in Tanzania. Food Additives and Contaminants 25(11), 1353 – 1364.

Klich, MA. (2000). Aspergillus fumigatus. In: Identification of common Aspergillus species. Centraalbureau voor Schmmelcultures, Utrecht, The Netherlands. 50 – 51.

Kuruwita-Mudiyanselage, T. D. (2008). Smart Polymer Materials, Ph.D. Thesis, Graduate College, Bowling Green State University, Ohio, USA.

Lamboni, Y., Hell, K. (2009). Propagation of mycotoxigenic fungi in maize stores by post-harvest insects. International Journal of Tropical Insect Science 29, 31–39.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., luber, G., Nyamongo, S., Baker, L., Dahiye, A., Misore, A., Kevin, D. and the Kenya aflatoxin investigating group (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environmental Health Perspective 113 (12), 1763-1767.

Liu, J. G. and Yu, J. (2006). Aflatoxin in stored maize and rice in Liaoning province, China. Journal stored prod. Res., 42:468-479.

Lutfy, O.F., Mohd Noor, S.B., Abbas, K.A., Marhaban, M.H. (2008). Some control strategies in agricultural grain driers: A review. Journal of Food, Agriculture and Environment 6, 74-85.

Manoch, L., Chana, C., sangchote, S., and Banjoedchoedchu, R. (1988). Some mycotoxic fungi from agricultural products and food stuff in Thailand. Proc. Japanese Assoc. mycotoxicol., 1: 45- 46.

Manwiller, A. (1987). Aflatoxin Kenya. In: Zuber M, Lillehoj E, Renfro B (eds). Aflatoxin in maize. Proceedings of Workshop, 7-11Apr. 1986. El Batan, Mexico.

Mathias, R and Macharia, M. “East Africa raises alarm over toxic grains” Daily Nation 4th October 2013, upcountry edition.

Medina-Martinez, M.S., and Martinez, A.J. (2000). Mold occurrence and aflatoxin B_1 and fumonisinB_1 determination in corn samples in Venezuela. J. Agric. FoodChem., 48: 2833 – 2836.

Mestres, C., Bassa, S., Fagbohoun, E., Nago, M., Hell, K., Vernier, P., Champiat, D., Hounhouigan, J., and Cardwell, K.F. (2004). Yam chip food sub-sector: hazardous practices and presence of aflatoxins in Benin. Journal of Stored Products Research 40, 575-585.

Miller, D. (1979). Effect of H-SPAN on water retained by soils after irrigation. Soil Sci. Soc. Am. J. 43: 628-629.

Ministry of Agriculture (2008). The role of post harvest in the control of aflatoxins in cereals and pulses. Ministry of Agriculture Headquarters, Nairobi, Kenya.

Moreno, O. J., and Kang, M. S. (1999). Aflatoxin in maize: the problem and genetic solutions. Plant Breed., 118: 1 – 16.

Morenoa, E.C., Garciab, G. T., Onoc, M.A., Vizonid, E., Kawamurae, O., Hirookaf, E.Y., and Onoa, S.Y.E. (2009). Co-occurrence of mycotoxin in corn samples from the Northern region of Parana state, Brazil. J. foodchem 2009.02.037.

Murdock, L.L., Shade, R.E., Kitch, L.W., Ntoukam, G., Lowenberg-Deboer, J., Huesing, J.E., Moar, W., Chambliss, O.L., Endondo, C. and Wolfson, J.L. (1997). Postharvest storage of cowpea in sub-Saharan Africa. In B. B. Singh et al., Eds, Advances in Cowpea Research. Co-publication IITA and JIRCAS. IITA, Ibadan, Nigeria, pp. 302-312.

Muriuki, G., Siboe, G. (1995). Maize flour contaminated with toxigenic fungi and mycotoxins in Kenya. African Journal of Health Sciences 2, 236– 241.

Murphy, P., Hendrich, S. and Bryant C. (2006). Food Mycotoxins – An Update. Journal of Food Science 71 (5), 51 – 65.

Muthomi, J. W., Mureithi, B. K., Chemining'wa, G. N., Gathumbi J. K. and Mutitu, E. W. (2012). Aspergillus and aflatoxin B1 contamination of maize and maize products from Eastern and North-Rift regions of Kenya, KARI Annual Conference.

Muthomi, J., Njenga, L., Gathumbi, J. and Chemining'wa G. (2009). The occurrence of aflatoxins in maize and distribution of mycotoxin-producing fungi in Eastern Kenya. Plant Pathology Journal 8 (3), 113 – 119.

Mutungi, C., Lamuka, P.,Arimi, S., Gathumbi, J. K., and Onyango, C. (2008). The fate of aflatoxin during processing of maize into muthokoi – a traditional Kenyan food. Food Control 19, 714 – 721.

Mwihia, J. T., Straetmans, M., Ibrahim, A., Njau, J., Muhenje, O., Guracha, A., Gikundi, S., Mutonga, D., Tetteh, C., Likimani, S., Breimani, R.F., Njenga, K. and Lewis, L. (2008). Aflatoxin levels in locally grown maize from Makueni District, Kenya. East African Journal 85 (7).

Nassir, M. S., and Jolley, M. E. (2002). Fungicidal comparison for maize. J. Agric. Food Chem., 50: 3116 – 3121.

Njapau, H.,Muzungaile, E.M. and Changa, R.C (1998). The effect of village processing techniques on the content of aflatoxin in corn and peanuts in Zambia. J. Sci. Food Agric. 1998; 76:450-456.

Okoth, A.S, Kola, A.M, (2012). Market samples a source of chronic aflatoxin exposure in Kenya. African Journal of Health Sciences 20, 56-61.

Onsongo J. 2004. Outbreak of aflatoxin poisoning in Kenya. EPI/IDS Bull 5:3–4. Cited by Azziz-Baumgartner, E., K. Lindblade, K. Gieseker, H. Schurz Rogers, S. Kieszak, H. Njapau, R. Schleicher and L.F. Mccoy. (2005). Case-control study of an acute aflatoxicosis outbreak – Kenya. (2004). Environmental Health Perspective 113, 1779-1783.

Orzeszyna, H., Garlikowski, D, and Pawowski A. (2005). Using of geocomposite with superabsorbent synthentic polymers as water rentention element in vegetative layers. Journal of international agrophysics 20, 201-206.

Park, D and Liang, B. (1995). Perspectives on aflatoxin control for human foods and animal feed. Trends in Food Science and Technology, 4 (10), 334-348.

Park, D.L. (2002). Effect of processing on aflatoxin. Advances in Experimental Medicine and Biology 504, 173-179.

Pitt, J.I. and Samson, R.A (2000). Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* classification. Hardwood academic publishers, Reading, Uk, pp. 51-72.

Probst, C., Njapau, N. and Cotty, P. (2007). Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. Applied and Environmental Microbiology 73 (8), 2762-2764.

Rahmani, A., Jinap, S. and Soleimany, F. (2010). Validation of the procedure for the simultaneous determination of aflatoxins ochratoxin A and zearalenone in cereals using HPLC-FLD, Food Additives & Contaminants: Part A, 27: 12, 1683 - 1693.

Raju, K, Raju, M. and Mohan Y. (2003). Synthesis of superabsorbent copolymers as water manageable materials. Polymer. Int., 52: 768-772.

Rapper, K.B., and Fennell, D.I. (1965). The genus Aspergillus, Baltimore: Williams and Walkins Company. 686.

Reddy, B. and Raghavender, C. (2007). Outbreak of aflatoxicoses in India. African Journal of Food Agriculture Nutrition and Development 7 (5).

Roy, A.K., and Chourasia, H. K. (2001). Mycotoxin contamination in herbal seed samples under storage and their prevention, pp 131 – 144. Seed technology and seed pathology.

Saleem, M.J., Hannan, A., Nisa, A. U and Qaisar, T. A (2012). Occurrence of aflatoxins in maize seed under different conditions. International Journal of Agriculture and Biology, 14: 1473 – 476.

Sétamou, M., Cardwell, K.F., Schulthess, F., Hell, K. (1998). Effect of insect damage to maize ears, with special reference to *Mussidia nigrivenella* (Lepidoptera: Pyralidae), on *Aspergillus flavus* (Deuteromycetes: Monoliales) infection and aflatoxin production in maize before harvest in the Republic of Benin. Journal of Economic Entomology 91, 433-438.

Shephard, G. (2008). Risk assessment of aflatoxins in food in Africa. Food Additives and Contaminants 25(10), 1246 – 1256.

Siwela, A.H., Siwela, M., Matindi, G., Dube, S. and Nziramasanga, N. (2005). Decontamination of aflatoxin-contaminated maize by dehulling. Journal of the Science of Food and Agriculture 85, 2535-2538.

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R., Breiman, R., Brune, *et al*. (2006). Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. Environmental Health Perspectives 114, 1898–1903.

Sturton, S. L., Bilanski, W. K. and Menzies, D. R. (1983). Moisture exchange between corn and the dessicant in an intimate mixture, Can. Agric. Eng. 25: 139-141.

Suhaib, A.B., Azra, N. K. and Bashir, A. G. (2012). Identification of some Aspergillus Species isolated from Dal Lake, Kashmir by traditional approach of morphological observation and culture. African Journal of Micriobiology research 6 (29), 5824 – 5827.

Sumbali, G. (2001). Mold infestation and mycotoxin accumulation in cereal grains – a global scanario, PP: 100-115. Seed Technology and Seed Pathology, Pointer Publishers, Jaipur, India.

Thoruwa, T. F. N., Smith, J. E. and Grant A. D. (2007). Developments in solar drying using forced ventilation and solar regenerated desiccant materials. http://www.ku.ac.ke/images/stories/docs/publications/engineering/novel-low-cost-cacl2-based-desiccant.pdf. Retrieved 10/10/2013.

Turner, P.C., Syra, A., Gong, Y.Y., Diallo, *et al.* (2005). Reduction in exposure to carcinogenic aflatoxins by post harvest intervention measures in West Africa. A community based intervention study. Lancet. 2005; 365:1950-1956.

Udoh, J.M., Cardwell, K.F., Ikotun, T. (2000). Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. Journal of Stored Products Research 36, 187-201.

Wagacha, J. and Muthomi, J. (2008). Mycotoxin problem in Africa: Current Status, implications to food safety and health and possible management strategies. International Journal of Food Microbiology 124, 1-12.

Whitaker, T.B. (2003). Detecting mycotoxins in agricultural commodities. Molecular Biotechnology 23, 61-71.

Williams, J., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., Aggarwal, D., 2003. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. American Journal of Clinical Nutrition 80, 1106–1122.

Wilson, D. and Abramson, M. (1992). Storage of cereal grains and their products. St. Paul (MN): American Association of Cereal Chemists, Inc.; 1992; 341-391.

World Health Organisation (WHO) Global Strategy for Food Safety: safer food for better health. Food Safety Programme 2002a. world Health Organisation (WHO), Geneva, Switzerland.

Yadgiri, B., Reddy, V., Tulpule, G., Srikantia, S. and Gopalan, C. (1970). Aflatoxin and Indian childhood cirrhosis. Amer. J. Clin. Nutr. 1970; 23**:** 94-98.

Yan, L.Y., Yan, L.Y., Jiang, J.H., Ma, Z.H. (2008). Biological control of aflatoxin contamination of crops. Journal of Zhejiang University - Science B 9, 787-792.

APPENDICES

Appendix 1: Questionnaire

QUESTIONNAIRE ON THE AFLATOXIN CONTAMINATION IN LOWER EASTERN REGION IN KENYA.

GENERAL DETAILS

Start by introducing yourself to the farmer

Name of farmer (optional) ……

Date: ……..……. Gender; Male/Female: ……Age…….

Marital status: ……..

Respondent owner/manager/employee/ other: …… Crops' acreage…… …..

Duration in maize farming……yield per hectare……..

Level of education …… (No formal education, elementary, primary, high school, Further education).

Purpose of farming (cash crop, subsistence, other specify)……..

In case of cash crop where do you sell the produce (local market, NCPB, other specify) …….

Geographical location (District/Divisional Agricultural officers)

County: ……. ….. District ……...…….

Altitude……. Agro-Ecological zone…….

Longitude…….Latitude….……

Maize harvesting and drying practices

When/at what stage do you harvest your maize ……..

How is shelling done……..(By hand, use of sheller, beating with a stick, other specify)

How do you dry the maize (directly on the ground, placing on tarpaulins/sisal bags, other specify……

Do you dry before shelling or after shelling……

How long does it take to dry the maize whichever method you use ……

How do you know the maize is dry …….

Maize storage practices

Storage structures used (traditional granaries, in polystyrene bags, in sisal bags, in plastic containers, in clay containers, others specify……..

Storage form (shelled, unshelled)……

Storage period ……

Pest problems in storage and what you do against the pest ……..

……

Moisture content of the maize during harvest (samples collected) ……

Consumption/utilization

Do you consume the maize directly, ground to flour, maize grit or other specify ……..

Do you sort out to remove discolored grains before consumption? (Yes/No)

Farmer's knowledge about mycotoxin problem

Are you aware of the mycotoxin problem (identify maize kernels with the problem and show it to the farmer)……..

If you are aware of the problem how do you rate the problem (Not serious, moderately serious, serious problem)……..

What practice do you carry out to mitigate the problem……..

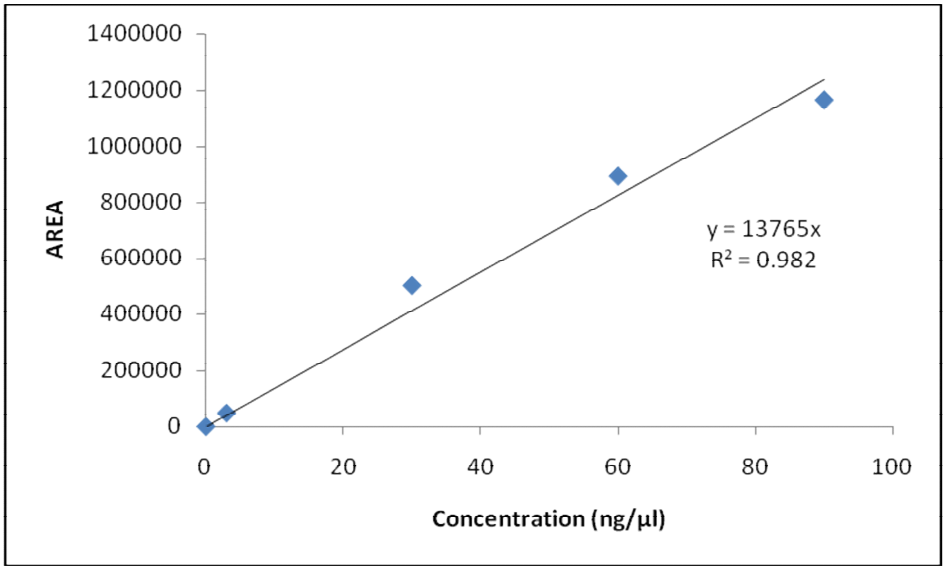
Are you aware of any varieties that are tolerant to mycotoxin contamination? (List the varieties in order of susceptibilities)……..

Your comments

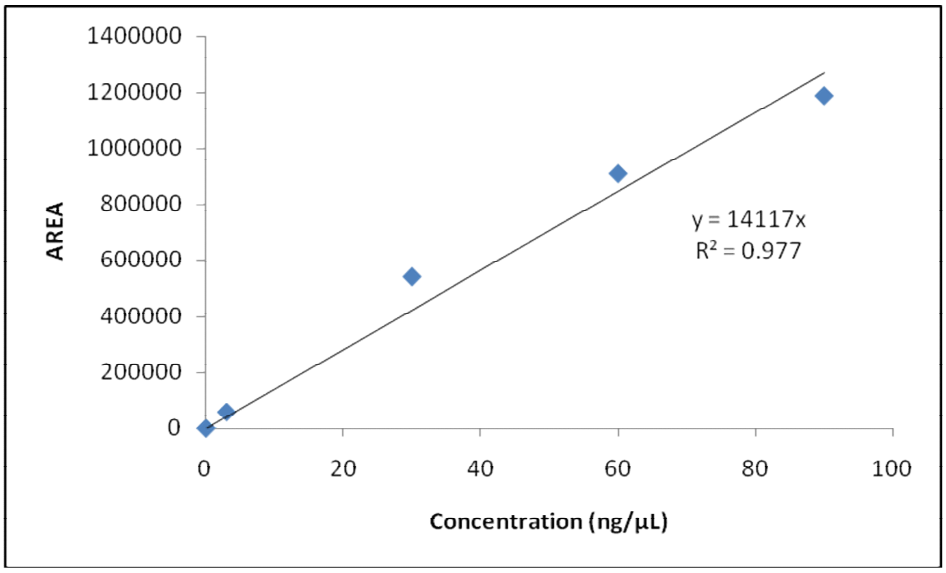
……

Thanks

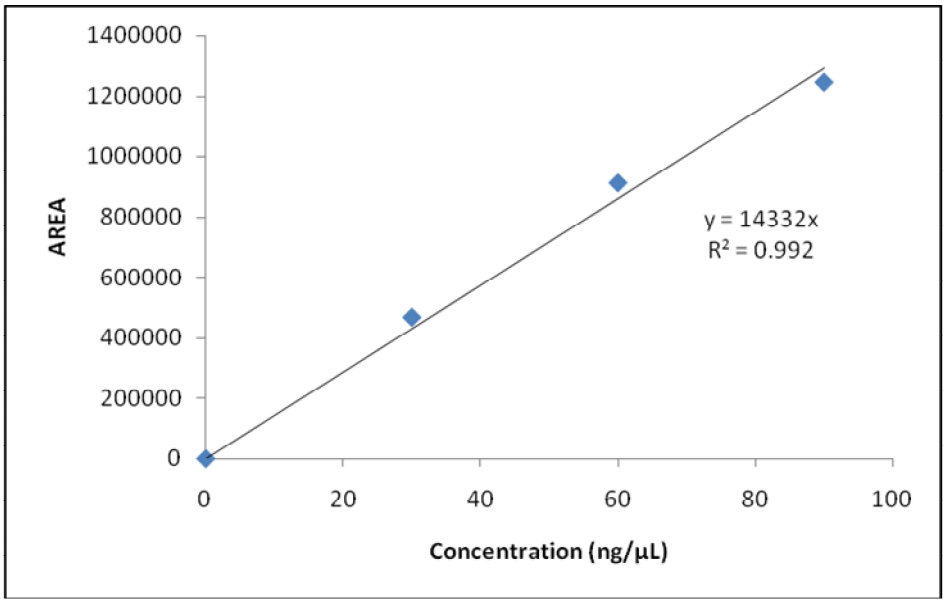
Appendix 2: Standard Calibration curves and total ion chromatograms and spectrums of standards and selected treatments.



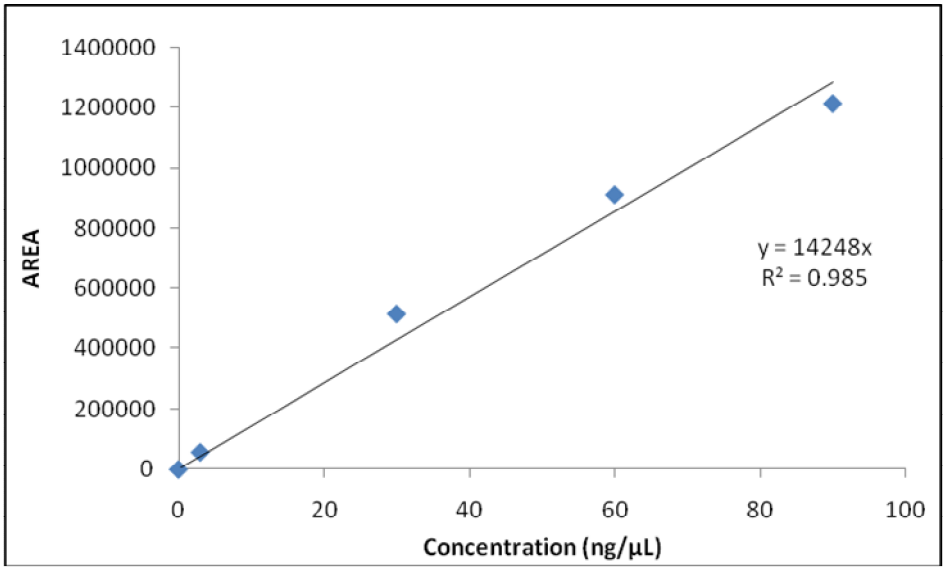
Appendix 2.1: Sample calibration curve for AFB1 standard.



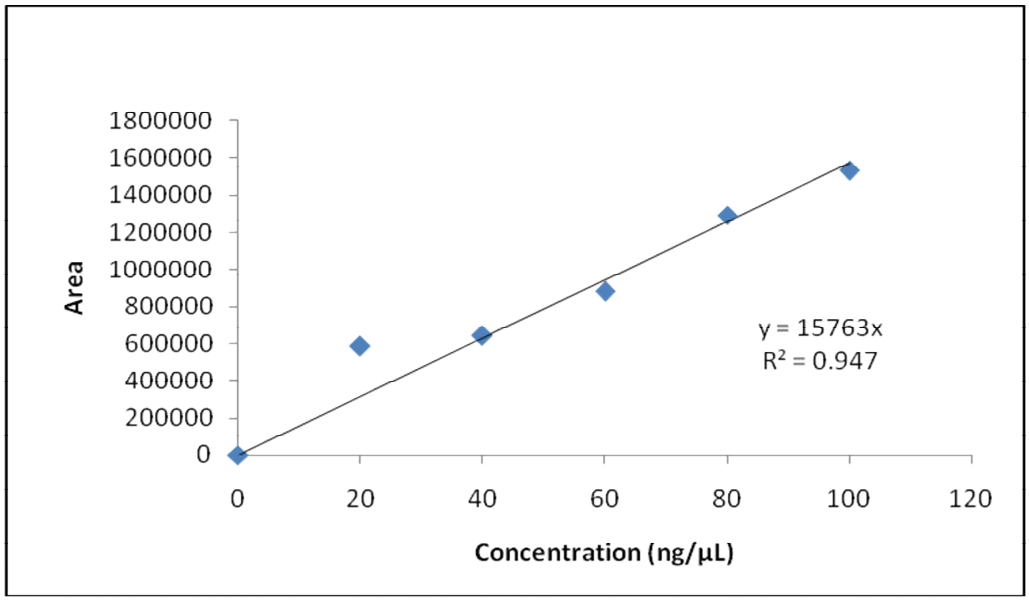
Appendix 2.2: Sample calibration curve for AFB2 standard.



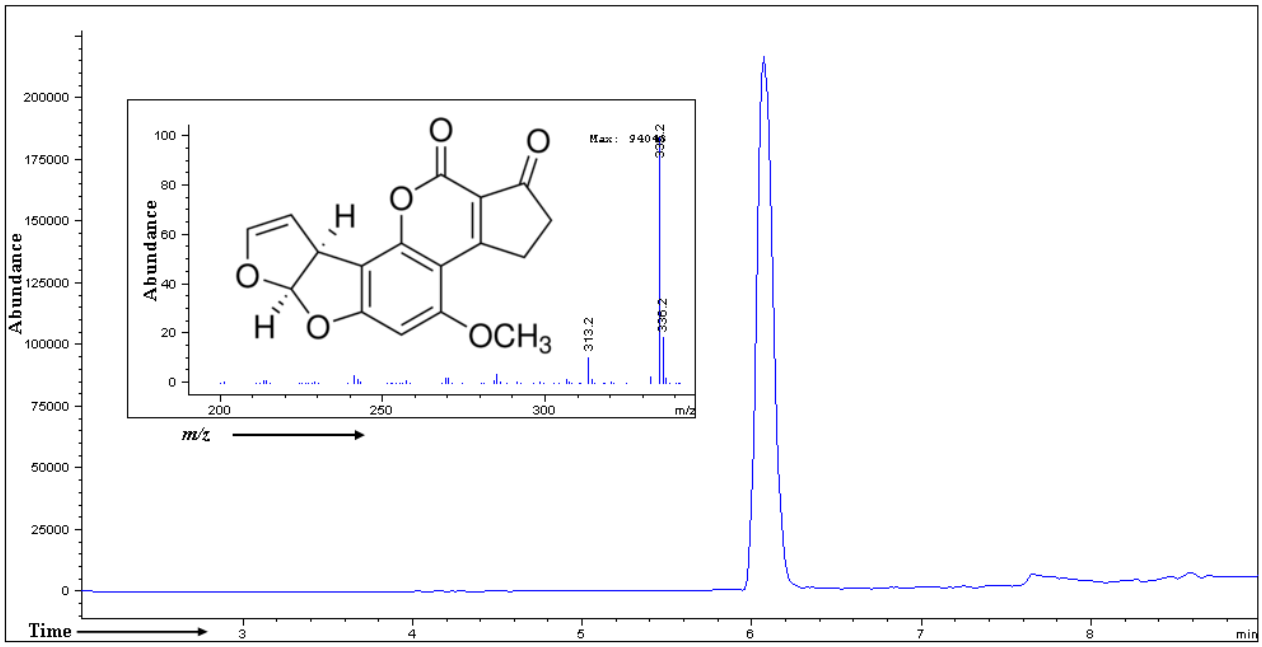
Appendix 2.3: Sample calibration curve for AFG1 standard.



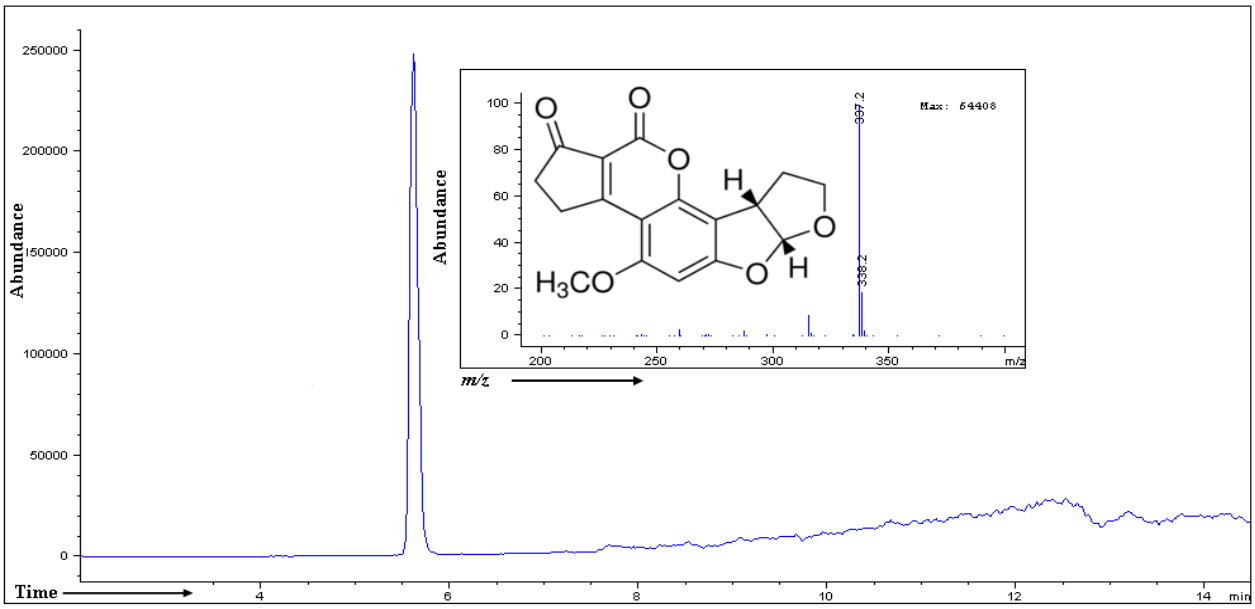
Appendix 2.4: Sample calibration curve for AFG2 standard.



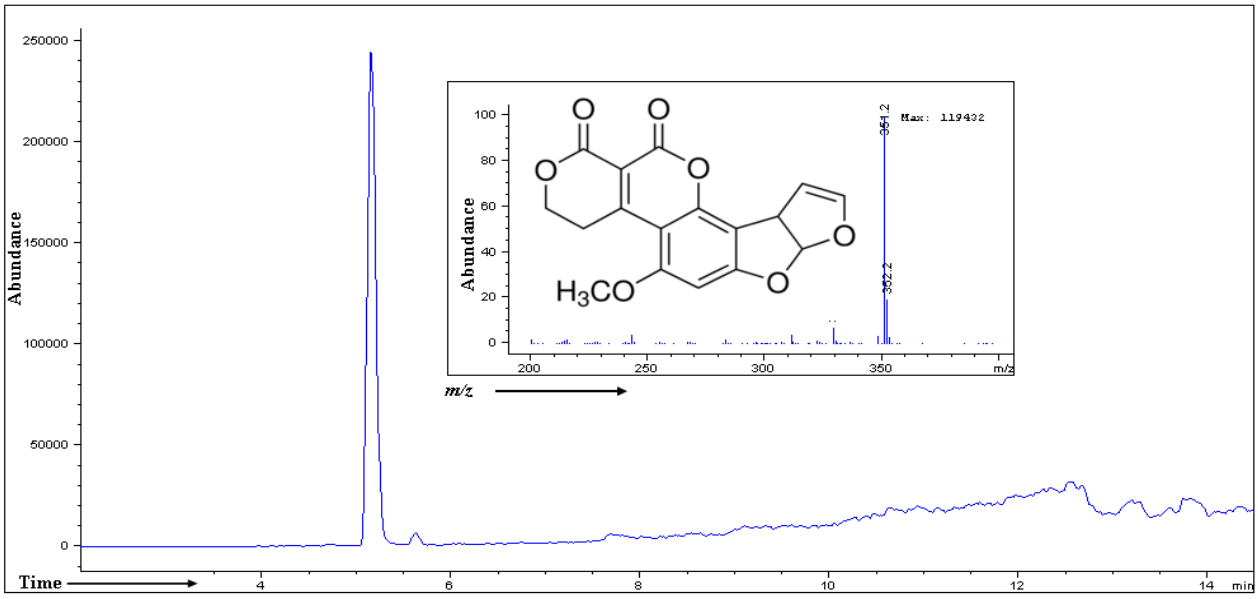
Appendix 2.5: Sample calibration curve for internal standard.



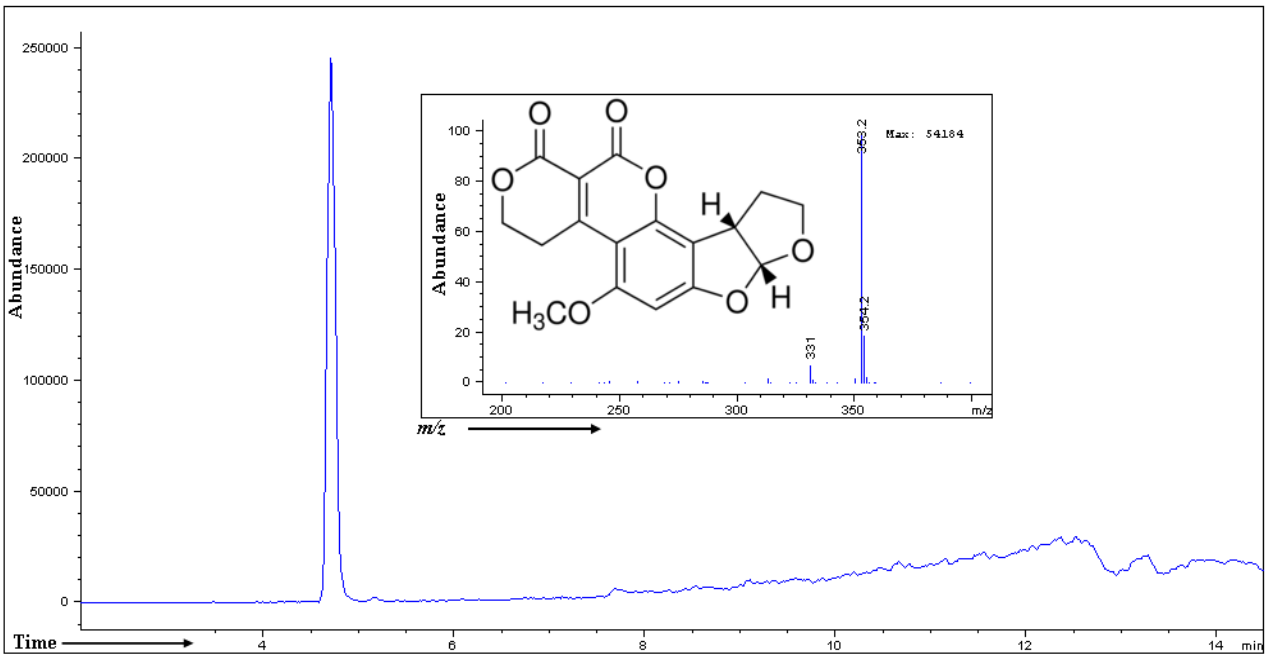
Appendix 2.6: Total ion chromatogram and mass spectrum for aflatoxin B_1 standard (m/z = 313 and retention time 6.157mins)



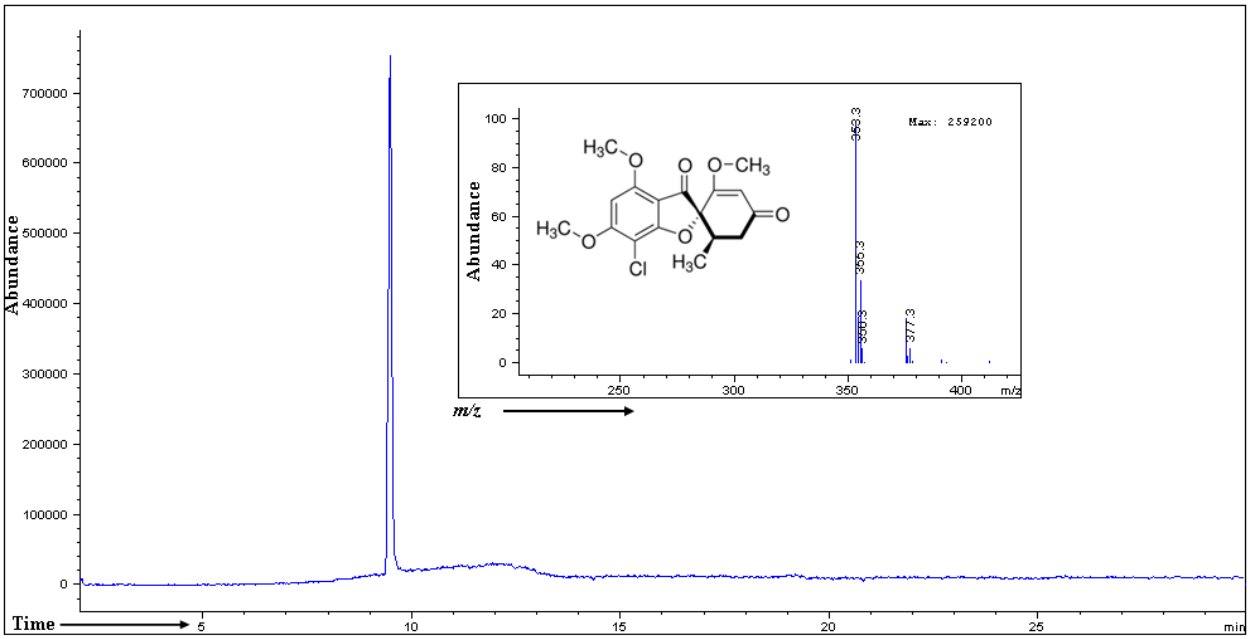
Appendix 2.7: Total ion chromatogram and mass spectrum for aflatoxin B_2 standard (m/z = 315 and retention time 5.824mins)



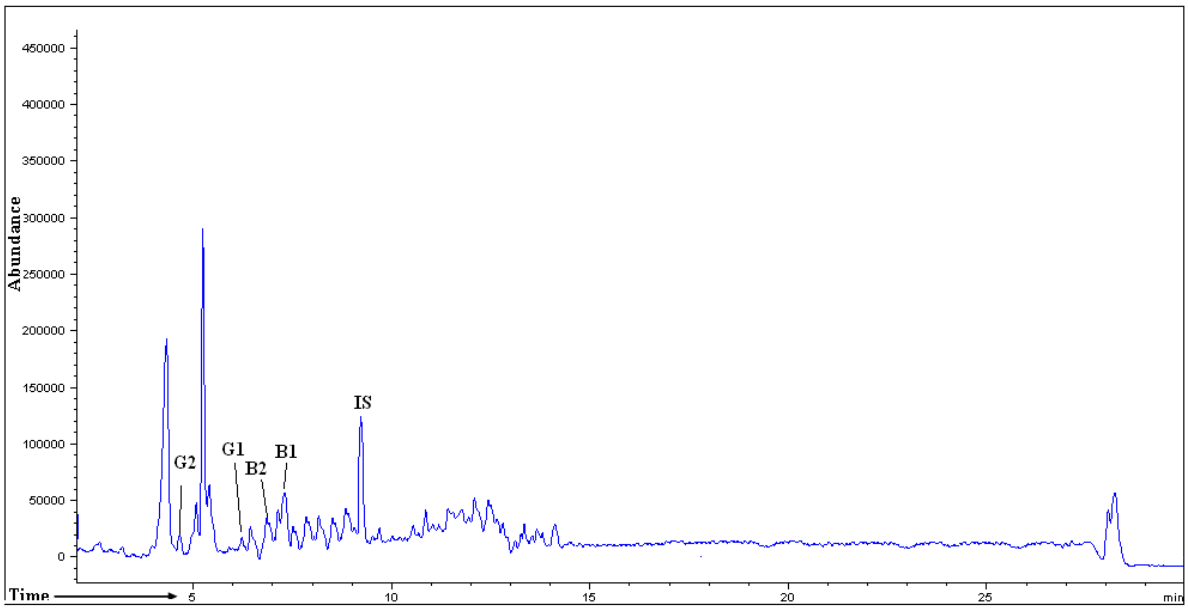
Appendix 2.8: Total ion chromatogram and mass spectrum for aflatoxin G_1 standard (m/z = 329 and retention time 5.229mins)



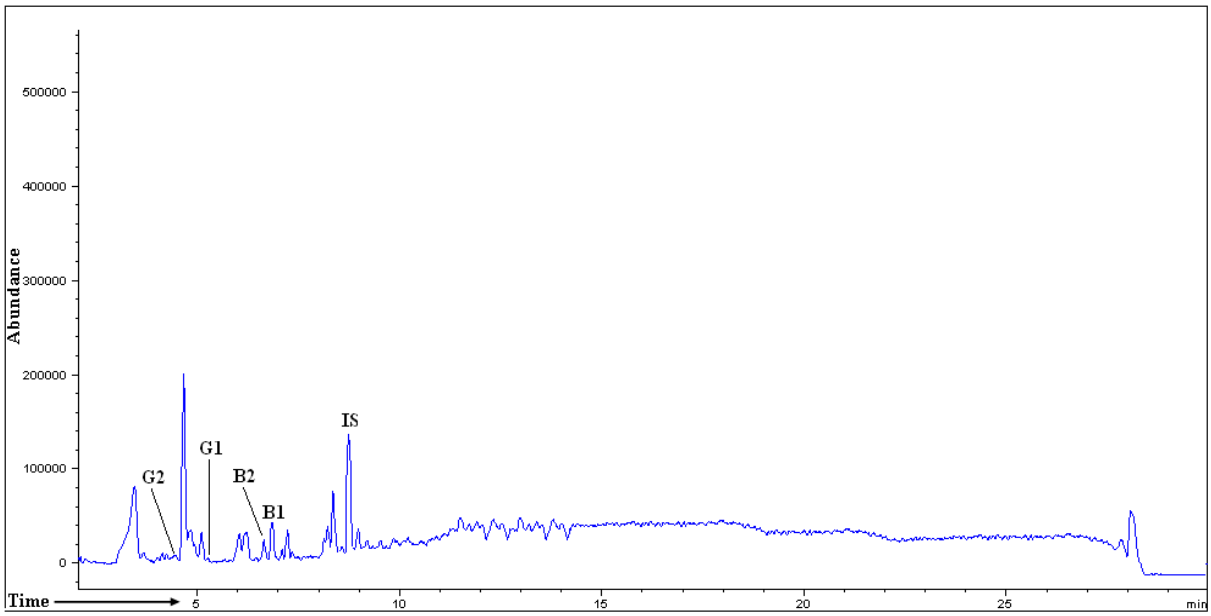
Appendix 2.9: Total ion chromatogram and mass spectrum for aflatoxin G_2 standard (m/z = 331 and retention time 4.87mins)



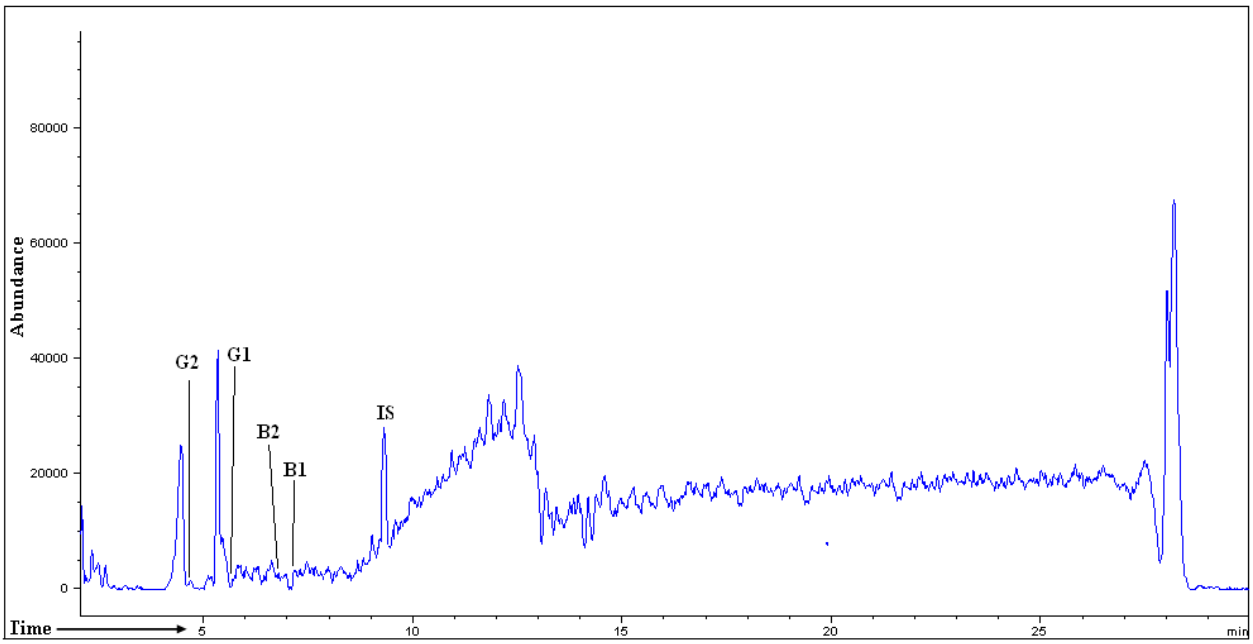
Appendix 2.10: Total ion chromatogram and mass spectrum for Griseofulvin (Internal Standard) (m/z = 354 and retention time 9.712mins)



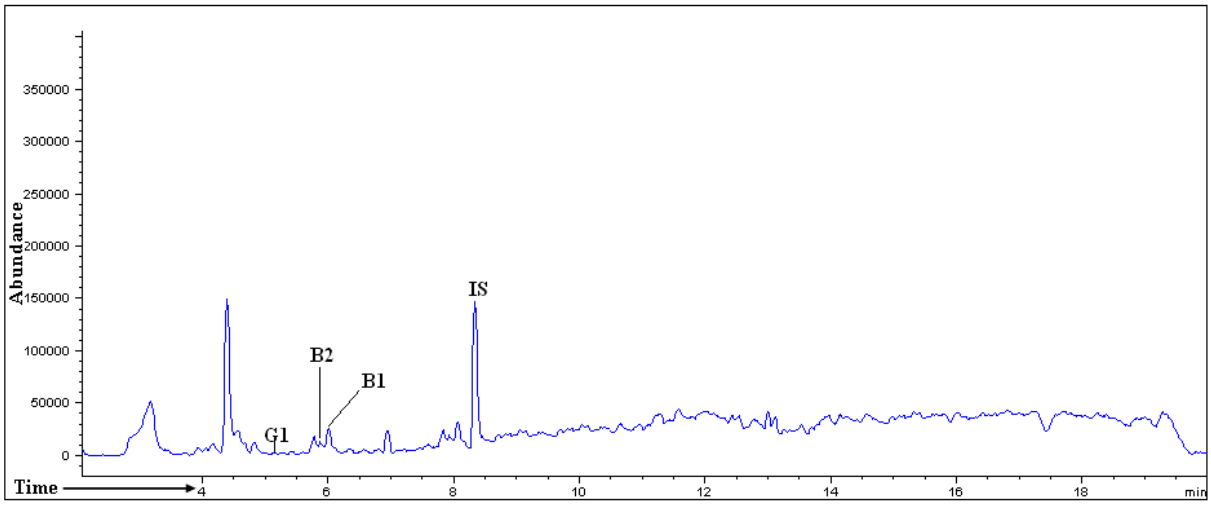
Appendix 2.11: Representative total ion chromatogram for control at 40 °C



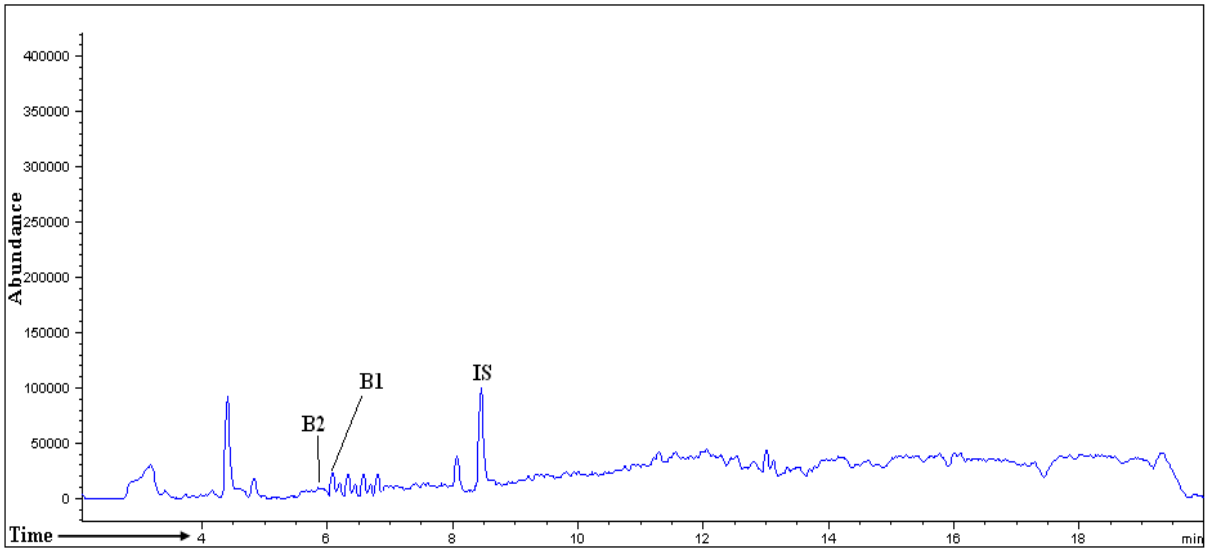
Appendix 2.12: Representative total ion chromatogram for sample 1:10 at 30 °C



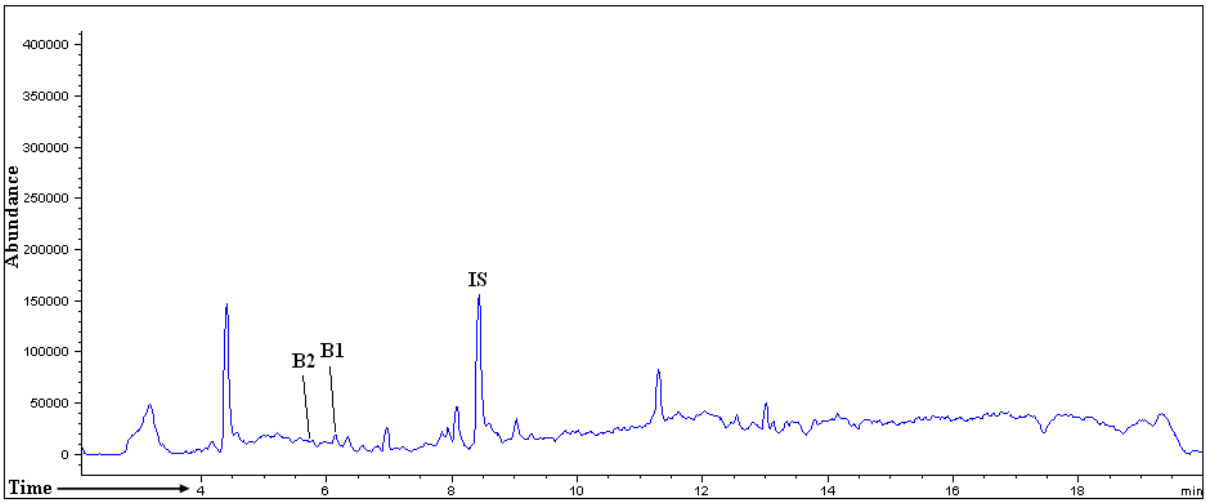
Appendix 2.13: Representative total ion chromatogram for control at 20 °C



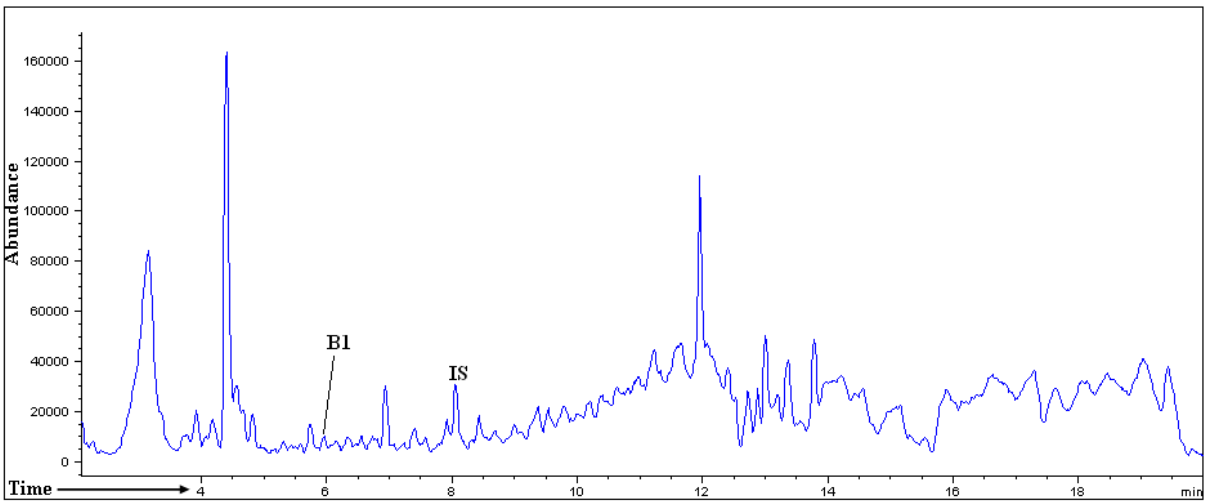
Appendix 2.14: Representative of total ion chromatogram for sample 13 from Kitui County of lower eastern in Kenya



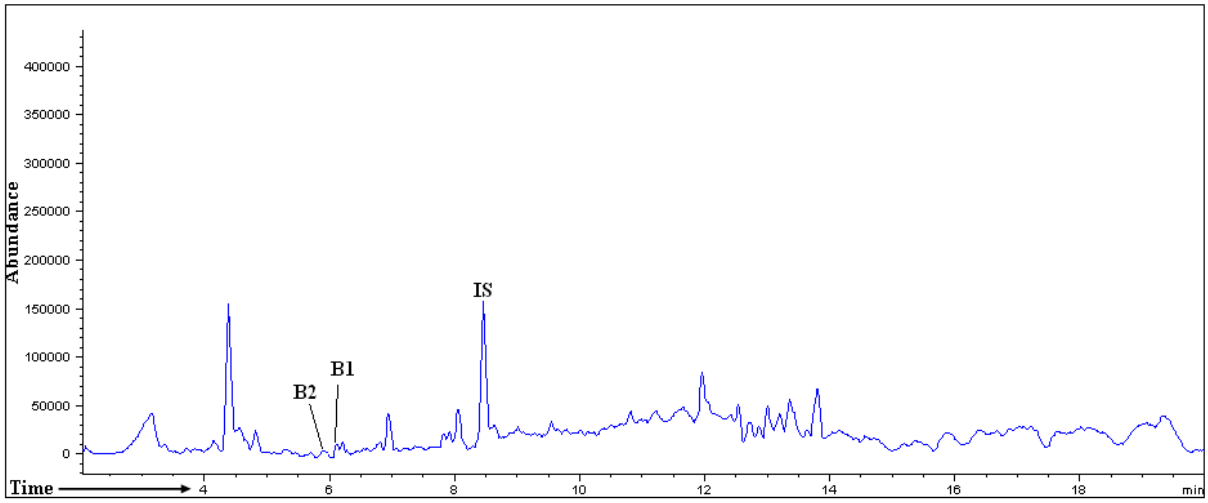
Appendix 2.15: Representative of total ion chromatogram for sample 16 from Kitui County of lower eastern in Kenya



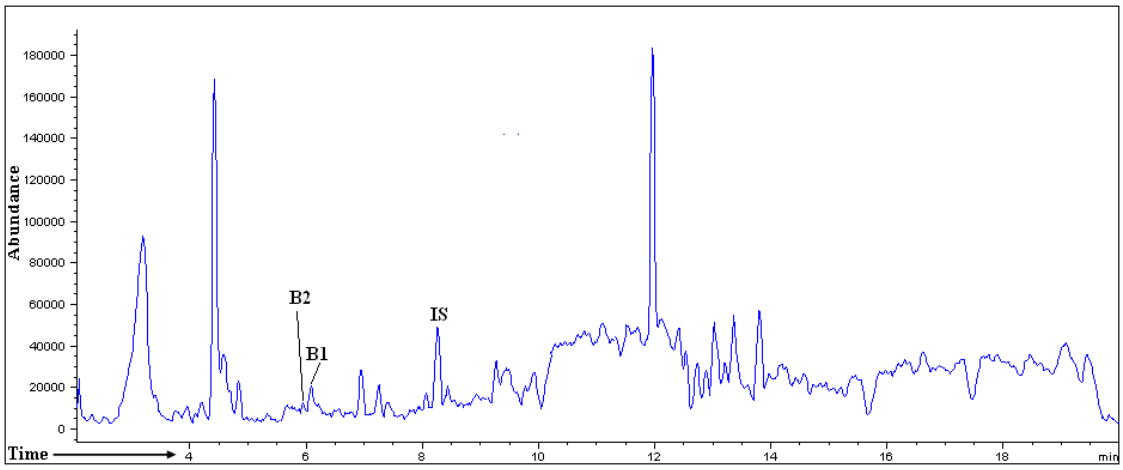
Appendix 2.16: Representative of total ion chromatogram for sample 19 from Machakos County of lower eastern in Kenya



Appendix 2.17: Representative of total ion chromatogram for sample 24 from Machakos County of lower eastern in Kenya



Appendix 2.18: Representative of total ion chromatogram for sample 6 from Makueni County of lower eastern in Kenya



Appendix 2.19: Representative of total ion chromatogram for sample 8 from Kitui county of lower eastern in Kenya

Appendix 3: Procedure used to calculate the actual amount of aflatoxin in the maize samples

The following procedure was used to extract aflatoxin from the maize samples;

1. Ten grams of maize four was weighed from a stock sample and put into falcon tube.
2. 40mls of AcN 86:16 water was added,
3. 40µL of internal standard – (Griesofluvin 5mg/ml) was added,
4. Shake for 30 minutes and leave to settle for 30 minutes
5. 6mls of the supertanant was filtered through multistep 228 Aflapat column
6. 4mls of the eluate was evaporated to dryness in the hood
7. 400µL of methanol 20:80 water was added to re-dissolve the contents
8. Samples analyzed in the Lc-Ms for 20 minutes.

Maize samples in 40 °C control experiment were used to give guidelines on how the rest of the samples were done.

B1 in control

Retention time = 6.157 (appendix 2, figure 11), Area covered 2.56E+5 (average of 3 replicates), volume injected to Lc/Ms was 10µL

If 10µL covered area of 2.55067433e5, 1µL will cover 2.55067433e4

Using equation from the standard calibration curve (Appendix 2.0 figure1) whereby y=13765x, then x=y/13765, but y=2.55067433e4.

X=2.55067433e4/13765 =1.853014ng/µL

But, 4mls was evaporated and concentrated to 400µL concentration factor of 10.

Therefore in 4mls will have a concentration of 0.1853014ng/µL.

If in 4mls has a concentration of 0.0888296, 6mls will have 6/4 x 0.1853014 = 0.27795ng/µL

0.27795ng/µL is equivalent to 0.27795/0.001ml = 1277.95ng/ml

277.95ng/ml is in 6mls, what about 40.04mls?

Therefore, = 40.04/6 x 277.95 = 1854.87ng/ml,

Thus, 10g contains 1854.87ng of aflatoxin implying that the concentration in a gram is 185.487ng/g equivalent to **185.487ppb**.

B2 in control

Retention time = 5.824 (appendix 2, figure 11), Area covered 6.17E+4, (average of 3 replicates), volume injected to Lc/Ms was 10µL.

If 10µL covered area of 6.171759E4, 1µL will cover 6.17176E3

Using equation from the standard calibration curve (appendix 2, figure 2) whereby y=14117x, then x=y/14117, but y=6.17176E3.

X=6.17176E3/14117 =0.437186ng/µL

But, 4mls was evaporated and concentrated to 400µL concentration factor of 10.

Therefore in 4mls will have a concentration of 0.0437186ng/µL implying that 6mls will have 6/4x0.0437186 = 0.065578ng/µL.

0.065578ng/µL is equivalent to 0.065578/0.001ml = 65.578ng/ml.

6mls contains 65.578ng/ml, what about 40.04mls?

This equals to 40.04/6x65.578ng/ml = 437.842ng/ml

Therefore 10g contains 437.842ng/ml meaning 1 g will have 43.7842ng/g=**43.7842ppb**

G1 in control

Retention time = 5.229 (appendix 2, figure 11), Area covered 3.46+04 (average of 3 replicates), volume injected to Lc/Ms was 10µL

If 10µL covered area of 3.45945E4, 1µL will cover 3.45945e3

Using equation from the standard calibration curve (appendix 2 figure 3) whereby y=14332x, then x=y/14332, but y=3.45945e3.

X=3.45945e3/14332 =0.24138ng/µL

But, 4mls was evaporated and concentrated to 400µL concentration factor of 10.

Therefore in 4mls will have a concentration of 0.24138ng/µL, implying that 6mls will have 6/4x0.24138=0.0362069ng/µL

0.0362069ng/µL is equivalent to 0.0362069/0.001ml = 36.2069ng/ml.

6mls contains 36.2069ng/ml what about 40.04mls?

This equals to 40.04/6x 36.2069 = 241.6208ng/ml

Therefore 10g contains 241.6208ng/ml meaning 1g will have 241.6208/10 = 24.16208ng/g equivalent to **24.16208ppb**.

G2 in control

Retention time = 4.87 mins(appendix 2, figure 11) Area covered 9.91E3 (average of 3 replicates), volume injected to Lc/Ms was 10µL

If 10µL covered area of 9.906.57 then, 1µL will cover 9.90657E2

Using equation from the standard calibration curve (appendix 2 figure 4) whereby y=14248x, then x=y/14248, but y=9.90657E2.

X=9.90657E2/14248 =0.06953ng/µL

But, 4mls was evaporated and concentrated to 400µL concentration factor of 10.

Therefore in 4mls will have a concentration of 0.006953ng/µL, implying that 6mls = 6/4x0.006953 = 0.010429ng/µL

0.010429ng/µL is equivalent to 0.010429/0.001ml = 10.429ng/ml.

6mls contains 10.429ng/ml, what about 40.04?

This equals to 40.04/6x10.429 = 69.596ng/ml

10g contains 69.596, meaning 1g will have 69.596/10 = 6.9596ng/g = **6.9596ppb**

Final Calculations aflatoxin concentration in the above maize samples using internal standard to correct errors.

Average area covered by IS control 40 °C =2.58703e5 (average of 3 replicates) volume injected was 10µL and the standard calibration curve Equation, Y=15763x (appendix 2 figure 5)

Calculations;

If 10µL = 2.58703e5 then 1µL = 2.58703e4.

Using calibration curve equation, y=15763x, then x=y/15763. But y=2.58703e4.

Therefore, x=2.58703e4/15763= 1.61204ng/µL.

Using formulae; An/[An] = **F** x (IS/[IS])

Where IS = Area IS

An = Area Analyte

[IS] = Conentration IS

[An] = Concentration Analyte

Considering AFL B1 above at 10µL area covered is 2.550674e5 and concentration at same level = 1.8530ng/µL.

Then, 2.550674e5/1.8530ng/µL = F x 2.58703e5/1.641204 thus **F=0.8733**

Therefore, using the formulae **[An Corr] = F x [An]**, the correct AFL concentration for B1 will be **0.8733 x 185.487.**

Which equals to **161.98ng/g = 161.98ppb**.

Calculations for B2 above at 10µL area covered is 6.17175e4 and concentration at same level is 0.437186.

Using above equation, then 6.17175e4/0.437186=F x 2.58703e5/1.641204 thus **F= 0.8956**

Therefore, using the formulae **[An Corr] = F x [An]**, the correct AFL concentration for B2 will be **0.8956 x 43.92.** Which equals to **39.33ng/g = 39.33pbb**

Calculation for G1 above at 10µL area covered 3.45945e4 and concentration at same level 0.24138

Using above equation then, 3.45945e4 /0.24138=F x 2.58703e5/1.641204 thus **F = 0.9092**

Therefore, using the formulae **[An Corr] = F x [An]**, the correct AFL concentration for G1 will be **0.9092 x 10.19.**

Which equals to **9.26ng/g = 9.26ppb**.

Calculations for G2 above at 10µL area covered 9.90657e3 and concentration of 0.06953.

Using the above equation, then 9.90657e3/0.06953= F x 2.58703e5/1.641204 thus

F = 0.9039.

Therefore, using the formulae **[An Corr] = F x [An]**, the correct AFL concentration for G2 will be **0.9039 x 6.96.** Which equals to **6.29ng/g = 6.29ppb**.

The above formulae were used to calculate aflatoxin concentration in all maize samples.

Appendix 4: Supporting plates for laboratory and survey work

Appendix 4.1: Maize samples in the oven drying at *icipe* laboratory

Appendix 4.2: Hydrogel being weighed in the weighing machine at icipe laboratory

Appendix 4.3: Maize samples being tested moisture content by Agromatic moisture meter

Appendix 4.4: Replications and experiment set up of maize samples cultured in the microbiology laboratory in the University of Nairobi Upper Kabete Campus

Appendix 4.5: Maize stored in bags in a farmer's house in Makueni county in Kenya

Appendix 4.6: Maize stored in a granary in Kitui County in Kenya

Appendix 4.7: storage structure- granary in Machakos county in kenya

Appendix 4.8: LC-MS analysis machine in *icipe* BCED laboratory

Appendix 5: Isolation and identification of fungal species in maize samples collected from lower eastern part of Kenya.

Data collection sheet **Date …….**

County ……. **Replication No. …..**

sample	*Aspergillus spp*	*Fusarium spp*	*Penicillium spp*	Others	Remarks
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					

Key;

+ means present, - means absent, () number infected per petri dish

Appendix 6: Isolation and identification of *Aspergillus* spp in maize samples collected from lower eastern part of Kenya

Data collection sheet **Date …….**

Region ……. **Replication No …..**

sample	*A. flavus*	*A. fumigatus*	*A. parasticus*	*A. niger*	*A.Versicolor*	others	remarks
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							

Key;

+ means present, - means absent, () number infected per petri dish

Appendix 7: A summary of Anova tables of Aflatoxins in maize samples from lower eastern counties in Kenya and treatments of SAP to Maize at different temperatures

County	DF	Sum Sq	Mean Sq	F Value	Pr (>F)
Kitui	23	81997	3565	2168	P<0.001
Machakos	23	34470	1478.7	3298	P<0.001
Makueni	23	104558	4546	2824	P<0.001
Treatments					
20 °C	10	204871	20487	5499	P<0.001
30 °C	10	157073	15707	1367	P<0.001
40 °C	10	38748	3875	4715	P<0.001