# EFFECT OF PRODUCTION PRACTICES, STORAGE MATERIALS AND MOISTURE CONTENT ON FUNGAL AND AFLATOXIN CONTAMINATION OF MAIZE AND MAIZE PRODUCTS

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## DECLARATION

I declare that this is my original work and has not been presented for the award of a degree in any other university.

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## DEDICATION

To my wife Magdaline Wamaitha and our children Winfred, Evalyne, Maureen, Joy and Esther for their emotional support and encouragement.

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## ABSTRACT

Maize is the staple diet of majority of Kenyan population with a consumption of 400g $person^{-1}day^{-1}$. Several outbreaks of mycotoxicosis have been reported in Kenya almost on an annual basis in Eastern province since 1978. These outbreaks have caused a lot of concern because they have worsened the food situation, since maize is the main staple in almost every household. This study was therefore conducted to determine maize production practices, fungal and mycotoxins contamination of maize in Eastern and North Rift regions of Kenya and the effect of moisture content and storage materials on fungal growth and mycotoxins production.

A survey was conducted in Makueni, Machakos, Kitui, Uasin Gishu and Trans Nzoia districts during March-May 2008 cropping season. The survey only covered Eastern province during the October-December cropping season. Information gathered included agronomic practices, harvesting, drying, storage materials and structures used, transportation, processing and weather conditions during harvesting and storage. Samples of whole maize grain, semi-processed grain, flour, soils and posho mill sweepings were collected and fungal isolation was done on Czapek Dox agar medium. The fungi were identified based on cultural and morphological characteristics. The effect of storage materials and moisture content was determined by inoculating maize adjusted to different moisture levels with *Aspergillus flavus* and stored using sisal, synthetic or polythene bags. Re-isolation of *Aspergillus flavus* was done on Czapek Dox agar and Aflatoxin $B_1$ was determined by Enzyme Linked Immunosorbent assay (ELISA).

The study found differences in production and handling practices between Eastern and North Rift regions. Some of the practices could predispose maize to contamination with mycotoxin producing fungi. Such practices included planting of uncertified seeds, harvesting maize before safe moisture content, drying grain on bare ground and storage in living houses and in synthetic or polythene bags. Most farmers had good awareness on mycotoxins. Whole maize grain, maize products and soils were contaminated with mycotoxin producing fungi such as *Fusarium, Aspergillus* and *Penicillium. Fusarium*

was the most commonly isolated pathogen. The *Aspergillus* species isolated included *A. flavus, A. niger, A. ochraceus, A. clavatus, A. fumigatus, A. terreus* and *A. versicolor*. Among the *Aspergillus* species, *A. flavus* had the highest frequency of isolation in both Eastern and North Rift regions. Maize sampled from Eastern region had a higher frequency of isolation for *A. flavus* than in the maize samples from North Rift region. Frequency of isolation for *A. flavus* was higher in semi-processed than in maize samples and flour had the lowest frequency of isolation. *Fusarium subglutinans* was the most predominant species in Eastern region while *F. proliferatum* was dominant in North Rift. Aflatoxin $B_1$ was detected in maize and maize products at levels above the national legal limit of 10µg/kg. The maize products and regions differed in the amount of aflatoxin $B_1$. Samples from Eastern regions had higher aflatoxin levels of upto 136.4 µg/kg. Contamination of grain was upto 77.4 µg/kg while semi-processed maize had higher levels of upto 136.4 µg/kg. Maize flour had levels of upto 40.9 µg/kg.

Moisture level and storage materials significantly affected the growth of *Aspergillus flavus* in inoculated maize during storage. Maize stored in sisal and synthetic bags had lower kernel infection with *A. flavus* than polythene bags. There were no significant differences in kernel infection between sisal and synthetic bags. The number of discoloured and mouldy grain and kernel infection were significantly affected by moisture level and storage materials. Moisture levels above 13% encouraged higher infection with *A. flavus*. The highest kernel infection with *A. flavus* was promoted by 18% moisture content.

The result of the study showed that some of the maize production and handling practices in Eastern Province predisposes the maize to fungal and mycotoxin contamination. In addition, the favourable conditions created by high temperatures and periodic drought, contributed to the higher fungal and aflatoxin contamination of samples from this region. The study also confirmed that unfavourable drying and storage practices like planting uncertified seeds, harvesting maize with high moisture content and storage in living houses was compounding the problem. Therefore, there is need for continued mycotoxin awareness campaigns to educate farmers, traders, transporters and processors.

# CHAPTER ONE: INTRODUCTION

## 1.1 Maize production and consumption in Kenya

Maize was introduced in Kenya in the 16th century from Portugal (Export Processing Zone Authority (EPZA), 2005) and is heavily dependent on rainfall. Only less than 20% of Kenya receives adequate rainfall for maize production. This area is mostly at higher altitudes in Rift Valley, Central and Western Kenya (Allan, 1971). Despite this, maize is grown in a wide range of agro-ecological zones that allows maize to grow irrespective of limiting temperature and rainfall environments (Wokabi, 1997).

Maize is the most important staple food for the majority of the Kenyan population (EPZA, 2005), and it is consumed as a dietary staple at the level of 400gm per person per day (Muriuki and Siboe., 1975; Shephard, 2008). Despite great efforts made to increase maize production, the demand has occasionally outstripped supply, requiring importation of large quantities of maize grain (Wokabi, 1997). Production is widely distributed, with commercial production mainly in the Rift Valley province. Maize constitutes 3% of Kenya's Gross Domestic Product (GDP), 12% of the Agricultural GDP and 21% of the total value of primary agricultural commodities (De Groote *et al.*, 2003). The crop is grown in almost all the agro-ecological zones and on two out of every three farms in Kenya. It accounts for 40% of daily calories and the country has a per capita consumption of 98 kg, which translates to between 30 and 34 million 90kg bags (2.7 to 3.1 million metric tonnes) of annual maize consumption.

The country produces an average of 28 million bags annually and the deficit is bridged by imports from neighbouring countries (Kibaara, 2005). During bumper harvests, Kenya exports its maize to Tanzania, Uganda, Rwanda, Zaire, Sudan and Ethiopia among other countries and imports from USA, South Africa, and Zambia when faced with deficits (EPZA, 2005). About 3.5 million small- scale farmers are involved in maize production mainly for household consumption and produce 75% of the total maize crop. Large scale farms account for the remaining 25% and are estimated to be just about one thousand farms (Ministry of Agriculture (MOA), 2001; EPZA, 2005).

In 2005, an area measuring about 1,760,914 Ha was planted with maize and the yield realised was 32,423,963 bags of 90 kgs. In the year 2006, 1,888,185 Ha were planted with maize and the production went up by 11% to stand at 36,086,406 (90 kgs) bags which translate to 3,247,777 metric tonnes (MOA, 2007). Production in 2007 stood at 32,542,143 bags or 2.928,723 tons from 1,615,304 hectares. Productivity of maize as a national staple food item declined in 2008 to 14.7 bags per hectare from an average of 20.1 bags/Ha in 2007. The area under maize increased by 11% to 1,793,757 Ha but the total production dropped by 19.2% to 26, 302,219 (90 kgs) bags. The trend was attributed to the high cost of farm inputs including fertilizers and diesel (MOA, 2009). In 2009, the area under the crop went up by 6.1% to stand at 1,903,440 Ha and total production increased by 20.7 % to 31,738,951 bags (MOA, 2010). The target for maize production in the Kenya currently stands at 2 tonnes per hectare, with an upward potential of up to 6 tonnes per hectare.

Maize production is affected by environmental factors, pests and diseases. Among the environmental factors, unreliable rainfall, drought and moisture stress are the most important (Odour, 2006). Poor soil fertility, especially lack of nitrogen and phosphorous, inadequate supply and high cost of improved certified seeds are also serious production constraints. During harvesting, it is important to control factors such as timeliness, clean-up and drying of the agricultural product. Such control is essential for preventing mycotoxin formation during storage.

Although farmers are well aware that timely harvesting reduces fungal infection of the crops in the field and consequent contamination of harvested produce by mycotoxins, they are compelled to harvest at inappropriate times by; unpredictable weather, labour constrains, need for cash, threat of thieves, rodents and other animals (Wagacha and Muthomi, 2008). Grain moulding is one of the most important factors contributing to deterioration of quality in stored maize. The infection of maize by several mycotoxin producing fungi can cause serious hazards for human and animal health. In 2004, locally produced maize associated with aflatoxin poisoning outbreak in Makueni was harvested

in February during peak rains, and the first illnesses were reported in late March and early April (Lewis *et al.*, 2005).

### 1.2 Problem statement and Justification

Maize has been adopted as a primary staple food for people living in Sub-Africa, particularly those living in rural areas (Kimanya *et al.*, 2008). It is vulnerable to degradation by mycotoxigenic fungi. 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). They are produced by fungal action during production, harvest, transportation, storage and food processing (Council for Agricultural Science and Technology (CAST), 2003; Murphy *et al.*, 2006). Their production is unavoidable and depends on a variety of environmental factors in the field and/or during storage. Stress caused by environmental extremes, shortage of food or competition from other micro organisms are the main production factors (Windham and Williams, 1998). Mycotoxin contamination is unavoidable and unpredictable, which makes it a unique challenge to food safety (Lopez-Garcia *et al.*, 1999). It attracts worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade (Wagacha and Muthomi, 2008). Mycotoxins are capable of causing disease and death in humans and animals (Bennett and Klich, 2003)

Mycotoxicosis occurs in human and animals through ingestion, inhalation and absorption through the skin (Park and Liang., 1993; Wagacha and Muthomi., 2008). High-level exposure may cause instant death while long-term effects include cancer, mutagenicity and nervous disorders (Kenya Plant Health Inspectorate Service (KEPHIS), 2006). Chronic exposure to low levels of aflatoxin in contaminated maize is likely to occur than acutely toxic exposure which is experienced during an outbreak, and this leads to cancer and represents a serious public health concern (Roebuck *et al.*, 1994). Mycotoxins are also important due to ubiquitous presence of aflatoxigenic fungi in all the agricultural commodities under field and storage conditions (Reddy and Raghavender., 2007). In the

fields as well as in the store, many pests and parasites attack maize during the storage period (Fandohan *et al.* 2003).

Insects are most often considered as the principal cause of grain losses. However, fungi are also important and rank second as the cause of deterioration and loss of maize (Fandohan *et al.*, 2003). It is reported that fungi can cause about 50 – 80% of damage on farmers' maize during storage period if conditions are favourable for their development (Fandohan *et al.*, 2003). Maize contamination by fungi does not only render grains unfit for human consumption by discolouration and reduction of nutritional value but can also lead to mycotoxin production. The Food and Agricultural Organization (FAO), estimates that between 25% and 50% of agricultural crops worldwide is contaminated by mycotoxins (Fandohan *et al.*, 2003; Lewis *et al.*, 2005; Wagacha and Muthomi, 2008). The direct impact of mycotoxins on the staple product quality constitutes an important danger for human health (Fandohan *et al.*, 2003).

At the farm level, mould growth can result in reduced crop yields and livestock productivity stemming from illness or death due to consumption of contaminated feed (Murphy *et al.*, 2006). Unfortunately, information on toxicity, stability and extent of occurrence is limited for many of the mycotoxins that have been identified (Lopez-Garcia *et al.*, 1999). The real problem is that maize cobs, although contaminated, may appear non-contaminated. This is because there are no outward physical signs of fungal infection. In food manufacturing, destruction of mycotoxins by conventional food processing is difficult because they are typically resistant and detection is complicated due to limitations in analytical methodology. Mycotoxins can be a hurdle to international trade, leading to increased regulation of foods and feeds that may contain them and removal from the market of commodities not meeting regulatory limits (Murphy *et al.*, 2006).

Several factors may enhance the occurrence of mycotoxin in the human diet. These include eating habits, existing marketing problems which encourage long storage periods; the pre and post harvest practices that encourage build up of moisture and thus encourage

mould growth; lack of preparedness in handling the large quantities of maize produced due to the success of hybrid maize technology, ignorance and poverty (Moturi, 2008). These recurring outbreaks of aflatoxin poisoning suggest that there are factors that favour fungal growth and production of mycotoxins. There are concerns that aflatoxins could be co-occurring together with the other mycotoxins and people could be consuming maize contaminated with mycotoxins, but because of the slow poisoning, the effects are not immediately visible.

It was therefore necessary to carryout a study that was to determine the occurrence of different types of mycotoxins in maize, the level of contamination and distribution. The study also determined the effect of moisture content and different storage materials used by farmers on fungal growth and mycotoxin production during storage.

The main objective of the study was to determine the distribution and types of mycotoxin producing fungi and the factors that lead to mycotoxin contamination.

The specific objectives were:

1. To determine the effect of production practices on the levels and distribution of fungal and aflatoxin contamination in maize

2. To determine the effect of grain moisture content and storage materials on fungal growth and aflatoxin production during storage.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Mycotoxins associated with maize

The natural fungal flora associated with foods is dominated by three fungal species belonging to the genera *Aspergillus, Fusarium*, and *Penicillium* (Kumar *et al.*, 2006; Krska, 2008). Mycotoxins of importance in foods include aflatoxins, zearalenone, fumonisins, ochratoxin, deoxynivalenol and trichothecenes (Kumar *et al.*, 2000; Shephard, 2008; Krska *et al.*, 2008).

Aflatoxins are a group of toxic, carcinogenic fungal metabolites produced by two species of fungi, *Aspergillus flavus* and *Aspergillus parasiticus* in several agricultural commodities including maize and groundnuts (Garber et al. 1997; Kaaya and Warren, 2005; Gonzalez *et al.*, 2008; Morenoa *et al.*, 2009). In 1993, the International Agency for Research on Cancer (IARC) classified $AFB_1$ and mixtures of aflatoxins as group 1 carcinogens (IARC, 2002; Kimanya *et al.*, 2008; Wagacha and Muthomi, 2008). The toxins can be produced in the field prior to harvest, or under poor storage conditions (Shephard, 2008; Mutungi *et al.*, 2008). The aflatoxin-producing *Aspergillus flavus* and *Aspergillus parasiticus* occur widely on inadequately dried food and feed grains in sub-tropical and tropical climates throughout the world (Food and Agricultural Organisation (FAO), 1994). *Aspergillus flavus* is commonly found in soil and crop debris, which act as the principle source of primary inoculum for infecting maize (Atehnkeng *et al.*, 2008; Jaime-Garcia and Cotty, 2004; Stack and Carlson, 2006).

Crop residues including corn cobs are the main source of organic matter in soils where crops are produced and these support large populations of the fungus (Jaime-Garcia and Cotty, 2004). The optimum conditions for growth and subsequent production of aflatoxins by *A. flavus* include moisture content above 14%. The optimum temperature ranges between 28°C to 35°C and water activity of 0.83-0.97. The oxygen to carbon dioxide ratio, physical integrity of the grain, initial levels of mould infection, pest activity and genetic properties of the grain also determine the degree of contamination (Diener *et*

*al.*, 1987, Mutungi *et al.*, 2008). Isolates of *A. flavus* vary greatly in aflatoxin production, with some producing copious amounts while others are non-toxigenic.

The aflatoxins were first isolated and characterised in 1960 after the death of more than 100,000 turkey poults (Turkey X disease) was traced to the consumption of a mould contaminated peanut meal in the United Kingdom (Bennett and Klich, 2003; Phillips *et al.*, 2007; Shephard., 2008). In this incident, the affected animals showed signs of severe liver necrosis as well as fatty degeneration, fibrosis and extensive bile duct hyperplasia. Upon investigation it was discovered that the turkeys had been fed on Brazilian peanut meal containing the mould *Aspergilllus flavus* and contained four metabolic by-products, namely, aflatoxin $B_1$, $B_2$, $G_1$, and $G_2$ (Phillips *et al.*, 2008). There are four major groups of aflatoxins which are $B_1$, $B_2$, $G_1$ and $G_2$ based on their fluorescence (Blue or green) under ultra violet light (CAST, 2003; Bennett and Klich. 2003; Kimanya *et al.*, 2008). *Aspergillus flavus* grain mould on corn is often characterized by visible light green mould on the surface of the kernels. This surface mould can develop anywhere on the ear, but is most often observed at the base of the ear (Stack and Carlson, 2003).

Aflatoxin $M_1$ is a metabolite of aflatoxin $B_1$ in mammals and it is found in the milk of animals eating feeds contaminated by aflatoxin B1 (Kaaya and Warren, 2005). When cows are fed on aflatoxin-contaminated feeds, they metabolically biotransform aflatoxin $B_1$ into a hydroxylated form $AFM_1$ (Bennett and Klich, 2003). Aflatoxin $B_1$ ($AFB_1$), a potentially lethal metabolite, is a known human carcinogen (Group 1 A) and implicated in hepatocellular carcinoma (International Agency for Research on Cancer (IARC) 1993; Phillips *et al.*, 2007). It is toxic to the liver, and has immunosuppressant, hepatocarcinogenic, teratogenic, and mutagenic effects on humans (Bennett and Papa, 1988; Park and Liang, 1993; Bennett and Klich, 2003; Tedesco *et al.* 2008; Gonzalez *et al.*, 2008; Kimanya *et al.*, 2008).

Fumonisins are carcinogenic mycotoxins produced by several *Fusarium* species (Noonim et al., 2009, Gelderblom *et al.*, 1998) and have been reported in many food commodities, especially maize (Marin *et al.*, 2004). The IARC declared Fumonisin $B_1$ as a Group 2

carcinogenic agent (Kimanya *et al.*, 2008). The major species that produce fumonisins in maize are *Fusarium verticilloides* (formally *F. moniliforme*) and *Fusarium proliferatum* (Chen *et al.*, 1992). *F. verticillioides* is a causal agent of symptomless infection of maize, in which all fumonisins can also be present although usually at low levels. Among the fumonisins characterised include $FA_1$, $FA_2$, $FB_1$, $FB_3$, $FB_4$, $FC_1$, $FC_3$, $FC_4$, $FAK_1$, $FP_1$, $FP_2$, $FP_3$, $FPH_1a$ and $FPH_1b$. Fumonisin $B_1$ is the most toxic and the most abundant comprising from 60 to 90% of the fumonisins found (Carmargos *et al.*, 2000). Attention has focused on fumonisins because of their widespread occurrence, acute toxicity to certain livestock and their potential carcinogenicity (Duvick, 2001).

Zearalenone, also called F-2 toxin, is a non-steroidal estrogenic mycotoxin produced as a secondary metabolite by several species of *Fusarium,* particularly *Fusarium graminearum* (or *Giberella zeae*), *F. semitectum, F equiseti, F. crookwellense* and *F. culmorum* (Mirocha *et al.*, 1971; Bennett and Klich., 2003). This toxin may also be produced by *F. tricinctum, F. moniliforme, F. oxysporum, F. sporotrioichides* and *F. laterium* (Mirocha *et al.*, 1971). Zearalenone production does not seem to occur in significant amounts prior to harvest. Alternating low temperatures in storage promote production of this toxin. Temperatures between 11.6°C to 13.8°C induce the enzymes involved in biosynthesis of this toxic substance, and optimum production occurs at 27.2°C (DeWolf *et al.*, 2003; Sangare-Tigori *et al.*, 2005). Zearalenone is found as a contaminant in maize but also may occur in oats, barley, wheat and sorghum (CAST, 2003). Zearalenone is a stable compound both to storage/milling and the processing/cooking of food. It does not degrade at high temperatures and wet milling increases the level of zearalenone in the gluten fraction by 2 – 7 fold concentration (Scientific Committee on Food (SCF), 2000).

Deoxynivalenol (DON) which is also known as vomitoxin, is one of 150 related compounds known as trichothecenes that are formed by a number of *Fusarium* spp and other fungi. It is generally found in various cereal crops such as wheat, barley, oats, rice, rye and maize (Instanes and Hetland, 2004). DON is produced by *Fusarium graminearum* (*Giberella zeae*) and *Fusarium culmorum* among other *Fusarium* species

on maize and wheat prior to harvest as well as during storage (Pestka and Smolinski, 2005; Ericksen, 2004). Both *Fusarium graminearum* and *Fusarium culmorum* have different optimum temperatures for growth (25°C and 21°C respectively) and this probably affects geographical distribution (Moss, 2002). DON can be found in many post-harvest products, it is believed to be resistant to standard processes like milling, baking, and heating (Pestka and Smolinski, 2005). DON is stable under weakly acidic conditions but is unstable under alkaline conditions (Yazar and Omurtag, 2008). DON is detected often at parts per million (ppm) level (Pestka, 2007; Yazar and Omurtag, 2008).

Mycotoxins T-2 and HT-2 generally occur together in the infected cereal products (Ericksen, 2004). They are generally found in various cereal crops such as wheat, corn, barley, oats, rye and processed grain products like malt, beer and bread (SCF, 2001). They have been reported to be produced by *F. sporotrichioides*, *F. poae, F. equiseti* and *F. acuminatum. F. sporotrichioides* of the sporotrichiella section is the most producer of T-2 and has no known teleomorph. It is basically a saprophyte species (non-pathogenic to plants) and is especially associated with cereals left in the fields after normal harvest (Yarzar and Omurtag, 2008). *F. sporotrichioides* grows at -2°C to 35°C and only at high water activities (Creppy, 2002). The optimum temperature production of T-2 is relatively low at 8 – 14°C, with yields being much lower or negligible at temperatures of 25°C and above (Yazar and Omurtag, 2008).

Nivalenol is one of the well known mycotoxins among naturally occurring trichothecenes (Ito *et al.*, 1986). *Fusarium cerealis* and *F. poae* are the main producers of nivalenol, but isolates of *F. culmorum* and *F. graminearum* are also able to produce nivalenol (Ericksen, 2004). Nivalenol occurs in various cereal crops such as wheat, corn, barley, oats, and rye (Ito et al., 1986). It occurs more often in years with dry and warm growing seasons.

Ochratoxin A (OTA) is a frequent natural contaminant of many foodstuffs such as cocoa beans, coffee beans, cassava flour, cereals, fish, peanuts, dried fruits, wine, poultry eggs and milk (Wagacha and Muthomi, 2008). It was discovered as a metabolite of *Aspergillus*

*ochraceus* in 1965 during a large screening of fungal metabolites that was designed to identify new mycotoxins (Bennett and Klich, 2003). Toxin production occurs over a wide temperature range. Optimal conditions for production are at a temperature range between 20-25°C and crop moisture of 16% or above. This toxin is produced by some species of *Penicillium* and *Aspergillus* in cereal grains. The major species implicated in the production includes *A. ochraceus*, *A. carbonarius, A. melleus, A. sclerotiorum, A. sulphurous,* and *A. glaucus. Aspergillus niger* is a less important OTA producer (Bennett and Klich, 2003). Ochratoxin A is also produced by *Penicilliun verrucosum* (Table 2.1) which is a common contaminant of barley and it is the only confirmed producer of ochratoxin in this genus (Bennett and Klich, 2003).

**2.2. Mycotoxin contamination and aflatoxicosis in Kenya**

From the African perspective, aflatoxins and fumonisins have been estimated to be widespread in major dietary staples. Although aflatoxins occur mostly in maize and groundnuts, the prevalence of fumonisins is 100% in all surveillance data reported on maize from different parts of Africa (Bankole *et al.*, 2006). Outbreak of mycotoxicosis in Kenya received publicity in 1978 and again in 1984-85 when a large number of dogs and poultry died due to aflatoxin poisoning (Manwiller, 1986). In 1978, the government chemist department collected 336 samples of food and feed. Of these, 52 samples exceeded 150ng/g of aflatoxin with the highest being 3000ng/g, which was found in dog meal (Manwiller, 1986). One of the reasons for high aflatoxin levels may have been that a bumper crop was produced in the 1977-78 season which was far in excess of the capacity of storage facilities. Maize was imported from several countries including the USA in 1984-85 which was a famine period. At least one ship load of incoming maize was ordered dumped in the ocean as high levels of aflatoxin made it unfit for use as human food or animal feed (Manwiller, 1986).

Table 2.1: Some of the mycotoxins, their source and potential toxicities

| Toxin | Producing fungi | Toxicity |
|---|---|---|
| Aflatoxin | *A. flavus, A. parasiticus, A. nomius, A. pseudotamarii and A. tamarii* | Hepatocarcinogen, and fatty liver |
| Fumonisin | *Fusarium verticillioides, F. proliferatum* | Disruption of spinolipid metabolism |
| Citreoviridin | *Penicillium viridicatum* | Cardiac beri-beri |
| Citrinin | *Penicillium vindicatun, P. citrinum* | Nephrotoxin |
| Cyclochlorotine | *Penicillium islandicum* | Hepatotoxin |
| Cytochalasin E | *Aspergillus clavatus* | Cytotoxicity |
| Maltoryzine | *Aspergillus oryzae* | |
| Ochratoxins | *Aspergillus ochraceus, A. verrucosum* | Hepatotoxin |
| Patulin | *Penicilliumc-expansum* *Penicillium patulum* | Brain and lung hemorrhage and carcinogenicity |
| PR Toxin | *Penicillium requeforti* | |
| Rubratoxin | *Penicillium rubrum* | Liver hemmorrhage and fatty infiltration |
| Sterigmatocystin | *Aspergillus flavus, A. versicolor* | |
| Tremorgens | *Penicillium* and *Aspergillus* | |
| T-2 and HT-2 | *F. sporotrichioides, F. poae, F. equiseti, F. acuminatum* | Cytotoxicity |
| Vomitocin (Deoxynivalenol) | *F. graminearum, F. culmorum* | Vomiting |
| Zearalenone | *Fusarium graminearum, F. culmorum, F. semitectum, F equiseti, F. crookwellense* | Hyper-estrogenic effect |
| Rugulosin | *Penicillium islandicum* | Nephrosis and damage of the liver |

Source: Suttajit, 2003; Yazar and Omurtag, 2008.

Aflatoxin poisoning has caused disease and death of many people in rural areas of Eastern and Central province (Lewis *et al.*, 2005). In 1981, 20 cases of aflatoxin poisoning were reported in Machakos district of Eastern Province and out of these, 12 people died (KEPHIS, 2006; Daily Nation, 2010). In the year 2001, twelve people were reported dead due to consumption of aflatoxin- contaminated maize in Meru. In this case the contaminated maize was destroyed and no further action was taken.

The worst outbreak was between March and July 2004 when 317 cases were reported with 125 deaths confirmed in Eastern and Central Provinces (Bennett and Klich., 2003; Lewis et al., 2005; KEPHIS, 2006, Probst et al., 2007; Shephard, 2008). This was the worst outbreak ever reported in the world only second to another that occurred in 1974 in India where 397 cases were recorded, with 108 being fatal (KEPHIS, 2006). Public health officials sampled maize from the affected area and found concentrations of aflatoxin $B_1$ as high as 4,400 ppb, which is 440 times greater than the 10 ppb limit for food suggested by Kenyan authorities (Reddy and Raghavender, 2007).

Although aflatoxicosis outbreaks have occurred periodically in Africa and Asia, the 2004 outbreak resulted in the largest number of fatalities ever documented (Reddy and Raghavender, 2007). In 2005, another outbreak in Makueni and Kitui districts affected 75 people with 32 deaths (Table 2.2). A report by the Ministry of Agriculture in Kenya indicates that in 2003, 68 people died of aflatoxin poisoning in Eastern Province. In 2006, another outbreak claimed 28 lives out of the 78 cases were reported in Eastern province (MOA, 2008). In 2007, 84 cases of aflatoxin poisoning were reported and these resulted in 21 deaths in Eastern Province (MOA, 2008). Out of these, 16 of the fatal cases occurred in Igembe District (KEPHIS, 2006). In 2008, the ministry of Agriculture reported 2 fatal cases out of the 6 reported cases. These cases occurred in remote villages and the case findings were based on those who reported to health centres, implying that the numbers could be higher (Lewis et al., 2005).

Table 2.2: Cases of aflatoxin poisoning reported in Kenya

| Year | Number of cases | Number of deaths | Areas of occurrence |
|---|---|---|---|
| 1981 | 20 | 12 | Machakos District |
| 2001 | - | 12 | Meru |
| 2003 | - | 68 | Eastern Province |
| 2004 | 317 | 125 | Thika, Kitui, Machakos, Makueni |
| 2005 | 75 | 32 | Kitui and Makueni |
| 2007 | 84 | 21 | Meru, Kitui, Makueni |
| 2008 | 6 | 2 | Eastern Province |
| 2010 | 24 | 3 | Kitui (Mutomo) and Makueni (Kibwezi) |

Source; lewis et al., 2005; KEPHIS, 2006; MOA, 2008; Daily Nation, 2010.

Key: - indicates data not available

## 2.3 Factors affecting fungal growth and mycotoxin production

Factors that contribute to mycotoxin contamination of food and feed in Africa include environmental, socio-economic and food production. Aflatoxins occur more frequently in tropical countries because of high temperature, moisture, unseasonal rains and flash floods. 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).

Environmental conditions especially humidity and temperatures favour fungal proliferation resulting in contamination of food and feed. During growth in the fields, maize and other cereals are exposed to mycoflora. Substrate moisture of more than 20%, air temperature of 25°C to 35°C and relative humidity of more than 90% provide "field fungi" excellent environmental conditions for development (Cvetnić *et al.*, 2004). The most frequent "field fungi" are *Fusarium* species which can colonise the straw, grain and ear before harvest. Physiological stress during the period just preceding maize harvest due to drastic oscillations in rainfall and relative humidity is likely to create favourable conditions for fumonisin production (Fandohan *et al.*, 2003). Aflatoxin contamination in

maize rises in drought conditions. Seasons with mid-late droughts such as 2004-05 present a high risk of aflatoxin in rainfed crops (Blaney, 2007).

Late planting of maize with harvesting in wet conditions favours diseases caused by *F. verticilliodes* and the prevalence of this fungus increases considerably with wet weather later in the season (Fandaohan *et al.*, 2003). Furthermore, temperature and moisture conditions during the growing season as well as during storage are often pointed out to affect maize infection by *Fusarium* spp and fumonisin synthesis (Fandohan *et al.*, 2003). *Fusarium verticillioides* generally grows in grain when moisture content is more than 18 – 20% (Fandohan *et al.*, 2003). In this connection, water activity (aw), the water available for fungal growth, plays a key role (Fandohan *et al.*, 2003). At water activities below 0.70, grains and oilseeds can be stored safely for long without danger of fungal deterioration (Wilson and Payne., 1994). At water activities above 0.85, many *Penicillium and Aspergillus* species, including species of the *A. flavus* group, are capable of growing in products stored in ambient conditions (Wilson and Payne., 1994).

Repeated planting of maize and other cereal crops in the same or nearby fields favours fungal infection by increasing the fungal inoculum and insect population that attack plants. *A. flavus* is ubiquitous in aflatoxin production. Fungal spores survive in the soils or in plant residues. It can infest maize by air-borne spores in the field during grain filling or storage and handling (Kumar *et al.*, 2000). Minimal tillage, nitrogen fertilizers, application of fungicides like azoxystrobin (fungicide) or herbicides such as glyphosate, and production of grains where maize had been grown the previous year have been reported as the main risk factors associated with increased DON accumulation (Murphy *et al.*, 2006).

Poor harvesting practices, improper storage and less than optimal conditions during transport and marketing can also contribute to fungal growth and proliferation of mycotoxins (Bhat and Vasanthi, 2003; Wagacha and Muthomi, 2008). Most small scale farmers dehusk the maize during harvesting and drop the cobs on the ground. The cobs are then transported to the homestead where drying is done. Some dry on polyethylene

sheets or mats while others spread it on bare ground (Kaaya and Warren, 2005). These drying methods are slow and may support growth and development of fungi thus increasing the potential for aflatoxin production. Some farmers also store grains or heap (unshelled maize) on the floor under the veranda or in the living house and such maize can have 100% aflatoxin contamination (Kaaya and Warren, 2005). Mechanical damage to maize kernels makes them much more vulnerable to invasion by storage moulds (Sauer *et al.*, 1987; Kumar, 2000). Cracks and breaks in maize are caused by harvesting and handling equipment, although insect feeding may also be responsible for breaks in the pericarp (Sauer *et al.*, 1987). When shelling maize, the practice of beating the maize cobs by small scale farmers leads to damage of kernels (Udoh *et al.*, 1999). Use of woven polypropylene bags during storage may enhance aflatoxin contamination because they do not protect the grains against aflatoxin contamination. The concentrations of aflatoxin present at harvest are usually not enough to poison, but can rapidly escalate in hot spots in storage if the grain is moist or not aerated properly (Blaney, 2007).

Insect damage is not a prerequisite for aflatoxin formation, although the incidence of *Aspergillus flavus* and *A parasiticus* is usually higher in damaged kernels. Insect damaged kernels provide infection routes and allow kernel drying to moisture levels more favourable for growth of *A. flavus* and aflatoxin production than for other fungi (Lopez-Garcia *et al.*, 1999). Insects that feed on maize ears in the field and stored maize predispose kernels to fungal infection through physical damage while storage pests open the kernels to fungal invasion (Avantaggio *et al.*, 2002). Therefore, insect damage of maize can serve as a good predictor of mycotoxin contamination, and can serve as an early warning (Wagacha and Muthomi, 2008). Insects carry spores from plant surfaces to the interior of the stalk or kernels or create infection wounds due to the feeding of the larvae on stalk or kernels (Munkvold, 2003). Insect-damaged kernels provide an opportunity for the fungus to circumvent the natural protection of the integument and establish infection sites in vulnerable interior (St. Leger *et al.*, 2000).

## 2.4. Economic importance of food borne mycotoxins

Mycotoxins are of economic importance due to the detrimental effects that they have on the health of both human and livestock. Mycotoxin contamination of crops may cause economic losses at all levels of food and feed production. The national economy would be affected adversely by losses incurred by crop and livestock producers and the multiplier effect this has on other industries as a result of the reduced spending power of producers (Charmley et al., 1995). Crops with large amounts of mycotoxins often have to be destroyed or the contaminated produce is sometimes diverted into animal feed resulting in monetary loss for the producers and traders (Bennett and Klich, 2003).

According to the Food and Agriculture Organisation (FAO), 25% of the world's food crops are affected by mycotoxin, which impact negatively on human health, food trade, food availability, and consumption (Hell et al., 2004; Kaaya and Warren, 2005). The US grain industry alone is estimated to incur economic loses to the tune of $923 million annually due to mycotoxin contamination. International trade in agricultural commodities such as maize, wheat, rice barley, soybeans, groundnuts and oil-seeds amounts to hundreds of millions of tonnes each year (FAO, 1988). Many of these commodities run a high risk of mycotoxin contamination and for some developing countries, where agricultural commodities account for as much as 50% of the total national exports, the economic importance of mycotoxins is considerable (Bhatt and Miller, 1991). The exact figures for world economic losses resulting from mycotoxin contamination may never be available but, apart from obvious losses of food and feed, losses from rejected shipment and lower prices for inferior quality can devastate a developing country's export market (Grisslier, 2007).

The toll on the effects on human health includes the cost of mortality on the cost of productive capacity lost when people die prematurely, the cost of morbidity, losses resulting from hospitalisation and the cost of health care services, both public and private (Bhat and Vasanthi, 2003). Finally there is an intangible cost of pain, suffering anxiety, and reduction of the quality of life. Costs incurred by inspection, sampling and analysis before and after shipments, losses attributable to compensation paid in case of claims,

farmer subsidies to cover production losses, research,, training and extension programme costs, costs of detoxification, all when combined could be very high (Coulibaly, 1989).

In Kenya, aflatoxin contamination in maize has been frequently reported almost on an annual basis particularly in Eastern province. When high levels of aflatoxin are noted in the maize, the government has been mopping up that maize and replacing it with clean maize and this interferes with the government's development plans because the funds are diverted from the intended projects to cater for the cost of buying maize. The food situation in the region and the country at large is worsened because the country does not produce adequate maize. In the year 2009, 31,781 bags of maize worth 65 million Kenyan shillings held by the National Cereals and Produce Board at Ishiara Depot in Embu district were found to contain high levels of aflatoxins rendering the maize unfit for human consumption (Daily Nation, 2nd July, 2010). The debate is still going on to decide on the best method of destroying it. The maize was to be destroyed by burning but an environmental assessment report by the National Environmental Management Authority (NEMA) says the steeping method which involves immersing the maize in acid after which it would be used for industrial purposes is the best method (Daily Nation, 2nd July, 2010).

This year (2010), 2.3 million bags of maize worth 5.3 billion Kenya shillings have been rendered unfit for human consumption by aflatoxin contamination in 29 districts of Eastern and Coast provinces (Standard Newspaper, 9th June, 2010). The government intends to buy that maize at Kshs. 1000.00 per bag from the farmers as a way of mopping it (Standard Newspaper, 9th June, 2010).

Fumonisins disrupt sphingolipids biosynthetic pathways in both plants and animal cells, with potentially profound consequences on cellular metabolism (Murphy *et al.*, 2006; Yazar and Omurtag, 2008). They have been found to be associated with several animal diseases such as leukoencephalomalacia in horses, and pulmonary oedema in pigs (Camargas *et al.*, 2000; Fandaohan *et al*, 2003; Noonim *et al.*, 2009). Although the effect of fumonisins on humans is not yet well understood, their occurrence in maize has been

associated with high incidences of oesophageal and liver cancer in human beings. Fumonisins are reported to be associated with oesophageal cancer in rural areas in South Africa and China (Fandaohan *et al.*, 2005). Consumption of mouldy sorghum and maize containing fumonisin $B_1$ has been associated with an outbreak of abdominal pain and diarrhoea in India (Fandaohan *et al.*, 2005). Although the effects of fumonisins on human are not yet understood, legislation is being put in place to regulate commercial exchanges of contaminated maize and maize-based foods (Duvick, 2001). Even though fumonisins are less acutely toxic than aflatoxins, they are found in high concentrations (mg $kg^{-1}$) in maize compared to concentrations (μg $kg^{-1}$) for aflatoxins (Noonim *et al.*, 2009)

Aflatoxin is the most potent natural toxin and carcinogen known that cause aflatoxicosis and liver cancer in animals (Park and Liang, 1993; Windham and Williams, 1998; Betran,, *et al.* 2002; Bennett and Klich, 2003). Aflatoxins can affect a wide range of commodities including cereals, oilseeds, spices, tree nuts, milk, meat, and other dried fruit (Bennett and Klich, 2003; Strosnider *et al.*, 2006). Maize and groundnuts are major sources of human exposure because of their great susceptibility and contamination and frequent consumption throughout the world (Strosnider *et al.*, 2006). Aflatoxins are implicated in liver cancer and an incident was reported when aflatoxins present in a foodstuff consumed by people in Malaysia in 1988 were strongly implicated as the cause of death for 13 children (Fandohan *et al.*, 2005). Aflatoxins have been reported to impair childhood growth in children from Benin and Togo (Fandohan *et al.*, 2005). Aflatoxins has long been linked to Kwashiakor, a disease considered a form of protein energy malnutrition, although some characteristics of the disease are known to be among the pathological effects caused by aflatoxins in animals (Shepherd, 2008). Aflatoxin losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and more subtle effects of immune system suppression, reduced growth rates, and losses in efficiency. Other adverse economic effects of aflatoxins include lower yields for food and fibre crops (Kaaya and Warren, 2005).

Two structural types of aflatoxins are known (B and G types), of which aflatoxin $B_1$ is considered the most toxic (Christensen, 1975). Exposure to aflatoxins occurs primarily

through ingestion of contaminated foods and can cause hepatic and gastrointestinal injury and have immunosuppressive, teratogenic, and oncogenic effects (Christensen, 1975). Chronic exposure can increase the risk for hepatocellular carcinoma (Christensen, 1975). Severe, acute liver injury with high morbidity and mortality has been associated with high dose exposures to aflatoxins (Christensen, 1975). Aflatoxins limit corn marketability, causing economic losses because of risk to animals and human health (Betran *et al.*, 2002).

Zearalenone is uterotrophic and estrogenic and has attracted recent attention due to concerns that environmental estrogens have the potential to disrupt sex steroid hormone functions (Murphy *et al.*, 2006; Cvetnić, et al., 2004). It is associated with reproductive problems in specific animals and possibly in humans (Wood, 1992). Free and conjugate forms of zearalenone have been found in the milk of cows under experimental conditions. Zearalenone has been shown to competitively bind to oestrogen receptors in uterus, mammary glands, liver and hypothalamus in different species (SCF, 2000). Occasional outbreaks of zearalenone mycotoxicosis in livestock are known to cause infertility (Murphy, 2006), usually causing vulvovaginitis, vaginal or rectal prolapse and loss of pregnancy (Cvetnić *et al.*, 2004). Zearalenone can be transmitted to piglets in sows' milk, causing estrogenism in pigs (CAST, 2003). Zearalenone may be an important etiologic agent of intoxication in young children or fetuses exposed to this mycotoxin, which results in premature thelarche, pubarche, and breast enlargement (CAST, 2003; Yazar and Omurtag, 2008). The toxin is found almost entirely in grains and in highly variable amounts ranging from a few nanograms per gram to thousands of nanograms per gram (Murphy *et al.*, 2006).

Deoxynivalenol (DON) disrupts the normal cell structure and function by inhibiting protein synthesis via binding to the ribosome and activating critical cellular kinases involved in signal transduction related to proliferation, differentiation, and apoptosis (Pestka and Smolinski, 2005). At the cellular level, the main toxic effects of DON are immunosuppressant or immunostimulation depending upon the dose and duration of exposure. These effects have largely been characterized in the mouse (Rotter *et al.*,

1996). The main effects of DON at low dietary doses appear to be decreased growth and anorexia, while higher doses induce vomiting (emesis), immunotoxic effects and changes in brain neurochemicals (Wijnands and Van Leusden, 2000). It causes extensive feeding problems in swine. Clinical signs associated with consumption of a DON-contaminated diet by hogs are vomiting and feed refusal, followed by loss of body weight. According to sensitivity between species, pigs are more sensitive to DON than mice, poultry and ruminants in part because of differences in metabolism of DON, with males being more sensitive than females (Yazar and Omurtag, 2008). Diets containing 12ppm, when consumed will provoke vomiting after 15 minutes (DeWolf *et al.*, 2003).

T-2 and HT-2 toxins are generally found in various cereal crops such as wheat, maize, barley, oats, and rye and processed grain products like malt, beer, and bread (SCF, 2001). T-2 contaminated products can cause severe effects in humans/ animals and at the same time, it may result in death (Holt *et al*, 1988; Moss, 2002)). General signs of T-2 toxins include nausea, emesis, dizziness, chills, abdominal pain, diarrhoea, dermal necrosis, abortion, irreversible damage to the bone marrow, reduction in white blood cells (aleukia), inhibition of protein synthesis, and is toxic for the haematological and lymphatic systems, producing immunosuppression (Pacin *et al.*, 1994). The immune system is the main target of T-2, and the effects include changes in leukocyte counts, delayed hypersensitivity, depletion of selective blood cell progenitors, depressed antibody formation, allograft rejection, and blastogenic response to lectins (Creppy, 2002) and cytotoxic effect in cell cultures (Holt *et al.*, 1988).

Ochratoxin is considered to be nephrotoxic, teratogenic, and immunotoxic, and has been classified as a class 2B carcinogen, probable human carcinogen (Murphy *et al.*, 2006). Ochratoxin A, affects kidney function. Ochratoxin A contaminated feed has its major economic impact on the poultry industry. Chickens, duckslings and turkeys are susceptible to this toxin. The clinical signs of avian ochratoxicosis involve reduction in weight gains, poor feed conversion, reduced egg production, poor egg shell quality and kidney poisoning (DeWolf, *et al.*, 2003).

## 2.5. Management of mycotoxicosis

Complete elimination of any natural toxicant from foods is an unattainable objective (Bennett and Klich, 2003). Mycotoxins are chemically diverse and for that reason, it would be impossible to develop one single control method that would ensure the reduction of every mycotoxin present in every agricultural commodity (Lopez-Garcia et al., 1999; Gressel, *et al.*, 2003; Duvick, 2001). Several approaches have been used to reduce mycotoxin contamination and these include cultural practices and crop management, host plant resistance through breeding and/or genetic engineering, and biocontrol for example, atoxigenic strains for control of toxigenic strains by out competition on nutrients (Brown *et al.*, 1998; Widstrom, 1987; Bhat and Vasanthi, 2003). Mycotoxin formation in crops can be limited before harvest through good agricultural practices such as rotating crops, irrigation to eliminate drought stress, controlling weeds, cultivating mould-resistant stocks, and introducing bio-controls such as non-mycotoxigenic fungal strains (Riley and Norred, 1999; Strosnider *et al.*, 2008). Post harvest measures include drying rapidly by mechanical means and keeping crops dry. Sorting by colour and washing with water will also reduce mycotoxins (Bhat and Vasanthi, 2003).

One possible approach is to manage the risks associated with mycotoxin contamination is the use of an integrated system (Lopez-Garcia *et al.*, 1999; Suttajit, 2003). Prevention through pre-harvest control is the first step in ensuring a safe final product but, when contamination does occur, the hazards associated with the toxin must be managed through post-harvest procedures, if the product is to be used as human food or animal feed. The first line of defence is the control of the vectors carrying the fungi that produce mycotoxins, both the stem borers that cause the systemic infection of the endophytic *Fusarium* spp, and the grain weevils, and especially in Africa the Lepidopteran earborer (Setamou *et al.*, 1998; Cardwell *et al*, 2000) that carries *Aspergillus* species. The second line of defence is to suppress the fungal attack, either by bio-control or by engineering resistance to the fungi that produce the mycotoxin. The third line of defence against mycotoxins is to prevent biosynthesis, and a fourth line is to degrade them in the grain before they enter the food chain (Gressel *et al.*, 2003). Association between mycotoxin

contamination and inadequate storage conditions has long been recognized. Studies have revealed that some seeds are contaminated with mycotoxins in the field (Lopez-Garcia *et al.*, 1999).

### 2.5.1. Reduction of fungal inoculum

Inoculum potential is a preliquisite for *Aspergillus* infection and subsequent aflatoxin production. Soil type and condition, as well as availability of viable spores, have been considered important factors in aflatoxin production (Wagacha and Muthomi, 2008). When the crop is harvested, some residues remain on the field. These provide an environment that is conducive to the survival of fungal spores and the subsequent infection of the infection of the next crop (Stack and Carlson, 2006). Adequate rotation may, therefore, aid the prevention of mycotoxin contamination (Lopez-Garcia *et al.*, 1999). It has been found that the rotation maize/nonhost crop of sorghum is better than maize/maize as the former is less favourable to *Fusarium* disease outbreak than the latter (Duvick, 2001). Repeated planting of maize and other cereal crops in the same or nearby fields favour fungal infection by increasing the fungal inoculum and insect population that attack maize plants.

### 2.5.2. Proper agronomic practices

Soil fertility and drought stress have been found to be contributing factors in pre-harvest aflatoxin contamination of maize (Gressel *et al.*, 2003). Moisture and temperature play the most important roles in the planning of any control strategy for fungal development (Askun, 2006). High moisture and high relative humidity are essential for spore germination and fungal proliferation (Williams *et al.*, 2004). Adequate efforts should therefore be made to avoid extreme conditions of either drought or excessive moisture. During harvesting, it is important to control factors such as timeliness, cleanup and drying of maize Lopez-Garcia *et al.*, 1999). This control is essential for preventing mycotoxin formation during storage. The planting date should be selected to take advantage of periods of higher rainfall that occur at some particular time (Lopez-Garcia *et al.*, 1999). As soon as the crop is fully grown, and the crop cycle is completed, harvesting should take place. Crops left on the field for longer periods of time may

present higher levels of toxin contamination (Lopez-Garcia *et al.*, 1999). Adequate drying is also essential to prevent fungal proliferation (Williams *et al.*, 2004).

### 2.5.3. Control of insect pests

Insect pests should be controlled to minimise damage of kernels. Although it has been reported that damage is not a preliquisite to aflatoxin formation, the incidence of *Aspergillus flavus* and *A. parasiticus* is usually higher in damaged kernels. Insects have been known to be associated with spread of the *Aspergillus flavus* and grain contamination by aflatoxin (Udo *et al.*, 1999). Insect-damaged kernels are routes for infection and are likely to dry to moisture levels that are more favourable for the growth of *Aspergillus flavus* and aflatoxin production than of other fungi. Maize weevil (*Stophilus zeamais*) was reported to contribute significantly to *A. flavus* infection in maize and subsequent production of aflatoxin (McMillan., 1981; Miller, 1995).

Weevils facilitate the growth of *A. flavus* and aflatoxin production in maize by increasing surface area susceptible to fungal infection and increasing moisture content as a result of weevil metabolic activity (Beti *et al.*, 1995). Control of insect infestation may, therefore, help to prevent *Aspergillus flavus* and *A. parasiticus* proliferation and subsequent aflatoxin production. *Aspergillus flavus* was also found to infest the crop systemically, often with stem borers and grain weevils as the vectors, or causing lesions through which the fungi enter the plant or seed (Greesel *et al.*, 2003). Stem borers cause systemic infection of endophytic *Fusariums* pecies; grain weevils, and especially in Africa the lepidopteran ear borer carry the *Aspergillus flavus* (Gressel *et al.*, 2003)

### 2.5.4. Good processing practices

Effects of processing on mycotoxin contamination in food products are increasingly being investigated throughout the world, and this strategy is showing great promise for mycotoxin reduction (Fandohan *et al.*, 2005). Contaminated mycotoxins in food and feeds should be removed, inactivated or detoxified by physical, biological and chemical means depending on the conditions (Suttajit, 2003). The use of physical methods, including cleaning, separation of screenings, washing, aqueous extraction, dehulling and

milling, has been shown to be effective to a certain extent in reducing mycotoxins in cereals (Fandohan *et al.*, 2005). Sorting and winnowing are operations linked to the preparation of maize based foods which have been found to effectively reduce mycotoxins. Systematic disposal of all visibly mouldy and damaged grains and impurities is a very useful detoxification process (Desjardins *et al.*, 1998; Rheeder *et al.*, 1992). In an experiment, it was found that dehulling significantly decreases aflatoxin levels (Mutungi *et al.*, 2008). Aflatoxin contents in the by-products, comprising hulls and fines, were 2-7 times higher than the levels in the whole grain maize (Mutungi *et al.*, 2008). Dehulling eliminates the pericarp (hull) underlying aleurone layer, hilum and a sizeable portion of the germ. These portions are usually the more highly contaminated with aflatoxins (Mutungi *et al.*, 2008).

Fumonisins are found more concentrated in the pericarp and germ of the grain than in the endosperm, so that removal of those outer parts by mechanical processes such as dehulling can significantly reduce the toxin in maize (Fandohan *et al.*, 2003). The fumonisins are highly water-soluble (Murphy *et al.*, 2006) and their concentration in maize grains can be significantly reduced through washing in water. Upto 74% of fumonisins were found to have been removed by simply washing maize grains, immersing them in water, and by removing the upper floating fraction (Shetty and Bhat, 1999). Current food processing techniques do not significantly contribute to DON remediation, either by reduction or detoxification, in human or animal foods (Murphy *et al.*, 2006). The combination of high pH (10.0) and high heat (100°C for 60 min and 120°C for 30 min) treatment of DON in aqueous buffer solution produced partial to complete destruction (Murphy *et al.*, 2006)

### 2.5.5. Detoxification of contaminated products

The mycotoxins in maize that are of greatest concern (namely aflatoxin, deoxynivalenol, zearalenone, ochratoxin A, and fumonisins) are also quite stable and, therefore, difficult to degrade (Riley and Norrad, 1999). Detoxification strategies have been arbitrarily divided into those that are primarily dependent on physical, chemical or microbiological processes that detoxify by destroying, modifying, or absorbing the mycotoxins so as to

reduce or eliminate the toxic effects (Riley and Norrad, 1999). However, the treatment has its own limitations, since the treated products should be healthsafe from the chemicals used and their essential nutritive value should not deteriorate (Suttajit, 2003). Sorting and disposing of visibly mouldy and damaged kernels before storage is an effective method for reducing but not eliminating the development of aflatoxins (Fandohan *et al.*, 2005; Strosnider *et al.*, 2006). A study of the distribution of aflatoxin in peanuts shows that a major portion of the toxin is often associated with the smaller and shrivelled seed, and thus removal can lower the overall concentration in the bulk (Williams *et al.*, 2004). Irradiation may also be an option for mycotoxin control. A completely satisfactory way of destroying mycotoxins that have already been formed has not been identified. However, irradiation may be considered as a method to control mycotoxin-producing moulds in certain products (Lopez-Garcia and Park, 1998).

One promising method is the use of high-affinity hydrated sodium calcium aluminosilicates to bind aflatoxin in feeds and food (Riley and Norrad, 1999). Innovative sorption strategies for the detoxification of aflatoxins have been developed. Enterosorption is the use of clay, such as NovaSil which is a processed montmorillonite clay with a high affinity for aflatoxins (Strosnider *et al*, 2006). NovaSil clay (NS) has been shown to prevent aflatoxicosis in a variety of animals when included in their diet (Phillips *et al.*, 2007). Results have shown that NS clay binds aflatoxins with high affinity and high capacity in the gastrointestinal tract, resulting in a notable reduction in the bioavailability of these toxins without interfering with the utilisation of vitamins and other micronutrients (Phillips *et al.*, 2007)

Use of chemicals for the inactivation and hazard reduction of selected mycotoxins has been evaluated through numerous studies. Most studies have however focused on aflatoxins and application to animal feeds. Ammoniation is the chemical method that has received the most research attention. It has been found to be an efficacious and safe way of decontaminating aflatoxin-contaminated feeds (Lopez-Garcia *et al.*, 1999). Nixtamalization, the traditional alkaline treatment of maize partially degrades aflatoxins and fumonisins, but the residue molecules can either be regenerated by digestive

processes or become more toxic (Price and Jorgensen, 1985; Mutungi *et al.*, 2008). The addition of oxidizing agents such as hydrogen peroxide has been shown to be an effective aid in nixtamalization. These chemicals degrade aflatoxins and fumonisin, thereby reducing toxicity (Lopez-Garcia, 1998).

### 2.5.6. Use of resistant and tolerant maize varieties

There has been extensive research on the promotion and development of plant varieties that are naturally resistant to fungal infection. Host resistance may present a promising strategy for the pre-harvest prevention of mycotoxin contamination (Lopez-Garcia *et al.*, 1999). A maize breeding population with aflatoxin resistance has been identified (Lopez-Garcia *et al.*, 1999). Studies of these specific populations have yielded useful information for the development of resistant lines. Among the strategies for reducing risk of fumonisin contamination in maize supplied to the market, development and deployment of *Fusarium* ear-mould resistant maize germplasm is a high priority. Breeding for ear mould tolerance and reduced mycotoxin levels is being practiced today in both commercial and public programs (Duvick, 2001). Plants have also been engineered with a large coterie of antifungal agents to prevent fungal growth such as phytoalexins (for example, stilbene) and enzymes (for example, chitinases and glucanases) but this strategy has rarely been used to prevent attack by toxigenic fungi (Gressel., 2003). An amylase inhibitor from a legume inhibits fungal growth and aflatoxin production (Fakhoury and Woloshuk, 2001), so that gene is a 'candidate' for engineering suppression of *Aspergillus*.

### 2.5.7. Biological control

One of the management strategies being developed for control of aflatoxins is biological control using the competitive exclusion mechanism. Biological control is based on competitive exclusion whereby a dominant population of a non-toxigenic strain of *A. flavus* is established in the soil before the crops are subjected to conditions favouring contamination (Dorner, 2008). Significant in-roads have been made in establishing various bio-control strategies such as development of atoxigenic bio-control fungi that can out-compete their closely related toxigenic strains in field environment, thus reducing the levels of mycotoxins in the crops (Cleaveland *et al.*, 2003; Wagacha and Muthomi,

2008). Natural population of *A. flavus* consists of toxigenic strains that produce copious amount of aflatoxin and atoxigenic strains that lack the capacity to produce aflatoxin. In the competitive exclusion mechanism, introduced atoxigenic strains, out compete and exclude toxigenic strains from colonizing grains thereby reducing aflatoxin production in contaminated grains.

This strategy is based on the application of non-toxigenic strains to competitively exclude naturally toxigenic strains in the same niche and compete for substrates (Yin *et al.*, 2008). For competitive exclusion to be effective, the biocontrol non-toxigenic strains must be predominant in the agricultural environments when the crops are susceptible to infection by the toxigenic strains (Yin *et al.*, 2008). Two products of non-toxigenic strains have received U.S. Environmental Protection agency (EPA) registration as biopesticides to control aflatoxin contamination in cotton and peanuts in several states of USA (Dorner, 2004; Yin *et al.*, 2008). So far, 50 candidate atoxigenic strains have been identified through screening (conference proceedings, 2005). Competitive exclusion has been successfully implemented on cotton seed in Arizona.

Peter Cotty of the Agriculture Reseach Service of the United States Department of Agriculture (USDA-ARS) and Ranajit Bandyopadhyay, a plant pathologist with the Africa-based International Institute of Tropical Agriculture (IITA) are reported to have identified a non-toxigenic strain of *A. flavus* native to Kenya and can now be used t0 control aflatoxin (Standard Newspaper, 9th June, 2010). The fungus out competes and drastically reduces the population of the poison-producing strains. According to the scientists, a single application of this biopesticide two to three weeks before maize flowering is sufficient to prevent contamination (Standard Newspaper, 9th June, 2010). These experts were also reported to have said that the toxigenic strains are also carried from the field to the stores. So, even if the grains are not stored properly or get wet during or after harvest, the maize does not get aflatoxin as they continue to prevent aflatoxin contamination during post harvest (Standard Newspaper, 9th June, 2010).

While soil application of a competitive non-toxigenic *Aspergillus flavus* strains is successful in reducing aflatoxin contamination in certain crops, direct application to aerial reproductive structures could be more effective for maize. A sprayable, clay-based water-dispersible granule formulation was developed to deliver non-toxigenic strain *Aspergillus flavus* strain K49 directly to maize ears. In field studies conducted to compare K49 colonization and effectiveness in reducing aflatoxin contamination when applied either as a soil inoculant or as a direct spray in plots infected with toxigenic strain F3W4, soil applied K49 reduced aflatoxin contamination by 65% and spray applications reduced contamination by 97% (Lyn *et al.*, 2009).

Use of biological agents to suppress growth of fumonisin-producing fungi has been reported. Desjardin *et al* (1998) observed inhibition of fumonisin formation by atoxigenic *F. verticillioides* strains although these caused higher disease incidence when applied through silk channel. Suppression of saprophytic colonization and sporulation of toxigenic *F. verticillioides* and *F. proliferatum* in maize by non-pathogenic *Fusarium* strains has also been reported (Luongo *et al.*, 2005). Control of fumonisin producing fungi by endophytic bacteria has also been reported (Bacon *et al.*, 2001). Competitive exclusion whereby the bacteria grow intercellularly precluding or reducing growth of intercellular hyphae was thought to be the mechanisim involved (Masoud and Koltoft, 2006).

Fungal strains of *Trichoderma* have also been demonstrated to control pathogenic fungi through mechanisms such as competition for nutrients and space, fungistasis, antibiosis, rhizosphere modification, myco-parasitism, biofertilization and the stimulation of plant defence mechanism (Benitez *et al.*, 2004). Some saprophytic yeast species (such as *Candida krusei* and *Pichia anomala*) have shown promise as biocontrol agents against *A. flavus* (Yin *et al.*, 2008). Similar to bacterial agents, these yeast strains were able to inhibit *Aspergillus* growth greatly in laboratory conditions (Masoud and Kaltoft, 2006). A novel yeast strain capable of degrading ochratoxin A and zearalenone has been isolated and characterized. The strain, named *Trichosporon mycotoxinivorans* (MTV) detoxifies

OTA by cleavage of the phenylalanine moiety from the isocumarin derivative ochratoxin α (Griessler, 2007)

### 2.5.8. Control by legislation and surveillance

Several countries have introduced legislation concerning mycotoxins and about 15 of these countries are in Africa. The objective is to protect the consumer from the effects of these mycotoxins (Fellinger, 2006; Barug *et al.*, 2003; Van Egmond, 2002). Most of the legislation pertains to aflatoxins, ergot alkaloids, deoxynivalenol and ochratoxins. Although various legislative measures have yet to be harmonised among countries, the codex Alimentarius Commission is making efforts to establish international levels for mycotoxins, and aflatoxins in particular (Bhat and Miller, 1991). Aflatoxin contamination of maize grains destined for human consumption and animal feed is heavily monitored and regulated in many countries to ensure a safe supply of food and feed (Food and Agriculture Organization (FAO), 2004; Atehnkeng *et al.*, 2008), but in the developing world and in sub-Saharan Africa in particular, official monitoring of mycotoxin contamination level is rare (Cardwell *et al.*, 2002).

The establishment of regulatory limits on traded foods, the enforcement of these limits through food monitoring, and the implementation of optimal drying and storage practices have mostly eliminated harmful exposures in developed countries. The application of these strategies in developing countries is difficult because of differences in production, such as the prominence of subsistence farming in developing countries (Strosnider *et al.*, 2006). Consequently, more than 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Shepherd, 2003; Williams *et al.*, 2004; Strosnider *et al.*, 2006)

Mycotoxins particularly aflatoxins are gaining increasing importance due to their deleterious effects on human and animal health. Human foods are allowed 4-30ppb aflatoxin, depending on the country involved (FDA, 2004; Henry *et al.*, 1999). Most countries have established regulatory limits for either aflatoxin B1, B2, G1, and G2, as well as regulatory limits for aflatoxin M1. Action levels or the maximum concentration of

aflatoxin in food or feed that presents no health hazard to humans or designated animal species have been developed by various countries (Stack and Carlson, 2003). The European Union has a maximum level of 2μg/kg for $B_1$ and 4 μg/kg for total aflatoxins in crops (van Egmond and Jonker, 2004; Murphy *et al.*, 2006; Yin *et al.*, 2008). The US based Food and Drug Administration (FDA) recommends that fumonisin levels should not be higher than 4μg/g in human foods (Fandaohan, 2003). In Switzerland, tolerance levels for fumonisins of 1μg/g in dry maize. The Joint FAO / WHO Export Committee on Food Additives (JEFCA), allocated a group Provisional Maximum Daily Intake (PMTDI) of 2 μg/g for fumonisins (Fandaohan, 2003). Surveillance and awareness creation could be a long-term strategy as has been advocated by WHO (Wagacha and Muthomi, 2008).

Surveillance and subsequent regulation of susceptible commodities, such as groundnuts and maize for aflatoxins and other mycotoxins, are routinely used as a primary intervention to safe guard the health of consumers as well as the economic interests of producers and traders. These surveillance data are frequently used to establish regulatory guidelines that define the limits of aflatoxins and other mycotoxins in foods (Phillips *et al.*, 2007). Mycotoxins such as aflatoxins are not visible neither do they have a particular flavour (Kaaya and Warren, 2005). Therefore, it is not easy to convince consumers about their existence in food. Information about the dangers and management aspects of mycotoxins, and susceptible produce should be disseminated to the majority of farmers, traders and consumers using simplified methods (Kaaya and Warren, 2005).

Table 2.3: Limits (ppb) of mycotoxins in milk and maize for human food and feed by different Regulatory authorities in various countries

| Commodity / use | Mycotoxin | FDA | EU | Codex | Kenya |
|---|---|---|---|---|---|
| Milk and milk products | Aflatoxin $M_1$ | 0.5 | 0.05 | 0.5 | |
| Maize (food) | Aflatoxin $B_1$ | 20 | 2 | 15 | 5 |
| Maize (food) | Total aflatoxins | 20 | 4 | - | 10 |
| Maize (food) | Fumonisins | 4000 | 2000 | - | |
| Maize products (food) | Fumonisins | 2000 | 1000 | - | - |
| Cereals and cereal products (feed) | Fumonisins | 5000 – 100,000 | 60,000 | - | - |
| Maize (Feed) | Aflatoxins | 100 - 300 | 20 | -- | - |
| Raw cereal grains (food) | Ochratoxin A | - | 3 – 5 | - | - |
| Unprocessed maize(food) | Deoxynivalenol | - | 1750 | - | - |
| Cereal flour (food) | Deoxynivalenol | 1000 | 750 | - | - |
| Cereals and cereal products (feed) | Deoxynivalenol | - | 8000 | - | - |
| Maize by-products (feed) | | - | 12,000 | - | - |
| Unprocessed maize(food) | Zearalenone | - | 200 | - | - |
| Maize products (food) | Zearalenone | - | 200 | - | - |

Source: Stack and Carlson, 2003; Murphy et al., 2006; Wrather and Sweets, 2007: Kenya Bureau of Standards (Kebs), 2005. Key: EU = European Union; FDA = Food and Drugs Administration; Codex in the USA = Codex Alimentarius Commission

### 2.5.9. Enhanced awareness on mycotoxins

One of the most practical and fundamental interventions at the subsistence-farm level in developing countries is the use of low technology approaches such as community education on food handling and storage (Phillips, 2008). Awareness campaigns should use systems that are in place already for disseminating to subsistence farmers (Strosnider *et al.*, 2006; James, 2005). Awareness of what mycotoxins are and the dangers that they pose to human and animal health could be done through Government bodies, private organizations, non-governmental organizations, national media networks such as radios and television programs as well as features in newspapers and magazines (Wagacha and Muthomi, 2008; Phillips *et al.* 2007).

Multiple means for spreading information should be used to reach a broad range of people, given the diversity of cultures and remoteness of villages (Strosnider *et al.*, 2006). These primary approaches have been shown to reduce significantly the level of aflatoxin contamination in post harvest foods and associated exposure in human populations at high risk for aflatoxicoses (Phillips, 2008). It was reported that during the 2004 aflatoxin poisoning in Kenya, individuals who received information on maize drying and storage through an awareness campaign run by the food and Agriculture Organisation and Kenya's Ministry of Health and Ministry of Agriculture had lower serum aflatoxin than those who did not receive the information (Strosnider *et al.*, 2006)

It is imperative for African countries to strengthen nationwide surveillance, increase food and feed inspection to ensure food safety, and local education and assistance to ensure that food grains and animal feeds are harvested correctly, dried completely, and stored properly (Wagacha and Muthomi, 2008). Continuous surveillance of high-risk agricultural commodities for contamination by selected mycotoxins and the monitoring of human population groups for diseases attributable to mycotoxins have to be carried out throughout the world to ensure a supply of safe food which is free from naturally occurring contaminants (Bhat and Miller., 1991). A regional experts meeting held in 2005 on aflatoxins problem with particular reference to Africa made certain recommendations that could be instrumental in addressing or reducing mycotoxins

contamination in the continent (Wagacha and Muthomi, 2008). The consultations are dependent on the concerted efforts of all actors along the food production chain. Multi-disciplinary approaches are therefore critical (Wagacha and Muthomi, 2008). The meeting recommended continued mycotoxin awareness as a public health issue, strengthened laboratory and surveillance capacities as well as establishing early warning systems

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1 Survey on maize production, handling and storage practices.

Surveys were carried in Eastern province and North Rift covering Kitui, Machakos, Makueni, Uasin Gishu and Trans Nzoia districts. In each district, two agro-ecological zones were selected that included hot spots of previous aflatoxin-poisoning outbreaks. In each agro-ecological zone, ten farmers and ten traders were selected at random and a structured questionnaire was administered through individual interviews. Information gathered included agronomic practices, harvesting, drying, storage structures and materials used, transportation, processing and weather conditions during production and storage. Agro-ecological zones LM4 and LM5 were selected in Machakos and Kitui while in Makueni, LM3 and LM5 were the selected agro-ecological zones. In Uasin Gishu and Trans Nzoia districts, the survey covered LH2, LH3, UM3 and UM4 agro-ecological zones in each district which cover the major maize producing areas. Apart from agro-ecological zones, data on altitude, rainfall and length of growing season was also obtained (Table 3.1)

Samples of whole maize grains, semi-processed grains, maize flour, soil from the ground in the homestead where maize is dried, soil or dust from stores, and posho mill dust was collected in Eastern Province for fungal isolation and mycotoxin analysis. In Uasin Gishu and Trans Nzoia district, only whole maize grain samples were collected from farmers and traders. Two samples of packed maize flour were collected from traders in each agro-ecological zone of Kitui, Machakos and Makueni districts, for fungal isolation and mycotoxin analysis. All the samples weighed about one kilogram. The moisture content of maize was determined using a moisture meter before sample collection. The samples were put in khaki papers and stored at 4°C until they were analyzed.

Table 3.1: Characteristics of the agro-ecological zones where the survey was carried out in the study area

| AEZ | Description | Altitude (m) Above sea level | Rainfall (mm) | Length of growing season (Days) | |
|---|---|---|---|---|---|
| | | | | Long rains | Short rains |
| LH2 | Wheat/maize-pyrethrum zone | 1830-2130 | 1000-1300 | 135-155 | 135-155 |
| LH3 | Maize/Wheat-Barley zone | 2070-2220 | 700-1400 | 115-175 | 75-135 |
| UM2 | Main coffee zone | 1400-1770 | 970-1200 | 115-150 | 110-130 |
| UM3 | Marginal coffee zone | 1400-1830 | 900-1050 | 105-130 | 100-115 |
| UM4 | Sunflower-Maize zone | 1340-1840 | 700-950 | 75-105 | 70-105 |
| UM6 | Ranching zone | 1300-1530 | 550-600 | 65-75 | 20-65 |
| LM3 | Cotton zone | 1160-1350 | 750-900 | 75-105 | 75-105 |
| LM4 | Marginal cotton zone | 1160-1280 | 700-850 | 55-85 | 75-85 |
| LM5 | Livestock-Millet zone | 790-1220 | 600-750 | 20-70 | 45-75 |

Source: Jaetzold et al., 2006

**3. 2. Isolation and identification of fungi**

The maize grains in a sample were thoroughly mixed and about 50g taken for mycological analysis. The kernels were surface sterilized by soaking in 3% sodium hypochlorite for three minutes and then rinsed off three times in sterile distilled water. A total of 100 kernels per sample were plated on a petri-dish plates containing about 20ml molten Czapek Dox Agar medium (Oxoid Agar 20g, sucrose 30g, $NaNO_3$ 2g, KCl 0.5g, $MgSO_4.7H_2O$ 0.5g, $FeSO_47H_2O$ 0.01g, $K_2HPO_4$ 1g, distilled water 1000ml) amended with 20 ppm of antibiotics tetracycline, streptomycin, penicillin and a fungicide

pentachloronitrobenzene (PCNB). Five kernels were aseptically plated in each plate and for each sample, there were 20 replicates making a total of 100 seeds that were plated according to procedure of International Seed Testing Association (Muthomi, et al., 2006). The plates were labelled and incubated at 25°C for five to seven days. Observations on fungal growth on the kernel and number of colonies of *Aspergillus*, *Fusarium* and other fungi in each Petri-dish were made. Identification was done based on cultural and morphological characteristics.

Colonies of *Aspergillus* were sub-cultured using Czapek Dox Agar and incubated at 25°C for a period between 7 to 14 days. Identification of *Aspergilllus* to species level was done based on morphological characteristics according to Raper and Fennell (1965) and Larone (1995) (Table 3.2 and 3.3). Colonies of *Fusarium* were sub-cultured on potato dextrose agar (PDA) and Spezieller nahrstoffarmer agar (SNA). Identification was done based on morphological features as outlined by Nelson et al (1983) and Leslie et al (2006). The colonies were observed under high power microscope and identification was made based on characteristics such as presence or absence of micro and macro-conidia, number of phialides, shape of the sporangiophores, branching of the mycelia and presence or absence of sclerotia.

One gram of soil and dust samples was dissolved in 9ml sterile distilled water and serially diluted up to a dilution of $10^{-4}$. One millilitre of $10^{-3}$ and $10^{-4}$ dilutions was plated in Czepak Dox Agar, by dispersing it across the bottom of sterile petri dish, and then adding 20ml of Czepak Dox Agar at 50°C to 60°C, amended with 20 parts per million of antibiotics streptomycin, penicillin, tetracycline and fungicide pentachloronitrobenzene (PCNB). The plates were arranged in a completely randomized design (CRD) and incubated for 5-7 days at room temperature. Different fungal isolates were identified by colony colour, shape and form. The types of different fungal colonies in each petri dish were counted and the number of colony forming units per gram (CFU/g) calculated using the formula:

$$\text{No. of colony forming units} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{number of dilutions.}}$$

Table 3.2: Cultural characteristics used in identifying the various *Aspergillus* species

| Species | Surface | Reverse |
|---|---|---|
| *Aspergillus clavatus* | Blue-green | White, brownish with age |
| *Aspergillus flavus* | Yellow green | Goldish to red brown |
| *Aspergillus fumigatus* | Blue-green to grey | White to tan |
| *Aspergillus glaucus* group | Green with yellow areas | Yellowish to brown |
| *Aspergillus nidulans* | Green, buff to yellow | Purplish red to olive |
| *Aspergillus niger* | Black | White to yellow |
| *Aspergillus terreus* | Cinnamon to brown | White to brown |
| *Aspergillus versicolour* | White at the beginning, turns yellow, tan, pale green or pink | White to yellow or purplish red |

Source: Larone, 1995.

### 3.3. Analysis of aflatoxins content in grains and semi-processed grain

The level of aflatoxin in the samples was determined by competitive direct Enzyme Linked Immunosorbent Assay (ELISA) using the procedure described by Gathumbi, (2001) and Mutungi *et al* (2008). Binding of the aflatoxin-enzyme conjugate to the antibody on the microtitre plates is inhibited by the presence of free aflatoxin in the standard or sample extract solution. Since only a fixed number of antibody binding sites are available on the coated microtitre plates, the amount of aflatoxin-enzyme conjugate bound is inversely proportional to the amount of free toxin in standard or sample extract solution.

Maize, semi-processed maize grains, and flour samples were mixed thoroughly and ground into fine powder. Five grams of the ground sample placed into 50ml test tubes and 25ml methanol / water (50:50) added. The solution and powder were mixed in a vortex stirrer for about 10 minutes. 10ml of the mixture was centrifuged at 1500g for 15 minutes and the supernatant recovered. Each micro-plate of the ELISA plates were coated by adding 100 µl of coating solution containing anti aflatoxin antibody dissolved in bicarbonate buffer at a rate of 1:15000 and incubated overnight in a moist chamber. The plates were emptied and free protein binding sites blocked addition of 200µl of 3%

bovine serum albumin in Phosphate Buffered Saline (PBS) ( NaCl 6.79g, $Na_2HPO_4$ 1.47g, $KH_2PO_4$ 0.43g in 1000ml distilled water; $P^H$ 7 – 7.5) for 20 minutes. The plates were then washed thrice with Tween solution (NaCl 8.5g, Tween 20 200 µL, distilled water 1000ml) and semi- dried with blotting paper.

Table 3.3: Morphological characteristics used in identifying the various *Aspergillus* species

| Species | Conidiophore | Phialides | Vesicles | Vesicles |
|---|---|---|---|---|
| *A. clavatus* | Long, smooth | Uniseriate | Huge, clavate shaped | Absent |
| *A. flavus* | Colourless, rough | Uni-/biseriate | Round, radiate head | Only in some strains, brown |
| *A. fumigatus* | Short (<300µm), smooth, colourless | Uniseriate | Round radiate, columnar | absent |
| *A. versicolor* | Long, smooth, colourless | Biseriate | Round loosely | Absent |
| *A. nidulans* | Short, (<250µm), smooth, colourless | Biseriate, short | Columnar head | Absent |
| *A. niger* | Long, smooth, colourless to brown | Biseriate | Round radiate head | Absent |
| *A. terreus* | Short, (<250µm) smooth, colourless | Biseriate | Round, compactly columnar heads | Absent |
| *A. glaucus* group | Variable length, smooth colourless | Uniseriate | Round radiate to very loosely columnar head | Absent |

Source: Larone, 1995.

Four aflatoxin standard concentration levels (1, 0.333, 0.111 and 0ngmL$^{-1}$) were used. Then 50 µL of the sample extract and 50 µL of the calibrated aflatoxin standards were incubated simultaneously with aflatoxin-enzyme conjugate solution in wells of the coated

micro-titre. The plates were covered with aluminium foil and incubated at 25°C in a humid chamber for 2 hours after which the micro-plate wells were emptied, washed thrice with tween-saline solution and semi-dried by tapping on blotting paper before adding 100 µL of enzyme-substrate solution comprising hydrogen peroxide buffer. The plates were kept in a dark cabinet for 30 minutes to allow colour to develop. The enzyme reaction was stopped by addition of 100 µL of 1M sulphuric acid simultaneously into all wells. The intensity of colour in both standard and test extract wells was determined by reading absorbance at 450nm using an ELISA reader (Uniskan II® Labsystems, Finland). The percentage inhibition for each standard and sample was as calculated (B/Bo%) where B is the average absorbance value for each aflatoxin standard sample extract dilution and (Bo) that of the reagent blank standard. Absorbance values of aflatoxin standards dilutions were used to construct a standard curve and the aflatoxin content of sample was determined by interpolating on the curve.

## 3.4. Determination of the effect of storage material and moisture content on fungal growth and aflatoxin contamination in maize

### 3.4.1 Experimental treatments and design

Storage experiments were conducted at the Agricultural Training Centre in Machakos district and at the field station, Kabete campus. The objective was to investigate the effect of grain moisture content and storage materials on fungal growth and mycotoxin production. Maize was bought from one farm in Trans Mara district where aflatoxin poisoning had not been reported. The initial moisture content of the maize was 15%. The moisture content of the maize was adjusted to 20%, 18%, 15%, 13% and 10% moisture levels. It was stored in sisal bags, polypropylene (nylon) bags, and polythene paper bags. The design was split plot with the moisture content serving as the main plot treatment and storage materials as the sub-plot treatments.

The maize with inoculated with *Aspergillus flavus* at a concentration of $10^5$ spores per millilitre. The maize was weighed into 3kg portions and 30ml of the adjusted spore suspension was added and mixed thoroughly by shaking in a polythene paper bag. To

avoid maize imbibing the water, the inoculated maize was exposed at room temperature for 30 minutes to allow the water to dry. The inoculated maize was then put in the storage containers which, could be made of sisal, propylene or polythene material. For each moisture level, three samples of maize were stored in different containers; sisal bags, propylene bags and polythene paper bags. There were three replications of the inoculated maize. The well labelled storage materials with maize were placed on pallets to avoid the temperature variations of the concrete floor. The maize was stored for a total of 77 days.

The first sampling was done 14 days after inoculation and storage with subsequent samplings at $35^{th}$, $56^{th}$ and $77^{th}$ day after storage. During sampling, samples were thoroughly mixed and about 200 grams of the stored maize collected using khaki papers and kept at 4°C until they were analysed for fungal growth and mycotoxin content. Before sampling, the moisture content of the stored maize was taken using a moisture meter. The number of visibly mouldy, rotten, and discoloured grains out of 100 grains picked at random was counted when a sample was being taken.

### 3.4.2. Inoculum preparation and inoculation

The spores were harvested from Petri-dishes with pure cultures of *A. flavus*. Conidia were washed from the grits by flooding with sterile distilled water. The suspension was filtered through 2 layers of sterile cheese cloth to remove the mycelia and other vegetative structures. The concentration of spores was determined using a haemocytometer. The concentration was adjusted to $10^5$ spores /ml. From each sample, kernels were surface sterilized in 3% sodium hypochloride after which they were rinsed off three times in sterile distilled water. A total of 20 kernels per sample were plated on Czepak Dox agar amended with 20ppm of antibiotics streptomycin, penicillin, tetracycline and fungicide pentachlonitrobenzene (PCNB). Five kernels were aseptically plated in each plate and incubated at room temperature for 7 – 10 days. The fungi were isolated and *Aspergillus* identified up-to species level based on cultural and morphological characteristics. Data collected include the total number of kernels infected, number of *Aspergillus* genus, *Aspergillus* species and *Fusarium* colonies

### 3.4.3. Determination of aflatoxin content

Aflatoxin content in each sample was determined using Enzyme Linked Immunosorbent Assay (ELISA) method as described in section 3.3.

## 3.5. Data analysis

Data collected during the survey was analysed using the Statistical Package for Social Scientists programme (SPSS 12.0 for windows). The data collected during the fungal isolation and mycotoxin analysis was subjected to analysis of variance using Genstat Programme (Lawes Agricultural Trust Rothamsted experimental Station, 1998, version 8). Differences among the means were compared using Fisher's protected least significant difference (LSD) test at 5% probability level (Steel and Torie, 1987; Clewer, A. G., and Scarisbrick, 1991)

# CHAPTER FOUR: RESULTS

## 4.1. Maize production and handling practices in Eastern and North Rift regions

### 4.1.1. Production practices

Majority of farmers in the Eastern region intercropped maize with beans, pigeon peas, cowpeas, sorghum and cassava (Table 4.1). The main intercrops were beans, pigeon peas and cowpeas. However, more farmers grew maize as pure stand in North Rift region and the only crop used as an intercrop was beans. The source of planting seeds in both regions was seed stockists although a large proportion from Eastern region selected own maize seeds and only a few farmers used own seed in North Rift (Table 4.2). In both regions, most farmers harvested their maize before the grains and stalks were completely dry. Majority of farmers in Eastern removed the sheaths and heaped the cobs on bare ground during harvesting of maize (Table 4.3). The main harvesting practice in the North Rift region was stooking of maize. Most of the farmers in both regions harvested their maize during dry weather conditions.

### 4.1.2. Handling practices

After harvesting, most farmers dried their maize before keeping in the stores. While in Eastern region majority of farmers dried their maize when still in the cobs, a slightly high proportion of farmers in North Rift dried their maize after shelling (Table 4.4). Most of the farmers in the Eastern region dried their maize on bare ground but there were those who spread on mats and polythene sheets. In the North Rift, majority of the farmers spread maize on mats, polythene sheets and tarpaulin to dry with a very small proportion drying their maize on bare ground (Table 4.4). Majority of traders from both regions used their personal experience to determine whether maize was properly dry before they bought. Only a small proportion of traders from North Rift who had used moisture meters to determine the moisture content of the maize before buying (Table 4.5). Among the traders who did further drying of their maize, the majority from both regions spread the maize on mats, polythene sheets or tarpaulins to dry. There was still a sizeable proportion that spread their maize on concrete floors on verandas or spare rooms to dry. Only a very small proportion of traders in North Rift spread their maize outside on bare earth surface (Table 4.5). In Eastern province, most of the maize sold by traders was from outside the

district and from several sources. Some of the maize that sold in the Eastern region was from as far as Tanzania and had passed through either Loitokitok or Taveta (Table 4.6). The major source of maize sold by traders in North Rift was from middlemen and local farmers. Almost half of the traders in Eastern got their supply of maize from middlemen while about one third of the traders bought some of their maize from local farmers. In the North Rift, more than half of the traders relied on middlemen for supply of maize and all of them had bought maize from local farmers.

Maize was transported to the premises using various modes like vehicles, donkey / oxen carts, bicycles and in small quantities by people on foot (Table 4.7). Majority of the maize sold by traders in Eastern region was transported on vehicles. Some traders had received maize ferried using bicycles, carts, donkey backs and by people (Table 4.7). Donkey backs and bicycles carried most of the maize sold by traders in north Rift although lorries supplied a reasonably high proportion of traders (Table 4.7). Maize carried as luggage by people and carts supplied only a small proportion of traders. The maize was carried in containers such as baskets, paper bags, and sacks depending on the quantity and distance.

Table 4.1: Percentage of farmers who intercropped maize with other crops.

| District | Pure stand | Beans | Pigeon peas | Cowpeas | Sorghums/millets | Cassava |
|---|---|---|---|---|---|---|
| | | | Eastern region | | | |
| Makueni | 40.0 | 50.0 | 45.0 | 40.0 | 25.0 | 10.0 |
| Kitui | 5.0 | 90.0 | 95.0 | 90.0 | 0.0 | 0.0 |
| Machakos | 20.0 | 70.0 | 65.0 | 65.0 | 15.0 | 0.0 |
| **Mean** | **21.7** | **70.0** | **68.3** | **65.0** | **13.3** | **3.3** |
| | | | North Rift region | | | |
| Trans Nzoia | 45.0 | 55.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Uasin Gishu | 71.4 | 28.6 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Mean** | **58.2** | **41.8** | **0.0** | **0.0** | **0.0** | **0.0** |

Table 4.2: Percentage of farmers who acquired their seed maize from different sources.

| District | From stockists | From open market | Own Seeds | From Neighbours |
|---|---|---|---|---|
| | | Eastern region | | |
| Makueni | 70 | 10 | 45 | 15 |
| Kitui | 80 | 5 | 70 | 20 |
| Machakos | 70 | 0 | 85 | 0 |
| **Mean** | **73.3** | **5.0** | **66.7** | **11.7** |
| | | North Rift region | | |
| Trans Nzoia | 95 | 0 | 5 | 0 |
| Uasin Gishu | 83.2 | 0 | 16.8 | 0 |
| **Mean** | **89.1** | **0** | **10.9** | **0** |

Table 4.3: Percentage of farmers who harvested at different stages of crop growth using different methods and weather conditions at harvesting time

| | Stage of harvesting | | Harvesting method | | Weather Conditions at harvesting | |
|---|---|---|---|---|---|---|
| District | physiological maturity | completely dry | Without sheath | Stooking | Dry | Wet |
| | | | Eastern region | | | |
| Makueni | 80.0 | 20.0 | 95.0 | 5.0 | 55.0 | 45.0 |
| Kitui | 95.0 | 5.0 | 90.0 | 0.0 | 100.0 | 0.0 |
| Machakos | 20.0 | 80.0 | 100.0 | 0.0 | 70.0 | 30.0 |
| **Mean** | **65.0** | **35.0** | **95.0** | **1.7** | **75.0** | **25.0** |
| | | | North Rift region | | | |
| Trans Nzoia | 60.0 | 40.0 | 0.0 | 100.0 | 50.0 | 50.0 |
| Uasin Gishu | 71.4 | 28.6 | 0.0 | 85.7 | 71.4 | 28.6 |
| **Mean** | **65.8** | **34.3** | **0.0** | **92.9** | **60.7** | **39.3** |

Table 4.4: Percentage of farmers who dried maize in different forms and places in Easter and North Rift regions

| District | Form of drying | | Drying place | |
|---|---|---|---|---|
| | Shelled grains | In cobs | Bare ground | Mats |
| | | Eastern region | | |
| Makueni | 0.0 | 100.0 | 50.0 | 50.0 |
| Kitui | 5.0 | 95.0 | 80.0 | 20.0 |
| Machakos | 5.0 | 95.0 | 65.0 | 35.0 |
| **Mean** | **3.3** | **96.7** | **65.0** | **35.0** |
| | | North Rift region | | |
| Trans Nzoia | 50.0 | 50.0 | 10.0 | 90.0 |
| Uasin Gishu | 57.1 | 42.9 | 9.6 | 90.4 |
| **Mean** | **53.6** | **46.5** | **9.8** | **90.2** |

Table 4.5: Percentage of traders who used different methods to determine moisture content and dried maize at different places in Eastern and North Rift regions.

| | Determination of moisture content | | Place of drying | | |
|---|---|---|---|---|---|
| | Moisture meter | Personal experience | Mats or polythene bags | Cemented floor | Bare earth |
| | | Eastern region | | | |
| Makueni | 0.0 | 100.0 | 55.0 | 45.0 | 0.0 |
| Kitui | 5.0 | 95.0 | 65.0 | 35.0 | 0.0 |
| Machakos | 0.0 | 100.0 | 66.4 | 33.6 | 0.0 |
| **Mean** | **1.7** | **98.3** | **62.1** | **37.9** | **0.0** |
| | | North Rift region | | | |
| Trans zoia | 10.0 | 90.0 | 70.0 | 15.0 | 15.0 |
| Uasin Gishu | 5.0 | 95.0 | 85.5 | 14.5 | 0.0 |
| **Mean** | **7.5** | **92.5** | **77.8** | **14.8** | **7.5** |

Table 4.6: Percentage of traders from Eastern and North Rift regions who sourced maize from different places

| Source | Makueni | Kitui | Machakos | **Mean** | T. Nzoia | U. Gishu | **Mean** |
|---|---|---|---|---|---|---|---|
| Taveta | 25.0 | 20.0 | 5.3 | **16.7** | 0.0 | 0.0 | **0.0** |
| Tanzania | 25.0 | 10.0 | 15.8 | **16.9** | 0.0 | 0.0 | **0.0** |
| Middlemen | 40.0 | 70.0 | 36.8 | **48.9** | 90.0 | 55.0 | **72.5** |
| Local farmers | 15.0 | 85.0 | 0.0 | **33.3** | 100.0 | 100.0 | **100.0** |
| Own farm | 5.0 | 10.0 | 0.0 | **5.0** | 0.0 | 0.0 | **0.0** |
| NCPB depots | 0.0 | 30.0 | 0.0 | **10.0** | 0.0 | 0.0 | **0.0** |
| Busia | 0.0 | 20 | 0.0 | **6.7** | 0.0 | 0.0 | **0.0** |
| Loitokitok | 20.0 | 5.0 | 26.3 | **17.1** | 0.0 | 10.0 | **5.0** |
| Kitale | 30.0 | 25.0 | 36.8 | **30.6** | 5.0 | 0.0 | **2.5** |

Table 4.7: Percentage of traders who transported maize by different means in Eastern and North Rift regions.

| District | Vehicles | Carts | Bicycles | People | Donkeys back |
|---|---|---|---|---|---|
| | | | Eastern region | | |
| Makueni | 95 | 0 | 10 | 15 | 25 |
| Kitui | 90 | 10 | 20 | 5 | 15 |
| Machakos | 94.7 | 5.3 | 5.3 | 0 | 0 |
| **Mean** | **93.2** | **5.1** | **11.8** | **5.0** | **33.3** |
| | | | North Rift region | | |
| Trans Nzoia | 45 | 20 | 90 | 15 | 100 |
| Uasin Gishu | 50 | 10 | 80 | 0 | 95 |
| **Mean** | **47.5** | **15.0** | **85.0** | **7.5** | **97.5** |

### 4.1.3. Storage practices

Storage was mostly in sisal bags, propylene (Synthetic) bags or polythene bags. Majority of farmers and traders stored their maize in synthetic bags and about a fifth of each used sisal or jute bags (Table. 4.8). Polythene bags were used by a very small percentage of traders in North Rift but none of the farmers used it (Table 4.8). In both regions, majority of the farmers stored their maize for more than four months although a small proportion in both regions stored it for a period of four to six months. In the North Rift, none of the farmers stored maize for a period of one month but in eastern, there were a few farmers who stored for a period of one month (Table 4.9). A high proportion of traders in both regions sold their maize within seven days of buying (Table 4.9). In general, most traders stored their maize for a period of less than four weeks. Only a small proportion of traders stored their maize for a period exceeding one month (Table 4.9).

Most farmers in the Eastern region stored maize in the houses, cribs, and improved stores. In the North Rift region, majority of the farmers used the improved stores for keeping their maize (Table 4.10). About one-fifth of the farmers stored their maize in the houses and a very small proportion stored the maize in traditional stores (Table 4.10). Most of the farm storage structures in Eastern region had walls constructed of either stones or bricks or wooden walls in most cases using sisal stalks (Table 4.11). In the North Rift region, most of the storage structures had walls constructed of timber and off-cuts. About one-third of the farmers had mud walled storage structures while small proportion of the farmers used structures constructed of either stone or bricks or iron sheets (Table 4.11).

In both regions, most of the farm stores were roofed with iron sheets, with a few exceptions where the farmers had roofed with thatching grass (Table 4.12). In the North Rift, a high proportion of the stores had either raised wooden floors or bare earth surface (Table 4.12). Compared to the eastern region, the proportion of farmers with concrete floors in North rift was lower. However, most of the stores in the Eastern region had raised wooden platform floors.

Table 4.8: Percentage of traders and farmers using different storage materials in Eastern and North Rift regions.

| | Farmers | | | Traders | | |
|---|---|---|---|---|---|---|
| District | Polythene bag | Sisal bag | Synthetic Bags | Polythene Bags | Sisal bag | Synthetic bag |
| | | | Eastern region | | | |
| Makueni | 0.0 | 35.0 | 90.0 | 5.0 | 15.0 | 90.0 |
| Kitui | 0.0 | 15.0 | 95.0 | 0.0 | 10.0 | 85.0 |
| Machakos | 20.0 | 35.0 | 84.2 | 0.0 | 26.3 | 45.0 |
| **Mean** | **6.7** | **20.0** | **89.7** | **1.7** | **15.4** | **73.3** |
| | | | North Rift region | | | |
| Trans Nzoia | 0.0 | 15.0 | 95.0 | 5.0 | 15.0 | 90.0 |
| Uasin Gishu | 0.0 | 23.8 | 100.0 | 0.0 | 25.0 | 76.2 |
| **Mean** | **0.0** | **19.4** | **97.5** | **2.5** | **20.0** | **80.6** |

Table 4.9: Percentage of farmers and traders who stored maize for different duration in Eastern and North Rift regions.

| | Farmers | | | Traders | | | |
|---|---|---|---|---|---|---|---|
| District | <1 month | 1 - 4 months | >4 months | <7days | 7 days - 4 weeks | 1 - 6 months | > 6 months |
| | | | Eastern region | | | | |
| Makueni | 10.0 | 15.0 | 75.0 | 60.0 | 40.0 | 0.0 | 0.0 |
| Kitui | 0.0 | 20.0 | 80.0 | 35.0 | 50.0 | 5.0 | 10.0 |
| Machakos | 5.0 | 0.0 | 95.0 | 63.2 | 26.3 | 0.0 | 10.5 |
| **Mean** | **5.0** | **11.6** | **83.3** | **52.7** | **38.8** | **1.7** | **6.8** |
| | | | North Rift region | | | | |
| Trans Nzoia | 0.0 | 20.0 | 80.0 | 40.0 | 10.0 | 35.0 | 15.0 |
| Uasin Gishu | 0.0 | 0.0 | 100.0 | 40.0 | 40.0 | 20.0 | 0.0 |
| **Mean** | **0.0** | **10.0** | **90.0** | **40.0** | **25.0** | **27.5** | **7.5** |

Table 4.10: Percentage of farmers who used different storage structures in Eastern and North Rift regions

| District | House | Cribs | Traditional granary | Improved stores |
|---|---|---|---|---|
| | | Eastern region | | |
| Makueni | 25.0 | 50.0 | 5.0 | 20.0 |
| Kitui | 60.0 | 15.0 | 0.0 | 25.0 |
| Machakos | 45.0 | 5.0 | 0.0 | 50.0 |
| **Mean** | **43.3** | **23.3** | **1.7** | **31.7** |
| | | North Rift region | | |
| Trans Nzoia | 20.0 | 0 | 10.0 | 70.0 |
| Uasin Gishu | 23.8 | 0 | 0 | 76.2 |
| **Mean** | **21.9** | **0** | **5** | **73.1** |

Table 4.11: Percentage of farmers storing maize in structures with walls constructed in different materials in Eastern and North Rift regions.

| District | Stone or bricks | Timber, off-cuts or sisal stalks | Iron sheets | Mud |
|---|---|---|---|---|
| | | Eastern region | | |
| Makueni | 20 | 75 | 0 | 5 |
| Kitui | 65 | 30 | 5 | 0 |
| Machakos | 45 | 55 | 0 | 0 |
| **Mean** | **43.3** | **53.3** | **1.7** | **1.7** |
| | | North Rift region | | |
| Trans Nzoia | 10 | 40 | 10 | 40 |
| Uasin Gishu | 0 | 76.2 | 4.8 | 19 |
| **Mean** | **5** | **58.1** | **7.4** | **29.5** |

In the Eastern region, majority of the traders were using structures with stone or brick walls as stores for their maize (Table 4.13) and only a very small proportion used timber or sisal stalk walled structures. In the North Rift region, almost half of the traders were using timber walled structures (Table 4.13) and a fairly large proportion using stone or brick walled structures. One fifth of the traders were storing maize in mud walled structures (Table 4.13). In both regions, a very small proportion of the farmers placed the storage containers on bare earth or bare cemented floor. Majority of the farmers in both regions placed the containers either on raised wooden floor surface or on wooden pallets to prevent direct contact with earthen or concrete floor (Table 4.14). In the Eastern region, most of the traders placed the bags with maize on wooden pallets (Table 4.14). However, others placed the bags with maize on bare concrete floors, although none placed the maize in direct contact with bare earth surface (Table 4.14). Majority of the traders in North Rift region placed the bags on the bare concrete floors and only about one third placed either on wooden pallets or on bare earth surface (Table 4.14).

Weevils were the most common storage pest in both regions. Traders in the North Rift had more incidences of encounter with larger grain borer than in Eastern region but the proportion that encountered a problem with rats was almost equal (Table 4.15). Trapping and keeping of cats was the main method that was being used for control of rats by some traders in Eastern and North Rift. Weevils were mainly being controlled by dusting with storage chemicals and also by sun drying and sieving. More traders in North Rift dusted their maize for control of weevils compared to Eastern region (Table 4.15). Traders who kept their maize for a short duration controlled weevils by sun drying and sieving and these were more in Eastern than in North Rift (Table 4.15). Fumigation, a control method that requires specialized personnel was only carried out by 5% of traders in Makueni district (Table 4.15).

Table 4.12: Percentage of farmers storing their maize in structures with floor and roofs constructed from different types of materials in Eastern and North Rift regions.

| | Floor | | | Roofing | |
|---|---|---|---|---|---|
| District | Cemented | Bare earth | Raised wooden | Grass | Iron sheets |
| | | | Eastern region | | |
| Makueni | 20.0 | 5.0 | 75.0 | 35.0 | 65.0 |
| Kitui | 60.0 | 0.0 | 40.0 | 20.0 | 80.0 |
| Machakos | 30.0 | 5.0 | 65.0 | 5.0 | 95.0 |
| **Mean** | **36.7** | **3.3** | **60.0** | **20.0** | **80.0** |
| | | | North Rift region | | |
| Trans Nzoia | 10.0 | 55.0 | 35.0 | 25.0 | 75.0 |
| Uasin Gishu | 33.3 | 23.8 | 42.9 | 4.8 | 95.2 |
| **Mean** | **21.65** | **39.4** | **39.0** | **14.9** | **85.1** |

Table 4.13: Percentage of traders storing their maize in structures with different roofing materials and walls in Eastern and North Rift regions.

| | Roofing | | Walls | | |
|---|---|---|---|---|---|
| District | Thatching grass | Iron sheets | Timber | Mud | Stones/Bricks |
| | | | Eastern region | | |
| Makueni | 0.0 | 100.0 | 5.0 | 0.0 | 95.0 |
| Kitui | 5.0 | 95.0 | 0.0 | 0.0 | 100.0 |
| Machakos | 5.3 | 94.7 | 0.0 | 0.0 | 100.0 |
| **Mean** | **3.4** | **96.7** | **1.7** | **0.0** | **98.3** |
| | | | North Rift region | | |
| Trans Nzoia | 0.0 | 100.0 | 45.0 | 25.0 | 30.0 |
| Uasin Gishu | 5.0 | 95.0 | 50.0 | 15.0 | 40.0 |
| **Mean** | **2.5** | **97.5** | **47.5** | **20.0** | **35.0** |

Table 4.14: Percentage of farmers and traders placing storage containers on different surfaces in the store in Eastern and North Rift regions.

| | Farmers | | | Traders | | |
|---|---|---|---|---|---|---|
| District | Earthen / cemented | raised wooden | Wooden pallets | Cemented floor | Wooden pallets | Bare earth |
| | | | Eastern region | | | |
| Makueni | 0.0 | 20.0 | 80.0 | 35.0 | 65.0 | 0.0 |
| Kitui | 5.0 | 65.0 | 30.0 | 65.0 | 35.0 | 0.0 |
| Machakos | 0.0 | 35.0 | 65.0 | 10.5 | 89.5 | 0.0 |
| **Mean** | **1.7** | **40.0** | **58.3** | **36.8** | **63.2** | **0.0** |
| | | | North Rift region | | | |
| Trans Nzoia | 5.0 | 55.0 | 45.0 | 55.0 | 40.0 | 5.0 |
| Uasin Gishu | 0.0 | 38.1 | 61.9 | 75.0 | 15.0 | 10.0 |
| **Mean** | **2.5** | **46.6** | **53.5** | **65.0** | **27.5** | **7.5** |

Table 4.15: Percentage of traders who encountered different types of pests and the control methods they employed in Eastern and North Rift regions.

| | pests | | | Control method | | | |
|---|---|---|---|---|---|---|---|
| District | Weevils | LGB | Rats | Trapping | Dusting | Fumigation | Sun drying and sieving |
| | | | | Eastern region | | | |
| Makueni | 90.0 | 0.0 | 10.0 | 0.0 | 15.0 | 5.0 | 20.0 |
| Kitui | 80.0 | 10.0 | 10.0 | 10.0 | 30.0 | 0.0 | 35.0 |
| Machakos | 89.5 | 0.0 | 10.5 | 0.0 | 21.1 | 0.0 | 31.6 |
| **Mean** | **86.5** | **3.3** | **10.2** | **3.3** | **18.7** | **1.7** | **28.7** |
| | | | | North Rift region | | | |
| Trans Nzoia | 50.0 | 35.0 | 15.0 | 5.0 | 65.0 | 0.0 | 10.0 |
| Uasin Gishu | 70.0 | 20.0 | 10.0 | 20.0 | 40.0 | 0.0 | 15.0 |
| **Mean** | **60.0** | **27.5** | **12.5** | **12.5** | **52.5** | **0.0** | **12.5** |

Key: LGB = Larger Grain Borer

### 4.1.4. Consumption practices of maize and maize products.

Maize was consumed as whole grains, semi-processed grains ("Muthokoi") or flour. Whole grains or semi-processed grains were boiled, most of the times mixed with grain legumes such as beans, cowpeas and pigeon peas to cook a mixture ("Githeri"). Maize flour was used to prepare "ugali" and porridge. In the Eastern region, boiled maize and legume mixture ("Githeri"), thick porridge ("Ugali") and semi-processed maize grains ("Muthokoi") were consumed by majority of farmers (Table 4.16). Porridge was consumed by slightly more than half of the farmers (Table 4.16). Thick porridge ("Ugali") was consumed in virtually every household in North Rift but the proportion that consumed boiled whole grain and legume mixture was lower than in Eastern (Table 4.16). Only a very small proportion of the farmers consumed maize in form of semi-processed grains ("Muthokoi").

Taking own maize for grinding into maize flour for use at home was the most common practice in both regions (Table 4.17). Very few farmers in both regions relied on flour bought from shops and open-air markets. In the Eastern region almost one quarter of the farmers used maize flour ground at home by hand operated mills. Farmers had different sources of semi-processed grains ("Muthokoi") including buying from shops and open-air market, taking own maize for dehulling at the posho mills and processing at home using mortar and pestle (Table 4.18). In the Eastern region, most of the farmers dehulled their own maize by taking to posho mills and also processed at home using mortar and pestle (Table4.18). Most traders were selling other products like maize flour either in open bags or packed by millers and semi-processed grains besides maize (Table 4.19). More traders in the Eastern region than in North Rift sold maize flour and semi-processed grains (Table 4.19). Very few traders in the North Rift region dealt with semi-processed grains.

The major source of semi-processed grains sold by traders in both regions was the posho mills while the maize flour was from suppliers, middlemen and posho mills (Table 4.19). Slightly more than half of the farmers in either regions at one time or another were required to source maize from elsewhere to bridge the deficit (Table 4.20. Majority of

these farmers relied on the markets for their supply of maize. Relief food and neighbours also played important roles as sources of maize for some families (Table 4.20).

Table 4.16: Percentage of farmers who consume maize in different forms in Eastern and North Rift regions.

| District | "Muthokoi" | "Ugali" | Porridge | "Githeri" |
|---|---|---|---|---|
| | | Eastern region | | |
| Makueni | 95.0 | 95.0 | 55.0 | 90.0 |
| Kitui | 100.0 | 100.0 | 85.0 | 100.0 |
| Machakos | 85.0 | 90.0 | 35.0 | 100.0 |
| **Mean** | **93.3** | **95.0** | **58.3** | **98.3** |
| | | North Rift region | | |
| Trans Nzoia | 5.0 | 100.0 | 75.0 | 95.0 |
| Uasin Gishu | 19.0 | 100.0 | 19.0 | 42.9 |
| **Mean** | **12.0** | **100.0** | **47.0** | **68.95** |

Table 4.17: Percentage of farmers who have different sources for maize flour in Eastern and North Rift regions.

| District | Bought from shops | Own maize ground at home | Own maize taken to posho mill | From open-air market |
|---|---|---|---|---|
| | | Eastern region | | |
| Makueni | 5.0 | 25.0 | 100.0 | 0.0 |
| Kitui | 20.0 | 45.0 | 80.0 | 0.0 |
| Machakos | 10.0 | 0.0 | 90.0 | 0.0 |
| **Mean** | **11.6** | **23.3** | **90.0** | **0.0** |
| | | North Rift region | | |
| Trans Nzoia | 10.0 | 0.0 | 100.0 | 5.0 |
| Uasin Gishu | 0.0 | 4.8 | 95.0 | 0.0 |
| **Mean** | **5.0** | **2.4** | **97.5** | **2.5** |

### 4.1.5. Awareness about aflatoxin

Awareness about aflatoxin poisoning among traders and farmers in both regions was very high. Most farmers and traders cited proper drying of maize as a good measure that could be taken to prevent aflatoxin poisoning (Table 4.21). More traders than farmers were aware that sorting of mouldy and discoloured grains could reduce the level of aflatoxin concentration in maize and therefore prevent poisoning (Table 4.21). Only a very small proportion of farmers from both regions were aware that proper washing before cooking could prevent aflatoxin poisoning (Table 4.21). Radio and extension officers were the main sources of information about aflatoxin poisoning for the farmers in both regions (Table 4.22). Other neighbours had received the information from their neighbours and a few had read in the newspapers (Table 4.22).

Table 4.18: Percentage of farmers who have different sources for semi-processed grains ("Muthokoi") in Eastern and North Rift regions

| District | Bought from shops | Processed at home | Own maize taken to the posho mill | Bought from open-air market |
|---|---|---|---|---|
| | | Eastern region | | |
| Makueni | 0.0 | 50.0 | 65.0 | 0.0 |
| Kitui | 0.0 | 70.0 | 65.0 | 0.0 |
| Machakos | 5.0 | 40.0 | 75.0 | 5.0 |
| **Mean** | **1.7** | **53.3** | **68.3** | **1.7** |
| | | North Rift region | | |
| Trans Nzoia | 0.0 | 5.0 | 0.0 | 0.0 |
| Uasin Gishu | 4.8 | 4.8 | 4.8 | 4.8 |
| **Mean** | **2.4** | **4.9** | **2.4** | **2.4** |

Table 4.19: Percentage of traders who sold maize flour and semi-processed grains ("Muthokoi") and different sources of maize flour and semi-processed grains in Eastern and North Rift regions

| | Maize products sold | | Source of "Muthokoi" | | Source of maize flour | | |
|---|---|---|---|---|---|---|---|
| District | "Muthokoi" | Flour | Suppliers | Posho mill | Suppliers | Middlemen | Posho mill |
| | | | Eastern region | | | | |
| Makueni | 40.0 | 40.0 | 0.0 | 45.0 | 25.0 | 0.0 | 20.0 |
| Kitui | 50.0 | 85.0 | 5.0 | 45.0 | 70.0 | 15.0 | 10.0 |
| Machakos | 47.4 | 57.9 | 10.5 | 36.8 | 52.6 | 5.3 | 15.8 |
| **Mean** | **45.8** | **61.0** | **5.2** | **42.3** | **49.2** | **6.8** | **15.3** |
| | | | North Rift region | | | | |
| Trans Nzoia | 0.0 | 20.0 | 0.0 | 0.0 | 10.0 | 5.0 | 15.0 |
| Uasin Gishu | 5.0 | 15.0 | 0.0 | 5.0 | 15.0 | 0.0 | 0.0 |
| **Mean** | **2.5** | **17.5** | **0.0** | **2.5** | **12.5** | **2.5** | **7.5** |

Table 4.20: Percentage of farmers who sourced maize from elsewhere

| District | Market | Relief food | Neighbours |
|---|---|---|---|
| | Eastern region | | |
| Makueni | 75.0 | 20.0 | 15.0 |
| Kitui | 60.0 | 20.0 | 20.0 |
| Machakos | 25.0 | 5.0 | 0.0 |
| **Mean** | **53.3** | **15** | **11.7** |
| | North Rift region | | |
| Trans Nzoia | 50.0 | 15.0 | 55.0 |
| Uasin Gishu | 19.0 | 0.0 | 14.3 |
| **Mean** | **34.5** | **7.5** | **34.7** |

Table 4.21: Percentage of farmers and traders who were aware about aflatoxin poisoning and measures taken to avoid poisoning.

| | Farmers | | | Traders | |
|---|---|---|---|---|---|
| District | Proper drying | Washing | Removal of mouldy grains | Avoid selling mouldy grains | proper drying |
| | | | Eastern region | | |
| Makueni | 100.0 | 10.0 | 20.0 | 60.0 | 80.0 |
| Kitui | 95.0 | 5.0 | 40.0 | 45.0 | 75.0 |
| Machakos | 90.0 | 5.0 | 30.0 | 63.2 | 73.7 |
| **Mean** | **95.0** | **6.7** | **30.0** | **56.1** | **76.2** |
| | | | North Rift region | | |
| Trans Nzoia | 55.0 | 5.0 | 30.0 | 45.0 | 75.0 |
| Uasin Gishu | 85.7 | 0.0 | 4.8 | 45.0 | 95.0 |
| **Mean** | **70.4** | **2.5** | **17.4** | **45.0** | **85.0** |

Table 4.22: Percentage of farmers sourcing of information from different media in Eastern and North Rift regions.

| District | Extension Officer | Radio | Newspaper | Neighbour |
|---|---|---|---|---|
| | | Eastern region | | |
| Makueni | 60.0 | 50.0 | 5.0 | 35.0 |
| Kitui | 70.0 | 75.0 | 10.0 | 30.0 |
| Machakos | 60.0 | 85.0 | 30.0 | 40.0 |
| **Mean** | **63.3** | **70.0** | **15.0** | **35.0** |
| | | North Rift region | | |
| Trans Nzoia | 25.0 | 95.0 | 10.0 | 10.0 |
| Uasin Gishu | 57.1 | 52.4 | 4.8 | 9.5 |
| **Mean** | **41.1** | **73.7** | **7.4** | **9.8** |

## 4.2. Fungal and aflatoxin contamination of maize and maize products

### 4.2.1. Fungal inoculum in the storage environment.

The fungal genera isolated from the soils collected from under the stores and from outside the houses were *Fusarium, Aspergillus* and *Penicillium* (Table 4.23). In the soils from under the stores, the number of *Fusarium* colony forming units (CFUs) per gram of soil in the sample was significantly different ($P \leq 0.05$) among the agro-ecological zones (AEZs). The soil sample collected from agro-ecological zone LM4 in Kitui district had significantly a higher number of *Fusarium* colony forming units than soils collected from LM3 and LM5 in Makueni and LM5 in Machakos. No differences were noted in the number of colony forming units for *Fusarium* in the sample from LM5 in Machakos district, LM4 and LM5 of Kitui district. The *Aspergillus* species isolated were *A. flavus, A. niger, A. terreus, A. ochraceus,* and *A. versicolor*. The number of CFUs for *Aspergillus* genera and species were not different among the agro-ecological zones (Table 4.23).

In the fungal isolations from soil collected from outside the homesteads, only the number of colony forming units for *Aspergillus* genera and *A. flavus* were significantly different among the agro-ecological zones (Table 4.24). *Fusarium* was isolated at a higher frequency than the other fungi. The number of CFUs of *Aspergillus* in the soil sample from LM5 in Kitui district was significantly ($P \leq 0.05$) higher than in the other samples except the sample from agro-ecological zone LM4 in Kitui. Among the *Aspergillus* species, only *A. flavus* was significantly different among the agro-ecological zones. The number of colony forming units for *Aspergillus flavus* in the sample from agro-ecological zone LM5 in Kitui district was significantly ($P \leq 0.05$) higher than in the samples from LM4 and LM5 in Machakos districts and LM5 in Makueni (Table 4.24). No significant differences were noted in the number of *A. flavus* CFUs in the soil from LM4 and LM5 Kitui and LM3 of Makueni. Samples from agro-ecological zones LM4 and LM5 of Machakos did not form any colony forming units.

The fungal genera isolated from one gram of posho mill dust were *Fusarium, Aspergillus* and others which were not identified (Table 4.25). *Fusarium* had the highest frequency of

isolation followed by *Aspergillus*. Among the *Aspergillus*, the species isolated were *A. flavus*, *A. niger*, and *A. versicolor*. There was no significant difference in the number of CFUs for all genera and species isolated.

Table 4.23: Number of colony forming units of different per gram of soil collected from under the stores in different agro-ecological zones of Eastern Province during the long rain season of 2008

| District / AEZ | *Fusarium* | *Aspergillus* | *A. flavus* | *A. niger* | *A. ochraceous* | *A. terreus* | *A. versicolor* |
|---|---|---|---|---|---|---|---|
| Kitui LM4 | 4670 | 940 | 190 | 700 | 40 | 0 | 0 |
| Kitui LM5 | 2660 | 730 | 550 | 180 | 0 | 0 | 0 |
| Machakos LM4 | 2470 | 290 | 200 | 70 | 0 | 0 | 40 |
| Machakos LM5 | 1800 | 270 | 60 | 0 | 30 | 180 | 0 |
| Makueni LM3 | 1280 | 1380 | 160 | 70 | 0 | 20 | 40 |
| Makueni LM5 | 430 | 490 | 30 | 0 | 0 | 0 | 0 |
| Mean | 2170 | 620 | 200 | 160 | 10 | 30 | 20 |
| LSD $_{(P\leq0.05)}$ | 2310 | NS | NS | NS | NS | NS | NS |
| CV% | 113.9 | 247.6 | 242.5 | 360.9 | 546.6 | 637.7 | 440.7 |

Table 4.24: Number of colony forming units of different fungi per gram of soil sample collected from out-side homesteads in different agro-ecological zones of Eastern region during the long rain season of 2008.

| District / AEZ | *Fusarium* | *Aspergillus* | Others | *A. flavus* | *A. niger* |
|---|---|---|---|---|---|
| Kitui LM4 | 4940 | 120 | 270 | 90 | 30 |
| Kitui LM5 | 970 | 140 | 240 | 120 | 20 |
| Machakos LM4 | 230 | 0 | 830 | 0 | 0 |
| Machakos LM5 | 270 | 0 | 60 | 0 | 0 |
| Makueni LM3 | 1730 | 50 | 620 | 40 | 20 |
| Makueni LM5 | 1050 | 40 | 230 | 20 | 20 |
| **Mean** | **1640** | **60** | **370** | **40** | **20** |
| $LSD_{(p\leq0.05)}$ | NS | 80 | NS | 80 | NS |
| CV% | 393.0 | 152.4 | 262.4 | 195.6 | 273.1 |

Table 4.25: Number of colony forming units of different fungi per gram of sweepings collected from posho mills in different agro-ecological zones of Eastern region during the long rain season of 2008.

| District | *Fusarium* | *Aspergillus* | Others | *A. flavus* | *A. niger* | *A. versicolor* |
|---|---|---|---|---|---|---|
| Kitui LM4 | 1203 | 492 | 272 | 306 | 2 | 83 |
| Kitui LM5 | 1472 | 132 | 202 | 39 | 84 | 0 |
| Machakos LM4 | 2493 | 123 | 3849 | 1258 | 263 | 8 |
| Machakos LM4 | 425 | 1530 | 3392 | 28 | 0 | 0 |
| Makueni LM3 | 4654 | 12 | 998 | 149 | 0 | 0 |
| Makueni LM5 | 7 | 149 | 1607 | 72 | 2 | 20 |
| **Mean** | **1864** | **428** | **248** | **330** | **64** | **18** |
| $LSD_{(p\leq0.05)}$ | NS | NS | NS | NS | NS | NS |
| CV% | 135.2 | 220.9 | 168.7 | 236.9 | 249.1 | 286.6 |

### 4.2.2. Total fungal contamination of maize grains

In Eastern region, maize samples from traders had a higher percentage of kernel infection than maize from farmers (Table 4.26). However, there were no differences in percentage of kernel infection in maize from different traders. The long rain season samples from farmers had a higher percentage of kernel infection than the short rain maize samples. Agro-ecological zones had no significant effect on the percentage of kernel infection during the short rain season. However, during the long rain season, samples from agro-ecological zone LM4 and LM5 of Machakos had significantly lower total kernel infection than all samples from Kitui and Makueni. The sample from LM4 in Kitui had a significantly higher infection than maize samples from agro-ecological zone LM4 and LM5 of Machakos district but, it had significantly lower infection than the sample from LM3 in Makueni district (Table 4.26). No significant differences were noted in total kernel infection among the agro-ecological zones LM3 and LM5 in Makueni and LM4 in Kitui district. Percentage of total kernel infection in maize from farmers and traders of North Rift region was not significantly different among the agro-ecological zones and districts. Maize from farmers and traders of North Rift had a higher total kernel infection rate than maize sampled from farmers in Eastern region but the difference among traders was negligible.

Table 4.26: Percentage of total kernel infection in maize sampled from farmers and traders in different agro-ecological zones of Eastern and North Rift regions during the long and short rainfall seasons of 2008.

| | Eastern Region | | |
|---|---|---|---|
| District / AEZ | Traders | Farmers (long rains) | Farmers (short rains) |
| Kitui LM4 | 83.0 | 79.6 | 55.5 |
| Kitui LM5 | 90.7 | 82.2 | 64.3 |
| Machakos LM4 | 95.7 | 60.2 | 67.3 |
| Machakos LM5 | 91.7 | 62.6 | |
| Makueni LM3 | 94.5 | 97.5 | 57.0 |
| Makueni LM5 | 96.2 | 88.8 | 53.3 |
| Mean | 91.7 | 78.5 | 59.5 |
| LSD $_{(p\leq0.05)}$ | NS | 16.9 | NS |
| CV% | 13.7 | 24.1 | 29.0 |

| | North Rift Region (long rains) | |
|---|---|---|
| | Traders | Farmers |
| Trans Nzoia LH2 | 95.5 | 85.4 |
| Trans Nzoia LH3 | 95.6 | 87.6 |
| Trans Nzoia UM3 | 91.2 | 94.2 |
| Trans Nzoia UM4 | 86.6 | 95.0 |
| Uasin Gishu LH2 | 94.1 | 87.6 |
| Uasin Gishu LH3 | 96.0 | 97.2 |
| Uasin Gishu UM3 | 92.7 | 95.2 |
| Uasin Gishu UM4 | 97.0 | 87.3 |
| Mean | 92.5 | 90.8 |
| LSD $_{(p\leq0.05)}$ | NS | NS |
| CV% | 9.0 | 9.5 |

### 4.2.3 Contamination of maize grain with *Fusarium* species

*Fusarium* species isolated from the maize sampled from both farmers and traders in Eastern province were *Fusarium graminearum, F. proliferatum, F. semitectum, F. subglutinans and F. verticillioides* (Table 4.27). Among the *Fusarium* species isolated in samples from farmers of Eastern Province, *Fusarium subglutinans* had the highest frequency of isolation in both seasons, but *Fusarium proliferatum* had the least in the long rains season and *Fusarium vertcillioides* in the short rains season. *Fusarium graminearum* was the second most isolated species in both seasons. Frequency of isolation for *Fusarium proliferatum* was significantly different among the AEZs in both long and short rain season samples but *F. verticillioides* was significantly different only in the long rains samples.

The maize sample from agro-ecological zone LM5 in Makueni had the highest percentage isolation of *Fusarium proliferatum* during the long rains season (Table 4.27). No differences were noted in the frequency of *Fusarium proliferatum* during the long rains season among the rest of the agro-ecological zones and districts. The frequency of isolation for *F. verticillioides* was significantly lower in the sample from agro-ecological zone of LM4 of Machakos than in samples from agro-ecological zone of LM3 and LM5 of Makueni district. There were no significant differences among the agro-ecological zones in the frequency of isolation of *F. verticillioides* for samples from agro-ecological zone of LM5 of Machakos, LM3 and LM5 in Makueni and LM4 and LM5 in Kitui.

Frequency of isolation for *Fusarium proliferatum* was significantly ($P \leq 0.05$) different among the agro-ecological zones in both seasons, while percentage isolation for *Fusarium vertcillioides* was only significantly ($P \leq 0.05$) different during the long rains season (Table 4.27). The sample from agro-ecological zone LM5 in Makueni had the highest percentage isolation of *Fusarium proliferatum* during the long rains season. The sample from agro-ecological zone LM4 in Machakos district had a significantly ($P \leq 0.05$) lower percentage of isolation than the sample from LM5 in Makueni.

During the short rain season maize, *Fusarium proliferatum* had the highest frequency of isolation in the sample from agro-ecological zone LM3 in Makueni district (Table 4.27). No differences in the frequency of isolation of *Fusarium proliferatum* were noted among the agro-ecological zones LM4 in Machakos, LM5 in Makueni and LM4 and LM5 in Kitui. There was no significant ($P \leq 0.05$) difference in frequency of isolation of *Fusarium* species among the agro-ecological zones in the maize sampled from traders in Eastern Province.

*Fusarium* species isolated from the maize grains sampled from traders and farmers in North Rift were *Fusarium graminearum, F. proliferatum, F. semitectum, F. subglutinans and F. verticillioide* (Table 4.28). Only *Fusarium proliferatum* and *Fusarium subglutinans* had significant differences in frequency of isolation among the agro-ecological zones in the maize collected from traders in North Rift. The highest percentage of isolation was for *Fusarium proliferatum* followed by *Fusarium subglutinans.* The sample from agro-ecological zone UM4 in Uasin Gishu had a significantly ($P \leq 0.05$) higher percentage isolation of *Fusarium subglutinans* than samples from all other agro-ecological zones. No difference was noted in isolation of *Fusarium subglutinans* among samples from LH2, LH3, UM3 and UM4 in Trans Nzoia and LH2, LH3 and Um3 in Uasin Gishu districts.

The sample from agro-ecological zone UM4 and LH2 in Trans Nzoia had significantly ($P \leq 0.05$) higher percentage isolation of *Fusarium proliferatum* than the sample from agro-ecological zone UM4 in Uasin Gishu (Table 4.28). No differences in the frequencies of isolation were noted among the other samples. None of the species was significantly ($P \leq 0.05$) different among the agro-ecological zones in the maize sampled from farmers in North Rift region (Table 4.29).

Table 4.27: Percentage isolation of different *Fusarium* species from maize kernels sampled from different agro-ecological zones of Eastern region during long and short rainfall seasons in 2008

Long rain 2008

| District / AEZ | *subglutinans* | *graminearum* | *proliferatum* | *semitectum* | *verticillioides* |
|---|---|---|---|---|---|
| Kitui LM4 | 12.1 | 12.7 | 3.1 | 4.6 | 10.8 |
| Kitui LM5 | 11.9 | 16.2 | 2.3 | 3.0 | 11.2 |
| Machakos LM4 | 14.7 | 11.4 | 0.5 | 2.3 | 3.0 |
| Machakos LM5 | 16.7 | 10.7 | 2.0 | 0.8 | 7.4 |
| Makueni LM3 | 23.7 | 10.4 | 2.8 | 5.5 | 14.4 |
| Makueni LM5 | 21.1 | 12.4 | 6.9 | 4.0 | 15.8 |
| **Mean** | **16.7** | **12.3** | **2.9** | **3.4** | **10.6** |
| LSD $_{(p\leq0.05)}$ | NS | NS | 3.6 | NS | 8.38 |
| CV% | 78.6 | 82.0 | 136.7 | 126.4 | 88.2 |

Short rain 2008

| District / AEZ | *subglutinans* | *graminearum* | *proliferatum* | *semitectum* | *verticillioides* |
|---|---|---|---|---|---|
| Kitui LM4 | 17.9 | 7.5 | 2.2 | 5.0 | 2.0 |
| Kitui LM5 | 14.6 | 4.4 | 3.4 | 2.7 | 1.1 |
| Machakos LM4 | 13.4 | 9.9 | 1.2 | 7.4 | 0.3 |
| Makueni LM3 | 19.1 | 5.8 | 6.6 | 1.4 | 0.3 |
| Makueni LM5 | 16.7 | 7.1 | 2.2 | 2.0 | 0.3 |
| **Mean** | **16.3** | **6.9** | **3.12** | **3.7** | **0.8** |
| LSD $_{(p\leq0.05)}$ | NS | NS | 3.15 | NS | NS |
| CV% | 66.5 | 132.7 | 111.9 | 167.5 | 261.7 |

AEZ = agro-ecological zone LSD = least significant difference

NS = not significant ($p\leq0.05$)

Table 4.28: Percentage isolation of different *Fusarium* species from maize kernels sampled from traders in Eastern and North Rift regions in 2008

Eastern region

| District / AEZ | *subglutinans* | *graminearum* | *proliferatum* | *semitectum* | *verticillioides* |
|---|---|---|---|---|---|
| Kitui LM4 | 21.7 | 12.5 | 5.31 | 8.3 | 15.2 |
| Kitui LM5 | 21.7 | 13.2 | 4.3 | 10.8 | 24.3 |
| Machakos LM4 | 11.7 | 12.3 | 2.7 | 12.1 | 25.7 |
| Machakos LM5 | 14.1 | 15.1 | 3.7 | 7.2 | 17.2 |
| Makueni LM3 | 22.2 | 10.4 | 7.8 | 10.2 | 24.6 |
| Makueni LM5 | 21.9 | 8.0 | 11.2 | 12.2 | 22.1 |
| **Mean** | **19.1** | **11.9** | **5.84** | **10.1** | **21.3** |
| LSD $_{(p\leq0.05)}$ | NS | NS | NS | NS | NS |
| CV% | 56.5 | 102.1 | 113.7 | 107.1 | 61.4 |

North Rift region

| District / AEZ | *subglutinans* | *graminearum* | *proliferatum* | *semitectum* | *verticillioides* |
|---|---|---|---|---|---|
| Trans Nzoia LH2 | 8.5 | 0.0 | 65.0 | 13.5 | 1.0 |
| Trans Nzoia LH3 | 8.4 | 3.0 | 37.4 | 24.4 | 0.4 |
| Trans Nzoia UM3 | 16.8 | 3.5 | 54.4 | 7.8 | 1.3 |
| Trans Nzoia UM4 | 5.4 | 0.9 | 68.7 | 5.9 | 1.7 |
| Uasin Gishu LH2 | 17.3 | 5.8 | 51.9 | 13.9 | 2.0 |
| Uasin Gishu LH3 | 7.0 | 8.0 | 45.3 | 32.7 | 0.0 |
| Uasin Gishu UM3 | 8.3 | 5.3 | 41.7 | 16.3 | 3.0 |
| Uasin Gishu UM4 | 46.2 | 3.8 | 20.4 | 7.2 | 2.4 |
| **Mean** | **14.7** | **3.57** | **50.5** | **13.2** | **1.6** |
| LSD $_{(p\leq0.05)}$ | 29.0 | NS | 38.1 | NS | NS |
| CV% | 126.2 | 122.1 | 48.3 | 99.4 | 137.4 |

AEZ = agro-ecological zone LSD = least significant difference

NS = not significant ($p\leq0.05$)

Table 4.29: Percentage isolation of different *Fusarium* species from maize kernels sampled from farmers in different agro-ecological zones of North Rift region for the long rainfall season in 2008.

| District / AEZ | *graminearum* | *proliferatum* | *semitectum* | *subglutinans* | *verticillioides* |
|---|---|---|---|---|---|
| Trans Nzoia LH2 | 1.8 | 58.2 | 7.8 | 3.0 | 1.6 |
| Trans Nzoia LH3 | 0.4 | 67.4 | 8.2 | 1.6 | 0.8 |
| Trans Nzoia UM3 | 2.7 | 69.7 | 6.8 | 5.2 | 2.5 |
| Trans Nzoia UM4 | 1.0 | 79.7 | 5.3 | 1.7 | 0.3 |
| Uasin Gishu LH2 | 6.0 | 64.0 | 6.8 | 2.2 | 2.4 |
| Uasin Gishu LH3 | 7.2 | 60.8 | 21.2 | 1.8 | 0.6 |
| Uasin Gishu UM3 | 2.0 | 70.2 | 9.0 | 6.0 | 1.5 |
| Uasin Gishu UM4 | 4.4 | 55.4 | 10.3 | 4.0 | 0.6 |
| Mean | 3.4 | 64.5 | 9.6 | 3.3 | 1.3 |
| LSD $_{(p\leq0.05)}$ | NS | NS | NS | NS | NS |
| CV% | 166.1 | 28.0 | 114.2 | 146.3 | 118.8 |

AEZ = agro-ecological zone LSD = least significant difference

NS = not significant ($p\leq0.05$)

### 4.2.4 Contamination of maize grain with *Aspergillus* species

*Aspergillus* species isolated in maize collected from Eastern and North Rift regions were *Aspergillus flavus, A. niger, A. fumigatus, A. versicolor, A. terreus, A. clavatus,* and *A. ochraceus* (Table 4.30). For the long rains season maize, only *Aspergillus terreus* and *Aspergillus versicolor* were significantly ($P\leq0.05$) different among the various agro-ecological zones. The sample from agro-ecological zone LM4 in Kitui district had a significantly higher percentage isolation of *Aspergillus terreus* than samples from agro-ecological zones LM3 in Makueni, LM4 in Machakos and LM5 in Machakos. For the long rains season maize samples, *Aspergillus versicolor* was isolated in samples collected from agro-ecological zone LM4 in Machakos only.

Among the *Aspergillus* species isolated from the short rains season, only *A. niger* was significantly different over the agro-ecological zones (Table 4.30). The sample from agro-ecological zone LM5 in Kitui had significantly higher percentage isolation than the samples from other agro-ecological zones and districts except the sample from agro-ecological zone LM4 in Kitui district. There were no significant differences among the agro-ecological zone in percentage of isolation for all fungi isolated in maize from traders of Eastern region (Table 4.31). The sample from agro-ecological zone UM4 of Uasin Gishu had the highest frequency of isolation of *A. flavus*. Generally, the maize from farmers of Eastern region had a higher frequency of isolation for *Aspergillus* species than those from traders in Eastern province (Tables 4.30 and 4.31).

Among the *Aspergillus* species isolated from farmers in North Rift, only the frequency of isolation for *Aspergillus flavus* in maize samples collected from farmers of North Rift region was significantly different among the agro-ecological zones (Table 4.32). The maize sample from agro-ecological zone UM3 in Uasin Gishu had significantly higher percentage isolation of *A. flavus* than samples from all the other agro-ecological zones except in the sample from agro-ecological zone UM4 in Uasin Gishu district. There was no *A. flavus* isolated from the maize samples from LH2 and LH3 in Trans Nzoia. There was low frequency of isolation for all *Aspergillus* species in the maize samples from farmers in agro-ecological zones LH2, LH3 and UM3 of both districts (Table 4.32). Maize samples collected from farmers and traders in North Rift had very low frequencies of isolation for *Aspergillus* species compared to the samples from Eastern Province.

Table 4.30: Percentage isolation of different *Aspergillus* species from maize kernels sampled from farmers different agro-ecological zones in the Eastern Region during long and short rainfall seasons in 2008.

Long rains 2008

| District / AEZ | *flavus* | *fumigatus* | *niger* | *ochraceous* | *terreus* | *versicolor* |
|---|---|---|---|---|---|---|
| Kitui LM4 | 14.7 | 7.1 | 7.8 | 0.0 | 11.8 | 0.0 |
| Kitui LM5 | 15.8 | 6.5 | 12.0 | 0.0 | 9.5 | 0.0 |
| Machakos LM4 | 8.2 | 1.7 | 9.7 | 0.4 | 0.2 | 0.9 |
| Machakos LM5 | 6.6 | 1.2 | 9.5 | 0.5 | 0.9 | 0.0 |
| Makueni LM3 | 13.9 | 3.4 | 10.7 | 0.0 | 2.8 | 0.0 |
| Makueni LM5 | 12.9 | 2.1 | 12.0 | 0.0 | 5.9 | 0.0 |
| **Mean** | **11.7** | **3.7** | **10.3** | **0.2** | **5.2** | **0.2** |
| LSD $_{(p\leq0.05)}$ | NS | NS | NS | NS | 7.7 | 0.4 |
| CV% | 95.3 | 144.9 | 87.7 | 452.7 | 166.0 | 270.6 |

Short Rains

| District / AEZ | *flavus* | *fumigatus* | *niger* | *ochraceous* | *terreus* | *versicolor* | *clavatus* |
|---|---|---|---|---|---|---|---|
| Kitui LM4 | 7.5 | 0.2 | 9.2 | 0.0 | 1.0 | 0.0 | 0.0 |
| Kitui LM5 | 16.7 | 0.1 | 23.8 | 0.0 | 1.1 | 0.2 | 0.0 |
| Machakos LM4 | 13.0 | 0.4 | 19.3 | 0.3 | 0.3 | 0.2 | 0.1 |
| Makueni LM3 | 18.9 | 0.0 | 5.2 | 0.2 | 0.7 | 0.1 | 0.0 |
| Makueni LM5 | 11.6 | 0.2 | 9.0 | 0.2 | 0.7 | 0.6 | 0.2 |
| Mean | 13.5 | 0.2 | 13.3 | 0.1 | 0.8 | 0.2 | 0.1 |
| LSD $_{(p\leq0.05)}$ | NS | NS | 9.7 | NS | NS | NS | NS |
| CV% | 83.1 | 268.4 | 81.1 | 324.7 | 140.4 | 258.0 | 526.0 |

AEZ = agro-ecological zone LSD = least significant difference

NS = not significant ($p\leq0.05$)

Table 4.31: Percentage isolation of different *Aspergillus* species from maize kernels sampled from different traders in Eastern and North Rift regions during the long rainfall season in 2008

Eastern region

| District / AEZ | *flavus* | *fumigatus* | *niger* | *ochraceous* | *terreus* | *clavatus* |
|---|---|---|---|---|---|---|
| Kitui LM4 | 5.8 | 2.5 | 2.5 | 0.2 | 1.4 | 1.1 |
| Kitui LM5 | 9.3 | 4.7 | 4.4 | 0.0 | 3.8 | 0.0 |
| Machakos LM4 | 8.7 | 2.3 | 1.0 | 0.0 | 0.7 | 0.0 |
| Machakos LM5 | 16.7 | 1.6 | 3.3 | 0.0 | 0.1 | 0.0 |
| Makueni LM3 | 14.0 | 5.0 | 3.3 | 0.0 | 2.6 | 0.0 |
| Makueni LM5 | 7.9 | 2.9 | 5.0 | 0.0 | 1.8 | 0.0 |
| **Mean** | **10.2** | **3.2** | **3.2** | **0.1** | **2.0** | **0.2** |
| LSD $_{(p\leq0.05)}$ | NS | NS | NS | NS | NS | NS |
| CV% | 108.8 | 152.9 | 135.7 | 814.4 | 165.3 | 814.4 |

North Rift region

| District / AEZ | *flavus* | *fumigatus* | *niger* | *ochraceous* | *terreus* | *versicolor* | *clavatus* |
|---|---|---|---|---|---|---|---|
| Trans Nzoia LH2 | 0.5 | 0.5 | 0.5 | 0.0 | 0.0 | 0.0 | 0.5 |
| Trans Nzoia LH3 | 1.8 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 18.2 |
| Trans Nzoia UM3 | 1.2 | 0.0 | 1.3 | 0.3 | 0.0 | 0.5 | 1.2 |
| Trans Nzoia UM4 | 10.8 | 0.1 | 0.2 | 0.2 | 1.5 | 1.6 | 0.5 |
| Uasin Gishu LH2 | 1.9 | 0.0 | 0.8 | 0.1 | 1.3 | 0.3 | 0.6 |
| Uasin Gishu LH3 | 1.7 | 0.0 | 0.7 | 0.0 | 2.0 | 0.0 | 1.3 |
| Uasin Gishu UM3 | 1.3 | 0.3 | 0.0 | 0.0 | 0.7 | 0.0 | 1.3 |
| Uasin Gishu UM4 | 0.8 | 0.2 | 0.8 | 0.0 | 1.2 | 0.0 | 0.6 |
| **Mean** | **3.9** | **0.1** | **0.5** | **0.1** | **1.1** | **0.5** | **2.8** |
| LSD $_{(p\leq0.05)}$ | NS | NS | NS | NS | NS | NS | NS |
| CV% | 320.0 | 305.4 | 242.3 | 413.6 | 128.4 | 451.0 | 457.7 |

Table 4.32: Percentage isolation of different *Aspergillus* species from maize kernels sampled from farmers in different agro-ecological zones of North Rift region for the long rainfall season in 2008.

| District / AEZ | *flavus* | *fumigatus* | *niger* | *ochraceous* | *terreus* | *clavatus* |
|---|---|---|---|---|---|---|
| Trans Nzoia LH2 | 0.0 | 0.2 | 0.0 | 0.0 | 1.2 | 0.4 |
| Trans Nzoia LH3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.4 |
| Trans Nzoia UM3 | 0.2 | 0.0 | 0.2 | 0.0 | 1.3 | 0.5 |
| Trans Nzoia UM4 | 0.7 | 0.3 | 0.3 | 0.7 | 0.0 | 0.0 |
| Uasin Gishu LH2 | 0.4 | 0.0 | 0.2 | 0.0 | 0.4 | 1.0 |
| Uasin Gishu LH3 | 0.6 | 0.0 | 0.2 | 0.0 | 0.2 | 0.0 |
| Uasin Gishu UM3 | 5.6 | 0.0 | 0.0 | 0.0 | 0.3 | 0.5 |
| Uasin Gishu UM4 | 4.0 | 0.6 | 0.1 | 0.9 | 0.1 | 0.1 |
| **Mean** | **1.5** | **0.2** | **0.1** | **0.2** | **0.5** | **0.4** |
| LSD $_{(p\leq0.05)}$ | 4.3 | NS | NS | NS | NS | NS |
| CV% | 208.5 | 268.2 | 281.3 | 348.3 | 195.4 | 239.2 |

**4.2.5 Fungal contamination of processed maize.**

*Fusarium* and *Aspergillus* were among the fungal genera isolated from semi-processed grains from farmers and traders of Eastern region (Table 4.33). Semi-processed grains from both farmers and traders had a higher percentage isolation of *Fusarium* species than *Aspergillus* and other fungal genera. The frequency of isolation was not significantly different among the agro-ecological zones. *Aspergillus* species isolated in the samples collected from both farmers and traders were *A. flavus, A. niger, A. fumigatus and A. terreus* (Table 4.34). The most frequently isolated species was *Aspergillus flavus* in all the samples. Only frequency of isolation for *Aspergillus flavus* in samples collected from traders was significantly different among the agro-ecological zones.

*Fusarium* species isolated from the semi-processed grains were *F. proliferatum, F. subglutinans, F. verticillioides, F. graminearum* and *F semitectum* (Table 4.35). In the

samples collected from farmers, only *Fusarium graminearum* was significantly different among the agro-ecological zones. The samples from agro-ecological zone LM5 of Makueni and LM4 of Machakos had significantly higher frequency of isolation for *Fusarium graminearum* than samples from all the other agro-ecological zones.

In the semi-processed grain samples collected from traders, the frequency of isolation in descending order was *F. subglutinans, F. graminearum, F. verticillioides, F. proliferatum, and F. semitectum* (Table 4.36). Frequency of isolation was significantly different among agro-ecological zones for *Fusarium proliferatum* and *Fusarium graminearum* only. The sample from agro-ecological zone LM5 in Makueni was not infected with *Fusarium graminearum* and no significant differences were noted in frequency of isolation of *Fusarium graminearum* in the samples from LM3 in Makueni, and all maize samples from Kitui and Makueni. *Fusarium proliferatum* in semi-processed grains collected from traders was highest in samples from agro-ecological zone LM5 in Makueni. There was no difference in the frequency of isolation in the samples from agro-ecological zones LM3 and LM5 in Makueni, LM4 in Kitui and LM5 in Machakos district.

Table 4.33: Percentage isolation for different fungi genera from semi-processed grains ("Muthokoi") sampled from farmers and traders in different agro-ecological zones of Eastern region during the long rainfall season in 2008.

| | Farmers | | | Traders | | |
|---|---|---|---|---|---|---|
| District / AEZ | *Fusarium* | *Aspergillus* | Others | *Fusarium* | *Aspergillus* | Others |
| Kitui LM4 | 60.8 | 49.0 | 18.1 | 61.0 | 44.0 | 16.0 |
| Kitui LM5 | 97.5 | 15.5 | 13.5 | 73.0 | 59.0 | 21.5 |
| Machakos LM4 | 70.0 | 35.5 | 23.5 | 75.7 | 34.0 | 29.3 |
| Machakos LM5 | 70.0 | 8.5 | 19.5 | 70.5 | 24.5 | 22.5 |
| Makueni LM3 | 67.2 | 30.2 | 24.0 | 63.6 | 17.6 | 36.8 |
| Makueni LM5 | 68.8 | 44.0 | 33.0 | 98.0 | 20.0 | 16.0 |
| **Mean** | **68.5** | **36.4** | **22.3** | **70.3** | **34.2** | **25.7** |
| LSD $_{(p\leq0.05)}$ | NS | NS | NS | NS | NS | NS |
| CV% | 33.3 | 74.4 | 95.9 | 22.7 | 70.0 | 74.5 |

Table 4.34: Percentage isolation for different *Aspergillus* species from semi-processed grains ("Muthokoi") sampled from farmers and traders in different agro-ecological zones in the Eastern region during the long rainfall season in 2008.

| | Farmers | | | | Traders | | | |
|---|---|---|---|---|---|---|---|---|
| District / AEZ | *flavus* | *fumigatus* | *niger* | *terreus* | *flavus* | *niger* | *terreus* | *fumigatus* |
| Kitui LM4 | 33.5 | 10.1 | 10.9 | 4.2 | 30.0 | 3.0 | 8.5 | 13.5 |
| Kitui LM5 | 17.0 | 3.5 | 7.5 | 2.0 | 32.2 | 3.0 | 7.0 | 5.0 |
| Machakos LM4 | 26.5 | 4.0 | 11.0 | 7.0 | 36.3 | 12.0 | 10.3 | 11.0 |
| Machakos LM5 | 7.0 | 0.0 | 1.0 | 0.0 | 14.5 | 2.5 | 2.0 | 3.5 |
| Makueni LM3 | 19.6 | 6.8 | 8.0 | 1.2 | 5.2 | 2.8 | 1.6 | 8.0 |
| Makueni LM5 | 25.8 | 6.0 | 8.7 | 0.0 | 10.0 | 0.0 | 2.0 | 2.0 |
| Mean | 24.9 | 6.5 | 7.5 | 2.9 | 23.4 | 5.0 | 5.9 | 8.2 |
| LSD $_{(p\leq0.05)}$ | NS | NS | NS | NS | 36.2 | NS | NS | NS |
| CV% | 67.3 | 112.7 | 136.0 | 234.5 | 68.3 | 203.1 | 185.7 | 153.4 |

Table 4.35: Percentage isolation for different *Fusarium* species from semi-processed grains ("muthokoi") sampled from farmers in different agro-ecological zones in the Eastern region during the long rainfall season in 2008.

| District / AEZ | *subglutinans* | *graminearum* | *proliferatum* | *semitectum* | *verticillioides* |
|---|---|---|---|---|---|
| Kitui LM4 | 7.0 | 5.2 | 8.8 | 8.6 | 17.1 |
| Kitui LM5 | 10.0 | 3.0 | 5.5 | 8.5 | 6.5 |
| Machakos LM4 | 4.0 | 11.0 | 11.0 | 7.2 | 0.0 |
| Machakos LM5 | 0.0 | 1.0 | 1.0 | 0.0 | 0.0 |
| Makueni LM3 | 8.2 | 4.4 | 4.4 | 5.0 | 17.2 |
| Makueni LM5 | 16.5 | 23.0 | 8.8 | 7.5 | 5.0 |
| Mean | 8.0 | 8.3 | 11.8 | 6.8 | 10.2 |
| LSD $_{(p\leq0.05)}$ | NS | 15.4 | NS | NS | NS |
| CV% | 119.5 | 112.0 | 102.5 | 160.6 | 120.0 |

Table 4.36: Percentage isolation for different *Fusarium* species from semi-processed grains ("Muthokoi") sampled from traders in different agro-ecological zone in the Eastern region during the long rainfall season in 2008.

| District / AEZ | *graminearum* | *proliferatum* | *semitectum* | *subglutinans* | *verticillioides* |
|---|---|---|---|---|---|
| Kitui LM4 | 4.5 | 3.0 | 2.5 | 19.0 | 13.5 |
| Kitui LM5 | 1.5 | 11.0 | 8.0 | 11.0 | 19.0 |
| Machakos LM4 | 19.0 | 3.0 | 5.7 | 28.3 | 11.3 |
| Machakos LM5 | 20.0 | 6.0 | 2.7 | 23.3 | 18.0 |
| Makueni LM3 | 27.6 | 0.4 | 4.4 | 14.0 | 9.2 |
| Makueni LM5 | 0.0 | 20.0 | 2.0 | 12.0 | 44.0 |
| Mean | 14.8 | 5.0 | 4.6 | 19.4 | 15.0 |
| LSD $_{(p\leq0.05)}$ | 26.5 | 8.4 | NS | NS | NS |
| CV% | 78.5 | 74.1 | 120.4 | 50.1 | 97.5 |

*Fusarium* and *Aspergillus* were among the fungal genera isolated from maize flour sampled from farmers (Table 4.37). However, the number of CFUs per gram for *Fusarium, Aspergillus* and other fungal genera in maize flour from farmers was not significantly ($P\leq0.05$) different among the agro-ecological zones. The *Aspergillus* species isolated were *A. flavus* and *A. niger*. None of these species had CFUs which were significantly different among the agro-ecological zones. Significant differences among agro-ecological zones were noted in the number of CFUs per gram of maize flour in open bags sampled from traders was only noted in isolation of *Aspergillus* genus and *A. flavus* (Table 4.38). The number of CFUs per gram for *Aspergillus* in maize flour in open bags collected from traders was highest in the sample from agro-ecological zone LM5 in Makueni district. No significant ($P\leq0.05$) differences in the number of CFUs among the samples from agro-ecological zones LM3 in Makueni, LM4 and LM5 in Machakos and LM4 and LM5 in Kitui.

Among the *Aspergillus* species, only CFUs of *Aspergillus flavus* were significantly different among the agro-ecological zones. The highest number of colony forming units for *Aspergillus flavus* was in the sample from LM5 in Makueni district (Table 4.37). The number of CFUs in the samples from LM3 in Makueni, LM4 and LM5 in Machakos and LM4 and LM5 in Kitui were not significant ($P \leq 0.05$) different among the agro-ecological zones.

The number of CFUs for *Fusarium* per gram of packed maize flour from traders was significantly higher in the samples from agro-ecological zones LM5 of Makueni and LM4 of Machakos district than in samples from all other agro-ecological zones (Table 4.39). There were no significant differences in the number of CFUs among agro-ecological zones in the samples from LM4 and LM5 in Kitui and LM4 of Machakos. The number of *Aspergillus* CFUs was highest in the sample from agro-ecological zone LM5 in Makueni district. Packed flour from agro-ecological zone LM3 Makueni district had no contamination from *Aspergillus.* There were no significant differences in the number of CFUs among agro-ecological zones in the samples from LM4 in Kitui, LM5 of Kitui, and LM3 of Makueni district but they had significantly lower number of number of colony forming units the samples from agro-ecological zone LM5 Machakos, LM4 of Machakos and LM5 of Makueni district.

The number of CFUs of *Aspergillus flavus* was highest in the sample from agro-ecological zone LM5 in Makueni district (Table 4.39). The sample from Makueni LM3 had no contamination by *Aspergillus flavus.* The number of colony forming units in the samples from agro-ecological zone LM3 in Makueni, LM4 and LM5 in Kitui were not significantly different. The CFUs in these agro-ecological zones were significantly lower than agro-ecological zones LM5 in Makueni district and LM5 and LM4 in Machakos.

Table 4.37: Number of colony forming units for *Aspergillus* species and other fungi isolated from a gram of maize flour sample collected from farmers in different agro-ecological zones of Eastern region during long rainfall season in 2008.

| District | *Fusarium* | *Aspergillus* | Others | *A. flavus* | *A .niger* |
|---|---|---|---|---|---|
| Kitui LM4 | 1412.0 | 27.0 | 136.0 | 96.0 | 4.0 |
| Kitui LM5 | 260.0 | 213.0 | 311.0 | 207.0 | 5.0 |
| Machakos LM4 | 774.0 | 150.0 | 415.0 | 176.0 | 0.0 |
| Machakos LM5 | 850.0 | 100.0 | 131.0 | 102.0 | 0.0 |
| Makueni LM5 | 1243.0 | 85.0 | 270.0 | 87.0 | 0.0 |
| **Mean** | **862.0** | **117.0** | **246.0** | **138.0** | **2.0** |
| LSD $_{(p\leq0.05)}$ | NS | NS | NS | NS | NS |
| CV% | 162.6 | 149.5 | 159.1 | 142.7 | 322.5 |

Table 4.38: Number of colony forming units of *Aspergillus* species and other fungi isolated from one gram of maize flour sample in open bags collected from traders in different agro-ecological zones in the Eastern region during long rainfall season in 2008.

| District / AEZ | *Fusarium* | *Aspergillus* | 0thers | *A. flavus* | *A. niger* | *A. versicolor* |
|---|---|---|---|---|---|---|
| Kitui LM5 | 213.0 | 11.0 | 9.0 | 6.0 | 1.2 | 0.0 |
| Machakos LM4 | 668.0 | 38.0 | 148.0 | 17.0 | 0.0 | 0.0 |
| Machakos LM5 | 256.0 | 86.0 | 295.0 | 10.0 | 3.7 | 0.0 |
| Makueni LM3 | 835.0 | 75.0 | 216.0 | 99.0 | 0.0 | 1.3 |
| Makueni LM5 | 577.0 | 622.0 | 312.0 | 613.0 | 8.3 | 0.0 |
| **Mean** | 485.0 | 86.0 | 182.0 | 64.0 | 1.8 | 0.3 |
| LSD $_{(p\leq0.05)}$ | NS | 236.3 | NS | 159.6 | NS | NS |
| CV% | 120.2 | 114.1 | 195.8 | 105.6 | 267.4 | 432.8 |

Table 4.39: Number of colony forming units of *Aspergillus flavus* and other fungi in one gram of maize flour in packets collected from traders in different Agro-ecological zones of Eastern region during long rainfall season in 2008

| AEZ | *Fusarium* | *Aspergillus* | Others | *A. flavus* |
|---|---|---|---|---|
| Kitui LM4 | 1.7 | 1.7 | 0.0 | 1.7 |
| Kitui LM5 | 16.6 | 1.7 | 3.3 | 1.7 |
| Machakos LM4 | 5.0 | 10.8 | 1.6 | 10.8 |
| Machakos LM5 | 76.7 | 241 | 115 | 241.7 |
| Makueni LM3 | 56.7 | 0.0 | 41.7 | 0.0 |
| Makueni LM5 | 83.3 | 760.0 | 0.0 | 426.9 |
| **Mean** | 32.7 | 128.5 | 20.8 | 86.9 |
| LSD $_{(p\leq0.05)}$ | 19.4 | 57.8 | 19.6 | 57.8 |
| CV% | 11.3 | 8.5 | 17.9 | 12.6 |

AEZ = agro-ecological zone LSD = least significant difference

NS = not significant ($p\leq0.05$)

### 4.2.6. Aflatoxin content in maize and maize products

In the Eastern region, maize sampled during the long rain season had very low levels of aflatoxin content. Except for maize sampled from agro-ecological zone LM4 in Kitui that had 58.3 µg/kg, most of the other samples had aflatoxin levels of less than 2 µg/kg (Table 4.40). Most of the maize samples collected during the short rains season also had low levels of aflatoxin contents. Only two samples had aflatoxin content levels that exceeded the allowed limit in Kenya of 10 µg/kg. The highest content was 77.4 µg/kg contained in the sample from agro-ecological zone LM3 in Makueni followed by 48.3 µg/kg in the sample from agro-ecological zone LM4 in Kitui district. Maize samples from North Rift region had no contamination by aflatoxins.

Semi-processed grains from agro-ecological zone LM4 in Kitui had numerically the highest aflatoxin content with 136.4 µg/kg followed by the sample from LM4 in

Machakos at 100.8 µg/kg. Samples from all other samples areas had very low aflatoxin content levels (Table 4.40). Most of the maize flour from farmers had very low aflatoxin content and only one sample from agro-ecological zone LM4 in Kitui had exceeded the allowed Kenyan limit of 10 µg/kg.

Among the maize and maize products sampled from traders in Eastern region, only maize from Kitui, maize flour in open bags and semi-processed grains from Makueni had aflatoxin levels of more than 10 µg/kg (Table 4.40). Maize flour sold in packets from millers had no aflatoxin contamination. All the maize samples from traders in North Rift had no aflatoxin contamination.

Table 4.40: Aflatoxin content (µg/kg) in maize and maize products sampled from farmers and traders in different agro-ecological zones of Eastern and North Rift regions during the long and short rainfall seasons in 2008

| District/AEZ | Long rain season | Short rain season | Semi-processed grains | Flour (open bags) | Flour (packets) |
|---|---|---|---|---|---|
| | | | Farmers | | |
| Kitui LM4 | 58.3 | 0.1 | 136.41 | 40.9 | N/A |
| Kitui LM5 | 0.0 | 48.3 | 0.0 | 0.7 | N/A |
| Machakos LM4 | 2.1 | 0.7 | 100.8 | 0.4 | N/A |
| Machakos LM5 | 0.0 | N/A | 0.0 | 0.5 | N/A |
| Makueni LM3 | 0.0 | 77.4 | 2.2 | N/A | N/A |
| Makueni LM5 | 0.0 | 1.0 | 0.3 | 0.0 | N/A |
| | | | Traders | | |
| Kitui | 15.2 | N/A | 0.0 | 0.1 | 0.1 |
| Machakos | 0.0 | N/A | 0.2 | 3.3 | 3.3 |
| Makueni | 0.0 | N/A | 19.1 | 12.2 | 12.2 |

Key: N/A = not applicable; AEZ = Agro-ecological zone

## 4.3 Effect of storage material and moisture level on maize infection with storage fungi

### 4.3.1. Percentage of discoloured and mouldy grains

Moisture level, storage material and the interaction between the two had a significant effect on the proportion of discoloured and mouldy grains in the maize samples stored at Machakos and Kabete when sampled at 14 days after inoculation (Table 4.41). At Machakos, samples stored at 20% moisture content in polythene bags had a higher proportion than both synthetic and sisal bags. Increasing moisture content above 15% caused a significant increase in the proportion of discoloured and mouldy grains. There were no differences noted during storage among storage materials in this parameter at 10% to 15% moisture levels. At 18% and 20% moisture level, maize in polythene bag had significantly higher proportion of discoloured and mouldy grains than maize in synthetic and sisal bags. There was however, no significant difference in the proportion of discoloured and mouldy grains in maize stored using sisal and synthetic bags at 18% and 20% moisture levels. At Kabete, maize stored at 10%, 13% and 15% were not significantly different in proportion of discoloured and mouldy grains among all the storage materials (Table 4.41). At 18% moisture level, maize stored in polythene bags was significantly more discoloured and mouldy than maize in synthetic and sisal bags. At 20% moisture level, maize grains in sisal was significantly less discoloured and mouldy than maize in polythene and synthetic bags. On average, maize stored in sisal bags had a significantly lower proportion of discoloured and mouldy grains than the one stored in synthetic and polythene bags.

Storage materials and the interaction between moisture and storage had a significant effect on the proportion of discoloured and mouldy grains in the maize samples stored at Machakos at 35 days after inoculation. However, moisture content had no significant effect (Table 4.41). Maize samples stored at 20% moisture level in polythene bag had a higher proportion of discoloured and mouldy grains than maize grains in synthetic and sisal bags. Proportions of discoloured and mouldy grains in maize stored at 10% to 18% moisture levels were not significantly different in all storage materials. Increase in moisture level in maize stored in sisal and synthetic bags did not cause any significant

difference in the proportion of discoloured and mouldy grains at all moisture levels. Maize stored in sisal and synthetic bags had no significant difference in the proportion of discoloured and mouldy grains. An increase of moisture content above 13% in maize stored in polythene bags caused a significant increase in the proportion of discoloured and mouldy grains.

The proportion of discoloured and mouldy grains in maize stored at Kabete was significantly affected by moisture level, storage materials and their interaction at 35 days after inoculation (Table 4.41). At 15% to 20% moisture levels, maize stored in polythene bags had a higher proportion of discoloured and mouldy grains than maize stored in sisal and synthetic bags. No differences were noted among all storage materials at 10% and 13% moisture levels. Sisal and synthetic bags were not significantly different at all moisture levels. Increasing moisture content above 13% in maize samples stored in polythene bags caused a significant increase in the proportion of discoloured and mouldy grains. For samples stored in sisal bags, only those stored at 18% and 20% had significantly higher proportion of discoloured and mouldy grains. Generally, samples stored between 10% and 15% had a significantly lower proportion of discoloured and mouldy grains than samples stored at 18% and 20% moisture levels.

Moisture level, storage materials and their interaction had a significant effect on the proportion of discoloured and mouldy grains in the maize samples stored at Machakos and Kabete when sampled at 56 days after inoculation (Table 4.42). At Machakos, no differences were noted during storage at 10% to 15% moisture levels for all storage materials. Increase in the moisture level had no effect on the proportion of discoloured and mouldy grains in synthetic and sisal bags. No significant difference was noted during storage in maize stored in sisal bags and synthetic bags at all moisture levels. However, increasing moisture content above 15% caused a significant increase in the proportion of discoloured and mouldy grains in the maize stored in polythene bags. Maize stored at 18% and 20% moisture levels, maize stored in polythene bags had a significantly higher proportion of discoloured and mouldy grains than maize stored in sisal and synthetic bags.

At Kabete, samples stored at 18% and 20% moisture content in polythene bags had higher proportion of discoloured and mouldy grains than maize in sisal and synthetic bags. There were no significant differences noted among the storage materials at 10% - 15%moisture levels (Table 4.42). There were no significant differences in the proportion of discoloured and mouldy grains between sisal and synthetic bags at 18% and 20% moisture levels.

Moisture level, storage material and the interaction between the two had a significant effect on the proportion of discoloured and mouldy grains in the maize samples stored at Machakos and Kabete when sampled at 77 days after inoculation (Table 4.42 and 4.43). At Machakos, maize stored using synthetic and sisal bags had no significant difference in the proportion of discoloured and mouldy grains at all moisture levels. At 18% and 20% moisture levels, maize stored in polythene bags had more discoloured and mouldy grains than maize in sisal and synthetic bags. For maize stored using polythene bags, increase of moisture content above 10% caused a significant increase in the proportion of discoloured and mouldy grains at 13% to 20%.

At Kabete, there were no significant differences noted in all the maize stored at 10% to 15% irrespective of storage materials. The proportion of discolored and mouldy grains was significantly higher in maize stored at 20% for all storage materials. The proportion of discoloured and mouldy grains in maize stored in sisal and polythene bags increased significantly after moisture content was raised above 18% level.

Table 4.41: Percentage of discoloured and mouldy grains in maize sampled at 14 and 35 days after inoculation with *Aspergillus flavus* and storage at varying moisture contents using different storage materials at Kabete and Machakos.

| Moisture level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 14 days after inoculation | | | | | | | | |
| 10 | 10.7 | 11.0 | 8.3 | 10.0 | 5.7 | 8.0 | 6.0 | 6.6 |
| 13 | 12.1 | 11.0 | 14.7 | 12.6 | 7.0 | 6.0 | 3.3 | 5.4 |
| 15 | 12.3 | 16.7 | 22.3 | 17.1 | 10.0 | 9.3 | 10.0 | 9.8 |
| 18 | 31.0 | 41.7 | 29.7 | 34.1 | 11.7 | 21.3 | 6.0 | 13.0 |
| 20 | 28.0 | 63.3 | 31.3 | 40.9 | 26.3 | 24.3 | 19.0 | 23.2 |
| Mean | 18.8 | 28.7 | 21.3 | 8.7 | 12.1 | 13.8 | 8.9 | 11.6 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | 5.1 | | | | 2.5 |
| $LSD_{(p\leq0.05)}$ moisture level | | | | 8.7 | | | | 5.5 |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | 11.9 | | | | 6.7 |
| CV(%) | | | | 28.7 | | | | 28.2 |
| 35 days after inoculation | | | | | | | | |
| Moisture level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 7.0 | 10.0 | 9.0 | 8.7 | 8.0 | 7.3 | 5.7 | 7.0 |
| 13 | 5.7 | 7.7 | 5.7 | 6.3 | 5.7 | 11.7 | 7.3 | 8.2 |
| 15 | 11.0 | 19.0 | 13.3 | 14.4 | 9.0 | 15.7 | 7.3 | 10.7 |
| 18 | 17.0 | 29.7 | 19.0 | 21.9 | 9.3 | 19.3 | 12.7 | 13.8 |
| 20 | 15.7 | 38.3 | 14.7 | 22.9 | 21.3 | 29.7 | 20.0 | 23.7 |
| Mean | 11.3 | 20.9 | 12.3 | 14.8 | 10.7 | 16.7 | 10.6 | 12.7 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | 3.6 | | | | 1.8 |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | 4.4 |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | 13.8 | | | | 5.2 |
| CV(%) | | | | 31.6 | | | | 19.1 |

Table 4.42: Percentage of discoloured and mouldy grains in maize sampled at 56 and 77 days after inoculation with *Aspergillus flavus* and storage at varying moisture contents using different storage materials at Kabete and Machakos.

| Moisture level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 56 days after inoculation | | | | | | | | |
| 10 | 14.7 | 17.0 | 14.7 | 15.4 | 7.3 | 8.7 | 7.3 | 7.8 |
| 13 | 15.0 | 16.7 | 15.0 | 15.6 | 9.7 | 11.7 | 9.0 | 10.1 |
| 15 | 14.3 | 17.7 | 13.3 | 15.1 | 11.7 | 14.0 | 9.3 | 11.7 |
| 18 | 14.7 | 41.3 | 21.3 | 25.8 | 17.0 | 36.3 | 17.0 | 23.4 |
| 20 | 15.7 | 57.1 | 19.0 | 30.6 | 22.7 | 49.4 | 21.7 | 31.3 |
| Mean | 14.9 | 29.9 | 16.7 | 20.5 | 13.7 | 24.0 | 12.9 | 16.9 |
| $LSD_{(p\leq 0.05)}$ storage materials | | | | 6.8 | | | | 3.8 |
| $LSD_{(p\leq 0.05)}$ moisture level | | | | 9.5 | | | | 4.4 |
| $LSD_{(p\leq 0.05)}$ storage materials x moisture level | | | | 14.5 | | | | 7.9 |
| CV(%) | | | | 43.5 | | | | 29.7 |
| 77 days after inoculation | | | | | | | | |
| Moisture level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 12.3 | 10.3 | 11.0 | 11.2 | 5.0 | 7.0 | 7.0 | 6.3 |
| 13 | 12.3 | 23.7 | 12.3 | 16.1 | 8.0 | 11.3 | 6.7 | 8.7 |
| 15 | 15.0 | 23.7 | 15.0 | 17.9 | 12.0 | 15.7 | 8.3 | 12.0 |
| 18 | 20.0 | 51.0 | 21.3 | 30.8 | 12.3 | 25.7 | 10.0 | 16.0 |
| 20 | 12.7 | 69.7 | 17.7 | 33.3 | 24.7 | 70.7 | 21.7 | 39.0 |
| Mean | 14.5 | 35.7 | 15.5 | 21.9 | 12.4 | 26.1 | 10.7 | 16.4 |
| $LSD_{(p\leq 0.05)}$ storage materials | | | | 5.3 | | | | 3.3 |
| $LSD_{(p\leq 0.05)}$ moisture level | | | | 7.5 | | | | 6.6 |
| $LSD_{(p\leq 0.05)}$ storage materials x moisture level | | | | 11.6 | | | | 8.4 |
| CV(%) | | | | 31.8 | | | | 26.2 |

Table 4.43: Percentage of discoloured and mouldy grains in maize sampled at 14, 35, 56 and 77 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different storage materials at Kabete and Machakos.

| | Machakos | | | | | Kabete | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ML / SM | 14 | 35 | 56 | 77 | Mean | 14 | 35 | 56 | 77 | Mean |
| 10%synthetic | 10.7 | 7.0 | 14.7 | 12.3 | 11.2 | 5.7 | 8.0 | 7.3 | 5.0 | 6.5 |
| 10%Polythene | 11.0 | 10.0 | 17.0 | 10.3 | 12.1 | 8.0 | 7.3 | 8.7 | 7.0 | 7.8 |
| 10% Sisal | 8.3 | 9.0 | 14.7 | 11.0 | 10.8 | 6.0 | 5.7 | 7.3 | 7.0 | 6.5 |
| 13% synthetic | 12.1 | 5.7 | 15.0 | 12.3 | 11.3 | 7.0 | 5.7 | 9.7 | 8.0 | 7.6 |
| 13%Polythene | 11.0 | 7.7 | 16.7 | 23.7 | 14.8 | 6.0 | 11.7 | 11.7 | 11.3 | 10.2 |
| 13% Sisal | 14.7 | 5.7 | 15.0 | 12.3 | 11.9 | 3.3 | 7.3 | 9.0 | 6.7 | 6.6 |
| 15% synthetic | 12.3 | 11.0 | 14.3 | 15.0 | 13.2 | 10.0 | 9.0 | 11.7 | 12.0 | 10.7 |
| 15%Polythene | 16.7 | 19.0 | 17.7 | 23.7 | 19.3 | 9.3 | 15.7 | 14.0 | 15.7 | 13.7 |
| 15% Sisal | 22.3 | 13.3 | 13.3 | 15.0 | 16.0 | 10.0 | 7.3 | 9.3 | 8.3 | 8.8 |
| 18% synthetic | 31.0 | 17.0 | 14.7 | 20.0 | 20.7 | 11.7 | 9.3 | 17.0 | 12.3 | 12.6 |
| 18%Polythene | 41.7 | 29.7 | 41.3 | 51.0 | 40.9 | 21.3 | 19.3 | 36.3 | 25.7 | 25.7 |
| 18% Sisal | 29.7 | 19.0 | 21.3 | 21.3 | 22.8 | 6.0 | 12.7 | 17.0 | 10.0 | 11.4 |
| 20% synthetic | 28.0 | 15.7 | 15.7 | 12.7 | 18.0 | 26.3 | 21.3 | 22.7 | 24.7 | 23.8 |
| 20%Polythene | 63.3 | 38.3 | 57.1 | 69.7 | 57.1 | 24.3 | 29.7 | 49.4 | 70.7 | 43.5 |
| 20% Sisal | 31.3 | 14.7 | 19.0 | 17.7 | 20.7 | 19.0 | 20.0 | 21.7 | 21.7 | 20.6 |
| Mean | 22.9 | 14.8 | 20.5 | 21.9 | 20.0 | 11.6 | 12.7 | 16.9 | 16.4 | 14.4 |
| LSD SM x ML | 11.9 | 13.8 | 14.8 | 11.6 | 5.5 | 6.7 | 5.2 | 7.9 | 8.4 | 3.3 |
| CV (%) | 28.7 | 31.6 | 43.5 | 31.8 | 17.3 | 28.2 | 19.1 | 29.7 | 26.2 | 12.8 |

ML = moisture level, SM = storage materials, NS = not significant at ($P \leq 0.05$)

LSD = least significant difference.

### 4.3.2 Total kernel infection

At 14 days after inoculation, the percentage of total kernel infection with different fungi was not affected significantly by moisture content and storage materials in maize stored at Machakos (Table 4.44). A similar observation was made in maize stored at Kabete, except that the percentage total kernel infection was significantly affected by storage materials. Maize samples stored in sisal bags at Kabete had significantly lower percentage of total kernel infection than maize stored in synthetic and polythene bags. No difference was noted in the percentage of total kernel infection in maize stored using synthetic and polythene bags.

During the sampling of maize at 35 days after inoculation at Machakos, percentage total kernel infection was not significantly affected by moisture level, storage materials or interaction between the two (Table 4.44). Percentage total kernel infection was significantly affected by moisture content in maize stored at Kabete however, storage material had no effect. No differences were noted in percentage total kernel infection in maize stored at 10% to 15% moisture content. Increase of moisture content above 15% caused a significant increase in percentage total kernel infection relative to 10% moisture level. Maize samples stored at 20% moisture content had significantly higher percentage total kernel infection than maize stored at 10% to 15% moisture levels.

During sampling at 56 days after inoculation, percentage total kernel infection with fungi in maize stored at Machakos was significantly affected by moisture level but storage materials, and interaction between moisture content and storage materials had no significant effect (Table 4.45 and 4.46). No difference was noted during storage in percentage of total kernel infection in maize stored at 10% to 15% moisture content. Maize samples stored at 18% and 20% moisture content had significantly higher percentage total kernel infection than maize stored at 10% to 15% moisture levels. Storage material and moisture content had no significant effect on percentage of total kernel infection in maize stored at Kabete. During the sampling at 77 days after inoculation, total kernel infection in maize stored at Machakos was only significantly affected by moisture level. No significant difference was noted in maize stored at 10% to

15% moisture content. Maize stored at 18% and 20% moisture levels had higher percentage total kernel infection than maize stored at 10% to 15% moisture level. Maize samples stored at Kabete were not significantly affected by moisture content, storage materials or their interaction when sampled at 56 and 77 days after inoculation.

Table 4.44: Percentage of total kernel infection in maize sampled at 14 and 35 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different storage materials at Kabete and Machakos

| Moisture level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 14 days after inoculation | | | | | | | | |
| 10 | 91.7 | 98.3 | 96.7 | 95.6 | 96.7 | 98.3 | 81.7 | 92.2 |
| 13 | 100.0 | 100.0 | 98.3 | 99.4 | 93.3 | 95.0 | 86.7 | 93.3 |
| 15 | 96.7 | 98.3 | 98.3 | 97.8 | 95.0 | 100.0 | 88.3 | 94.4 |
| 18 | 98.3 | 100.0 | 98.3 | 98.9 | 98.3 | 100.0 | 98.3 | 98.9 |
| 20 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Mean | 97.3 | 99.3 | 98.3 | 98.3 | 97.7 | 98.7 | 91.0 | 95.8 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | 5.4 |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 3.9 | | | | 10.8 |
| 35 days after inoculation | | | | | | | | |
| Moisture level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 86.7 | 91.7 | 88.3 | 88.9 | 96.7 | 86.7 | 90.0 | 91.1 |
| 13 | 88.3 | 91.7 | 85.0 | 88.3 | 96.7 | 83.3 | 96.7 | 92.2 |
| 15 | 93.3 | 98.3 | 88.3 | 93.3 | 95.0 | 93.3 | 90.0 | 92.8 |
| 18 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 95.0 | 98.3 |
| 20 | 98.3 | 100.0 | 100.0 | 99.4 | 100.0 | 100.0 | 100.0 | 100.0 |
| Mean | 93.3 | 96.3 | 92.3 | 94.0 | 97.7 | 92.7 | 94.3 | 94.9 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | 6.5 |
| $LSD_{(p\leq0.05)}$ storage materials x moisture Level | | | | NS | | | | NS |
| CV(%) | | | | 8.4 | | | | 9.8 |

Table 4.45: Percentage of total kernel infection in maize sampled at 56 and 77 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different storage materials at Kabete and Machakos

| Moisture Level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 56 days after inoculation | | | | | | | | |
| 10 | 95 | 91.7 | 93.3 | 93.3 | 98.3 | 98.3 | 98.3 | 98.3 |
| 13 | 91.7 | 88.3 | 95.0 | 91.7 | 100.0 | 96.7 | 100.0 | 98.9 |
| 15 | 93.3 | 100.0 | 96.7 | 96.7 | 100.0 | 100.0 | 98.3 | 99.4 |
| 18 | 100 | 100.0 | 96.7 | 100.0 | 98.3 | 100.0 | 100.0 | 99.4 |
| 20 | 100 | 100.0 | 100.0 | 99.4 | 100.0 | 98.3 | 100.0 | 99.4 |
| Mean | 96.0 | 96.0 | 96.7 | 96.2 | 99.3 | 98.8 | 99.3 | 99.1 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | 5.5 | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 4.1 | | | | 5.8 |
| 77 Days after inoculation | | | | | | | | |
| Moisture Level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 93.3 | 88.3 | 88.3 | 90.0 | 95.0 | 100.0 | 100.0 | 98.3 |
| 13 | 95.0 | 85.0 | 78.3 | 83.9 | 100.0 | 98.3 | 95.0 | 97.8 |
| 15 | 95.0 | 88.3 | 88.3 | 90.6 | 95.0 | 96.7 | 96.7 | 96.1 |
| 18 | 93.3 | 100.0 | 100.0 | 97.8 | 93.3 | 100.0 | 96.7 | 96.7 |
| 20 | 100.0 | 86.7 | 100.0 | 95.6 | 100.0 | 96.7 | 100.0 | 98.9 |
| Mean | 94.0 | 89.7 | 91.0 | 91.6 | 96.7 | 98.3 | 97.7 | 97.6 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | 8.5 | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 12.4 | | | | 6.9 |

ML = moisture level, SM = storage materials, NS = not significant ($P\leq0.05$).

LSD = least significant difference.

Table 4.46: Percentage of total kernel infection in maize sampled at 14, 35, 56 and 77 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different storage materials at Kabete and Machakos

| | Machakos | | | | | Kabete | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ML / SM | 14 | 35 | 56 | 77 | Mean | 14 | 35 | 56 | 77 | Mean |
| 10%synthetic | 91.7 | 86.7 | 95.0 | 93.3 | 91.7 | 96.7 | 96.7 | 98.3 | 95.0 | 96.7 |
| 10%Polythene | 98.3 | 91.7 | 91.7 | 88.3 | 92.5 | 98.3 | 86.7 | 98.3 | 100.0 | 95.8 |
| 10% Sisal | 96.7 | 88.3 | 93.3 | 88.3 | 91.7 | 81.7 | 90.0 | 98.3 | 100.0 | 92.5 |
| 13% synthetic | 100.0 | 88.3 | 91.7 | 95.0 | 93.7 | 98.3 | 96.7 | 100.0 | 100.0 | 98.7 |
| 13%Polythene | 100.0 | 91.7 | 88.3 | 85.0 | 91.3 | 95.0 | 83.3 | 96.7 | 98.3 | 93.3 |
| 13% Sisal | 98.3 | 85.0 | 95.0 | 78.3 | 89.2 | 86.7 | 96.7 | 100.0 | 95.0 | 94.6 |
| 15% synthetic | 96.7 | 93.3 | 93.3 | 95.0 | 94.6 | 95.0 | 95.0 | 100.0 | 95.0 | 96.3 |
| 15%Polythene | 98.3 | 98.3 | 100.0 | 88.3 | 96.2 | 100.0 | 93.3 | 100.0 | 96.7 | 97.5 |
| 15% Sisal | 98.3 | 88.3 | 96.7 | 88.3 | 92.9 | 98.3 | 90.0 | 98.3 | 96.7 | 95.8 |
| 18% synthetic | 98.3 | 100.0 | 100.0 | 93.3 | 97.9 | 98.3 | 100.0 | 98.3 | 93.3 | 97.5 |
| 18%Polythene | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 18% Sisal | 98.3 | 100.0 | 100.0 | 100.0 | 99.6 | 98.3 | 95.0 | 100.0 | 96.7 | 97.5 |
| 20% synthetic | 100.0 | 98.3 | 100.0 | 100.0 | 99.6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 20%Polythene | 100.0 | 100.0 | 100.0 | 86.7 | 96.7 | 100.0 | 100.0 | 98.3 | 96.7 | 98.8 |
| 20% Sisal | 100.0 | 100.0 | 98.3 | 100.0 | 99.6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Mean | 98.3 | 94.0 | 96.2 | 91.6 | 95.0 | 95.8 | 94.9 | 99.1 | 97.6 | 96.8 |
| LSD SMxML | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| CV (%) | 3.9 | 8.4 | 4.1 | 12.4 | 4.1 | 7.4 | 6.2 | 2.0 | 4.5 | 2.2 |

ML = moisture level, SM = storage materials, NS = not significant ($P \leq 0.05$).

LSD = least significant difference.

### 4.3.3 Infection of maize grain with *Fusarium* species

Only moisture level significantly affected the percentage of kernel infected with *Fusarium* in maize stored at Machakos and Kabete at 14 days after inoculation (Table 4.47). Maize samples stored at 13%, 18%, and 20% moisture content had significantly higher infection than the maize sample stored at 10% moisture level. At Kabete, maize samples stored at 10% and 15% moisture contents had no significant differences in percentage of kernel infection with *Fusarium*. The samples stored at 18% and 20% had significantly higher percentage infection by *Fusarium* than 10% to 15%. Generally, the level of infection was higher in Machakos than Kabete

At 35 days after inoculation, moisture content and storage materials had no significant effect on percentage of kernel infection by *Fusarium* in Machakos and only moisture significantly affected maize stored at Kabete (Table 4.47). Samples stored at 10% and 15% moisture contents had no significant difference during storage. The samples stored at 18% and 20% had significantly higher percentage infection by *Fusarium* than maize samples stored at 10% to 15% moisture levels.

At 56 days after inoculation, moisture level, storage materials and their interaction had no significant effect on *Fusarium* infection in maize stored at Kabete, while at Machakos, only moisture had a significant effect (Table 4.48 and 4.49). At Machakos, samples stored at moisture levels of 10% to 15% were not significantly different but had significantly lower infection levels than the sample stored at 18% and 20%. At 77 days after inoculation, maize stored in Machakos and Kabete was not significantly affected by moisture level and storage materials. However, infection level was higher at Kabete than Machakos.

Table 4.47: Percentage of kernels infected with *Fusarium* species in maize sampled at 14 and 35 days after inoculation with *Aspergillus flavus* and stored at varying moisture levels using different storage materials at Kabete and Machakos.

| Moisture Level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 14 days after inoculation | | | | | | | | |
| 10 | 90.0 | 96.7 | 95.0 | 93.9 | 85.0 | 91.7 | 75.0 | 83.9 |
| 13 | 100.0 | 100.0 | 98.3 | 99.4 | 81.7 | 86.7 | 88.3 | 85.6 |
| 15 | 96.7 | 98.3 | 96.7 | 97.2 | 95.0 | 90.0 | 85.0 | 90.0 |
| 18 | 98.3 | 98.3 | 98.3 | 98.3 | 95.0 | 95.0 | 91.7 | 93.9 |
| 20 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.3 | 100.0 | 99.4 |
| Mean | 97.0 | 98.7 | 97.7 | 97.9 | 91.3 | 92.3 | 88.0 | 90.6 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | 3.7 | | | | 8.9 |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 5.1 | | | | 7.4 |
| 35 days after inoculation | | | | | | | | |
| Moisture Level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 83.3 | 86.7 | 80.0 | 83.3 | 95.0 | 85.0 | 83.3 | 87.8 |
| 13 | 80.0 | 83.3 | 76.7 | 80.0 | 95.0 | 75.0 | 81.7 | 83.7 |
| 15 | 91.7 | 90.0 | 85.0 | 88.9 | 88.3 | 85.0 | 86.7 | 86.7 |
| 18 | 96.7 | 100.0 | 96.7 | 97.8 | 100.0 | 100.0 | 88.3 | 96.1 |
| 20 | 98.3 | 100.0 | 100.0 | 99.4 | 100.0 | 100.0 | 100.0 | 100.0 |
| Mean | 90.0 | 92.0 | 87.7 | 89.9 | 95.7 | 89.0 | 88.0 | 90.9 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | 7.8 |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 9.3 | | | | 6.2 |

ML = moisture level, SM = storage materials, NS = not significant ($P\leq0.05$).

LSD = least significant difference.

Table 4.48: Percentage of kernels infected with *Fusarium* in maize sampled at 56 and 77 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different storage materials at Kabete and Machakos.

| Moisture level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 56 days after inoculation | | | | | | | | |
| 10 | 90.0 | 91.7 | 90.0 | 90.6 | 90.0 | 90.0 | 88.3 | 89.4 |
| 13 | 88.3 | 83.3 | 91.7 | 87.8 | 100.0 | 96.7 | 90.0 | 95.6 |
| 15 | 90.0 | 100.0 | 95.0 | 95.0 | 98.3 | 93.3 | 96.7 | 96.1 |
| 18 | 96.7 | 100.0 | 100.0 | 98.9 | 96.7 | 98.3 | 100.0 | 98.3 |
| 20 | 100.0 | 100.0 | 98.3 | 99.4 | 100.0 | 98.3 | 100.0 | 99.4 |
| Mean | 93.0 | 95.0 | 95.0 | 94.3 | 89.4 | 95.6 | 95.0 | 95.8 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | 4.4 | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 6.4 | | | | 2.0 |
| 77 days after inoculation | | | | | | | | |
| Moisture level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 93.3 | 86.7 | 85.0 | 88.3 | 93.3 | 98.3 | 93.3 | 95.0 |
| 13 | 65.0 | 80.0 | 71.7 | 72.2 | 98.3 | 95.0 | 95.0 | 96.1 |
| 15 | 95.0 | 86.7 | 88.3 | 90.0 | 91.7 | 86.7 | 96.7 | 91.7 |
| 18 | 93.3 | 100.0 | 100.0 | 97.8 | 90.0 | 100.0 | 96.7 | 95.6 |
| 20 | 100.0 | 98.3 | 100.0 | 99.4 | 100.0 | 95.0 | 100.0 | 98.3 |
| Mean | 89.3 | 90.3 | 89.0 | 89.6 | 94.7 | 95.0 | 96.3 | 95.3 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 17.5 | | | | 4.5 |

ML = moisture level, SM = storage materials, NS = not significant ($P\leq0.05$).

LSD = least significant difference.

Table 4.49: Percentage of kernels infected with *Fusarium* species in maize sampled at 14, 35, 56 and 77 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different storage materials at Kabete and Machakos

| | Machakos | | | | | Kabete | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ML / SM | 14 | 35 | 56 | 77 | Mean | 14 | 35 | 56 | 77 | Mean |
| 10%synthetic | 90.0 | 83.3 | 90.0 | 93.3 | 89.2 | 85.0 | 95.0 | 90.0 | 93.3 | 90.8 |
| 10%Polythene | 96.7 | 86.7 | 91.7 | 86.7 | 90.4 | 91.7 | 85.0 | 90.0 | 98.3 | 91.3 |
| 10% Sisal | 95.0 | 80.0 | 90.0 | 85.0 | 87.5 | 75.0 | 83.3 | 88.3 | 93.3 | 85.0 |
| 13% synthetic | 100.0 | 80.0 | 88.3 | 65.0 | 83.3 | 81.7 | 95.0 | 100.0 | 98.3 | 93.8 |
| 13%Polythene | 100.0 | 83.3 | 83.3 | 80.0 | 86.7 | 86.7 | 75.0 | 96.7 | 95.0 | 88.3 |
| 13% Sisal | 98.3 | 76.7 | 91.7 | 71.7 | 84.6 | 88.3 | 81.7 | 90.0 | 95.0 | 88.8 |
| 15% synthetic | 96.7 | 91.7 | 90.0 | 95.0 | 93.3 | 95.0 | 88.3 | 98.3 | 91.7 | 93.3 |
| 15%Polythene | 98.3 | 90.0 | 100.0 | 86.7 | 93.8 | 90.0 | 85.0 | 98.3 | 86.7 | 90.0 |
| 15% Sisal | 96.7 | 85.0 | 95.0 | 88.3 | 91.2 | 85.0 | 86.7 | 96.7 | 96.7 | 91.3 |
| 18% synthetic | 98.3 | 96.7 | 96.7 | 93.3 | 96.3 | 95.0 | 100.0 | 96.7 | 90.0 | 95.4 |
| 18%Polythene | 98.3 | 100.0 | 100.0 | 100.0 | 99.6 | 95.0 | 100.0 | 98.3 | 100.0 | 98.3 |
| 18% Sisal | 98.3 | 96.7 | 100.0 | 100.0 | 98.8 | 91.7 | 88.3 | 100.0 | 96.7 | 94.2 |
| 20% synthetic | 100.0 | 98.3 | 100.0 | 100.0 | 99.6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 20%Polythene | 100.0 | 100.0 | 100.0 | 98.3 | 99.6 | 88.3 | 100.0 | 98.3 | 95.0 | 95.4 |
| 20% Sisal | 100.0 | 100.0 | 98.3 | 100.0 | 99.6 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Mean | 97.8 | 89.9 | 94.3 | 89.6 | 92.9 | 90.6 | 90.9 | 95.8 | 95.3 | 93.2 |
| LSD SM x ML | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| CV (%) | 5.1 | 9.3 | 6.4 | 17.5 | 6.2 | 10.8 | 9.8 | 5.8 | 6.9 | 3.7 |

ML = moisture level, SM = storage materials, NS = not significant ($P \leq 0.05$)

LSD = least significant difference.

### 4.3.4 Infection of maize grain with *Aspergillus* species

Moisture level and storage materials had no significant effect on kernels infection with *Aspergillus* in maize stored at Machakos 14 days after inoculation (Table 4.50). At Kabete percentage kernel infection with *Aspergillus* species was significantly affected by storage materials but moisture had no effect. Maize samples stored in sisal bags had significantly lower percentage of kernels infected with *Aspergillus* than maize stored in

synthetic and polythene bags. Maize stored in polythene and synthetic bags registered similar levels of kernel infection with *Aspergillus* species. At 35 days after inoculation, moisture level and storage materials had no significant effect on percentage of kernels infected with *Aspergillus* at Machakos. At Kabete, kernel infection of maize with *Aspergillus* was significantly affected by interaction between moisture content and storage materials (Table 4.50). At 10% to 15% moisture levels no significant differences were noted among all storage materials in percentage kernel infection with *Aspergillus* genus. At 18% moisture level, the maize sample stored in polythene bag had a significantly higher percentage kernel infection with *Aspergillus* genus than maize stored in sisal and synthetic bags. The samples in sisal and synthetic bags had no significant differences at all moisture levels. At 20% moisture level, maize stored in sisal bag had a significantly higher infection than maize stored in polythene bags at Kabete.

At 56 days after inoculation, percentage isolation for the genus *Aspergillus* was not significantly affected by moisture content or storage materials in maize stored at Machakos and Kabete (Table 4.51). At 77 days after inoculation, percentage of infection with the genus *Aspergillus* in maize stored at Machakos was significantly affected by storage materials and the interaction between moisture level and storage materials but moisture level had no effect. Samples stored in sisal at 13%, 15%, 18% and 20% registered no difference during storage. For samples stored in polythene bags, maize at 13% and 15% had significantly higher infection than in other moisture levels (Table 4.51 and 4.52). Maize samples stored in synthetic bags had no significant differences in percentage of kernels infected with the genus *Aspergillus* at all moisture levels. Generally, samples stored in sisal bags had a significantly lower infection level than polythene and synthetic bags. There were no significant differences on infection between samples stored in synthetic and polythene bags. At Kabete, only moisture content had a significant effect in the percentage of kernel infection with the genus *Aspergillus*. The samples stored at 10% to 18% had no difference on the infection levels. At 20% moisture, the maize had significantly lower infection than maize stored at 13%, 15%, and 18%. The infection level was however similar to that of samples stored at 10% moisture content.

Table 4.50: Percentage of kernels infected with *Aspergillus* species in maize sampled at 14 and 35 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different storage materials at Kabete and Machakos.

| Moisture level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| | 14 days after inoculation | | | | | | | |
| 10 | 20.0 | 20.0 | 11.7 | 17.2 | 48.3 | 28.3 | 11.7 | 29.4 |
| 13 | 23.3 | 23.3 | 25.0 | 23.9 | 53.3 | 31.7 | 31.7 | 38.9 |
| 15 | 5.0 | 8.3 | 18.3 | 10.6 | 38.3 | 48.3 | 15.0 | 33.9 |
| 18 | 13.3 | 35.0 | 1.7 | 16.7 | 43.3 | 70.0 | 25.0 | 46.1 |
| 20 | 15.0 | 13.3 | 6.7 | 11.7 | 31.7 | 20.0 | 18.3 | 23.3 |
| Mean | 15.3 | 20.0 | 12.7 | 16.0 | 43.0 | 39.7 | 20.3 | 34.3 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | 13.5 |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 86.2 | | | | 51.5 |
| | 35 days after inoculation | | | | | | | |
| Moisture level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 11.7 | 23.3 | 21.7 | 18.9 | 23.3 | 21.7 | 25.0 | 23.3 |
| 13 | 26.7 | 35.0 | 23.3 | 28.3 | 16.7 | 35.0 | 30.0 | 27.2 |
| 15 | 28.3 | 45.0 | 21.7 | 31.7 | 28.3 | 25.0 | 20.0 | 24.4 |
| 18 | 36.7 | 43.3 | 45.0 | 41.7 | 18.3 | 61.7 | 40.0 | 40.0 |
| 20 | 35.0 | 53.3 | 4.7 | 31.0 | 28.3 | 16.7 | 45.0 | 30.0 |
| Mean | 27.7 | 40.0 | 23.3 | 30.3 | 23.0 | 32.0 | 32.0 | 29.0 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | 25.6 |
| CV(%) | | | | 59.4 | | | | 45.9 |

ML = moisture level, SM = storage materials, NS = not significant ($P\leq0.05$)

LSD = least significant difference

Table 4.51: Percentage of kernels infected with *Aspergillus* species in maize, sampled at 56 and 77 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different storage materials at Kabete and Machakos

| Moisture level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 56 days after inoculation | | | | | | | | |
| 10 | 40.0 | 16.7 | 26.7 | 27.8 | 35.0 | 33.3 | 33.3 | 33.9 |
| 13 | 18.3 | 18.8 | 20.0 | 18.9 | 45.0 | 38.3 | 50.0 | 44.4 |
| 15 | 41.7 | 40.0 | 15.0 | 32.2 | 23.3 | 45.0 | 28.3 | 32.2 |
| 18 | 30.0 | 56.7 | 38.3 | 41.7 | 35.0 | 46.7 | 30.0 | 37.2 |
| 20 | 45.0 | 50.0 | 25.0 | 40.0 | 36.7 | 5.0 | 28.3 | 23.3 |
| Mean | 35.0 | 36.3 | 25.0 | 32.1 | 35.0 | 33.7 | 34.0 | 34.2 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 45.9 | | | | 69.1 |
| 77 days after inoculation | | | | | | | | |
| Moisture level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 16.7 | 15.0 | 20.0 | 17.2 | 35.0 | 33.3 | 33.3 | 40.0 |
| 13 | 28.3 | 33.3 | 15.0 | 25.6 | 45.0 | 38.3 | 50.0 | 51.1 |
| 15 | 16.7 | 33.3 | 5.0 | 18.3 | 23.3 | 45.0 | 28.3 | 50.6 |
| 18 | 16.7 | 8.3 | 6.7 | 10.6 | 35.0 | 46.7 | 30.0 | 58.3 |
| 20 | 30.0 | 10.0 | 8.3 | 16.1 | 36.7 | 5.0 | 28.3 | 27.2 |
| Mean | 21.7 | 20.0 | 11.0 | 17.6 | 43.7 | 47.7 | 45.0 | 45.4 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | 6.1 | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | 17.3 |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | 14.5 | | | | NS |
| CV(%) | | | | 45.5 | | | | 40.7 |

ML = moisture level, SM = storage materials, NS = not significant ($P\leq0.05$)

LSD = least significant difference

Table 4.52: Percentage of kernels infected with *Aspergillus* species in maize sampled at 14, 35, 56 and 77 days after inoculation with *Aspergillus flavus* and storage at varying moisture levels using different materials at Kabete and Machakos

| | Machakos | | | | | Kabete | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ML / SM | 14 | 35 | 56 | 77 | Mean | 14 | 35 | 56 | 77 | Mean |
| 10%Nylon Bag | 20.0 | 11.7 | 40.0 | 16.7 | 22.1 | 48.3 | 23.3 | 35.0 | 40.0 | 36.7 |
| 10%Polythene Bag | 20.0 | 23.3 | 16.7 | 15.0 | 18.8 | 28.3 | 21.7 | 33.3 | 31.7 | 28.8 |
| 10% Sisal Bag | 11.7 | 21.7 | 26.7 | 20.0 | 20.0 | 11.7 | 25.0 | 33.3 | 48.3 | 29.6 |
| 13%Nylon Bag | 23.3 | 26.7 | 18.3 | 28.3 | 24.2 | 53.3 | 16.7 | 45.0 | 55.0 | 42.5 |
| 13%Polythene Bag | 23.3 | 35.0 | 18.3 | 33.3 | 27.5 | 31.7 | 35.0 | 38.3 | 58.3 | 40.8 |
| 13% Sisal Bag | 25.0 | 23.3 | 20.0 | 15.0 | 20.8 | 31.7 | 30.0 | 50.0 | 40.0 | 37.9 |
| 15%Nylon Bag | 5.0 | 28.3 | 41.7 | 16.7 | 22.9 | 38.3 | 28.3 | 23.3 | 48.3 | 34.6 |
| 15%Polythene Bag | 8.3 | 45.0 | 40.0 | 33.3 | 31.7 | 48.3 | 25.0 | 45.0 | 60.0 | 44.6 |
| 15% Sisal Bag | 18.3 | 21.7 | 15.0 | 5.0 | 15.0 | 15.0 | 20.0 | 28.3 | 43.3 | 26.7 |
| 18%Nylon Bag | 13.3 | 36.7 | 30.0 | 16.7 | 24.2 | 43.3 | 18.3 | 35.0 | 50.0 | 36.7 |
| 18%Polythene Bag | 35.0 | 43.3 | 56.7 | 8.3 | 35.8 | 70.0 | 61.7 | 46.7 | 81.7 | 65.0 |
| 18% Sisal Bag | 1.7 | 45.0 | 38.3 | 6.7 | 22.9 | 25.0 | 40.0 | 30.0 | 43.3 | 34.6 |
| 20%Nylon Bag | 15.0 | 35.0 | 45.0 | 30.0 | 31.3 | 31.7 | 28.3 | 36.7 | 25.0 | 30.4 |
| 20%Polythene Bag | 13.3 | 53.3 | 50.0 | 10.0 | 31.7 | 20.0 | 16.7 | 5.0 | 6.7 | 12.1 |
| 20% Sisal Bag | 6.7 | 4.7 | 25.0 | 8.3 | 11.2 | 18.3 | 45.0 | 28.3 | 50.0 | 35.4 |
| Mean | 16.0 | 30.3 | 32.1 | 17.6 | 24.0 | 34.3 | 29.0 | 34.2 | 45.4 | 35.7 |
| LSD $_{(p\leq0.05)}$ SM x ML | NS | NS | NS | 14.5 | 9.5 | NS | 25.6 | NS | NS | 19.3 |
| CV (%) | 86.2 | 59.4 | 45.9 | 45.5 | 21.6 | 51.5 | 45.9 | 69.1 | 40.7 | 32.2 |

ML = moisture level, SM = storage materials, NS = not significant ($P\leq0.05$).

### 4.3.5 Re - isolation of *Aspergillus flavus.*

At 14 days after inoculation, percentage of kernels infected with *Aspergillus flavus* in maize stored at Machakos and Kabete was not significantly affected by moisture content, storage materials and their interaction (Table 4.53). At 35 days after inoculation, the percentage of kernel infection with *A. flavus* in maize stored at Machakos was significantly ($p\leq0.05$) affected by moisture level and storage materials but their interaction had no significant effect. Samples stored at 10% and 13% moisture levels had no significant difference. However, the samples stored in polythene bags had significantly higher infection than those stored using synthetic and sisal bags. The

samples stored at 15% and 18% moisture had significantly higher percentage of infection by *A. flavus* than the sample stored at 10%, 13% and 20% moisture level (Table 4.53). The samples stored at 15% and 18% moisture level were not significantly different. The samples stored using sisal and synthetic bags had no significant difference in infection with *A. flavus*. At 56 days after inoculation, percentage kernels infected with *A. flavus* in maize stored at Machakos was not significantly affected by moisture content and storage materials (Table 4.54). However, the interaction between storage materials and moisture content had a significant effect in the percentage kernel infection with *Aspergillus flavus*. At 10% and 13% there was no significant effect for all storage materials (Table 4.54). All the samples in synthetic and sisal bags had no significant difference during storage at all moisture levels. Maize stored in polythene bags at 15% to 20% moisture levels had significantly higher infection by *Aspergillus flavus* than maize stored at 10% and 13% moisture levels.

At 77 days after inoculation, the percentage of kernel infection by *A. flavus* in maize stored at Machakos was significantly affected by moisture level, storage materials and their interaction (Table 4.54 and 4.55). At 10% moisture level, all storage materials had no significant difference in percentage of kernels infected with *Aspergillus flavus*. However at 13% moisture level, maize stored in sisal bag had significantly lower infected kernels than maize stored in synthetic and polythene bags. There was no significant difference between synthetic and polythene at 13% moisture level. At 15%, the maize stored in polythene bags had significantly higher infected kernels than maize stored in synthetic and sisal bags. There was no significant difference between synthetic and sisal at 15% moisture content. At 18% moisture content, samples in polythene and synthetic bag had significantly higher infection than sisal bag. There was no significant difference between all the other samples at 18% and 20% moisture level irrespective of storage materials. At all moisture levels, there was no significant difference in all samples stored in sisal bags. Sisal bags and synthetic bags had significantly lower infection levels than polythene bags, but no difference was registered between synthetic and sisal bags. Percentage of kernels infected by *A. flavus* in maize stored at Kabete was significantly affected by moisture level only. The percentage of kernels infected generally increased

with an increase in moisture with a peak at 18% and then dropped at 20% moisture content. The sample stored at 20% moisture level had significantly lower infection than all the others. Maize stored at 10% and 13% had similar percentage of infected kernels with *Aspergillus flavus*. At 15% and 18%, there was no significant difference in percentage of kernels infected with *Aspergillus flavus*.

Table 4.53: Percentage of kernels infected with *Aspergillus flavus* in maize sampled at 14 and 35 days after inoculation with the fungi and storage at varying moisture levels using different storage materials at Kabete and Machakos

| Moisture level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 14 days after inoculation | | | | | | | | |
| 10 | 15.0 | 3.3 | 3.3 | 7.2 | 28.3 | 18.3 | 8.3 | 18.3 |
| 13 | 8.3 | 6.7 | 13.3 | 9.4 | 40.0 | 16.7 | 25.0 | 27.2 |
| 15 | 18.3 | 8.3 | 11.7 | 12.8 | 26.7 | 31.7 | 6.7 | 21.7 |
| 18 | 3.3 | 1.7 | 1.7 | 2.2 | 35.0 | 28.3 | 25.0 | 29.4 |
| 20 | 5.0 | 1.7 | 0.0 | 2.2 | 18.3 | 13.3 | 8.3 | 13.3 |
| Mean | 10.0 | 4.3 | 6.0 | 6.8 | 29.7 | 21.7 | 14.7 | 22.0 |
| $LSD_{(p\leq 0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq 0.05)}$ moisture level | | | | NS | | | | NS |
| $LSD_{(p\leq 0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 136.2 | | | | 72.5 |
| 35 days after inoculation | | | | | | | | |
| Moisture level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 10.0 | 18.3 | 11.7 | 13.3 | 20.0 | 13.3 | 16.7 | 16.7 |
| 13 | 15.0 | 20.6 | 18.3 | 18.0 | 15.0 | 20.0 | 28.3 | 21.1 |
| 15 | 23.3 | 50.0 | 15.0 | 29.4 | 26.7 | 23.3 | 18.3 | 22.8 |
| 18 | 26.7 | 35.0 | 35.0 | 32.2 | 15.0 | 48.3 | 40.0 | 34.4 |
| 20 | 15.0 | 41.7 | 3.3 | 20.0 | 13.3 | 16.7 | 31.7 | 20.6 |
| Mean | 18.0 | 33.1 | 16.7 | 22.6 | 18.0 | 24.3 | 27.0 | 23.1 |
| $LSD_{(p\leq 0.05)}$ storage materials | | | | 11.9 | | | | NS |
| $LSD_{(p\leq 0.05)}$ moisture level | | | | 11.6 | | | | NS |
| $LSD_{(p\leq 0.05)}$ storage materials x moisture level | | | | NS | | | | NS |
| CV(%) | | | | 68.8 | | | | 45.5 |

Table 4.54: Percentage of kernels infected with *Aspergillus flavus* in maize sampled at 56 and 77 days after inoculation with the fungi and storage at varying moisture levels using different storage materials at Kabete and Machakos

| Moisture level (%) | Machakos | | | | Kabete | | | |
|---|---|---|---|---|---|---|---|---|
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 56 days after inoculation | | | | | | | | |
| 10 | 16.7 | 10.0 | 13.3 | 13.3 | 18.3 | 15.0 | 11.7 | 15.0 |
| 13 | 10.0 | 13.3 | 10.0 | 11.1 | 20.0 | 30.0 | 21.7 | 23.9 |
| 15 | 21.7 | 36.7 | 6.7 | 21.7 | 13.3 | 33.3 | 15.0 | 20.6 |
| 18 | 13.3 | 36.7 | 21.7 | 23.9 | 18.3 | 26.7 | 21.7 | 22.2 |
| 20 | 6.7 | 36.7 | 10.0 | 17.8 | 6.7 | 5.0 | 11.7 | 7.8 |
| Mean | 13.7 | 26.7 | 12.3 | 17.6 | 15.3 | 22.0 | 16.3 | 17.9 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | NS | | | | NS |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | 25.5 | | | | NS |
| CV(%) | | | | 66.2 | | | | 70.8 |
| 77 days after inoculation | | | | | | | | |
| Moisture level (%) | Machakos | | | | Kabete | | | |
| | Synthetic | Polythene | Sisal | Mean | Synthetic | Polythene | Sisal | Mean |
| 10 | 6.7 | 10.0 | 11.7 | 9.4 | 25.0 | 21.7 | 23.3 | 23.3 |
| 13 | 21.7 | 30.0 | 10.0 | 20.6 | 38.3 | 38.3 | 23.3 | 33.3 |
| 15 | 10.0 | 26.7 | 13.3 | 16.7 | 35.0 | 56.7 | 30.0 | 40.6 |
| 18 | 20.0 | 25.0 | 6.7 | 17.2 | 46.7 | 60.0 | 41.7 | 49.4 |
| 20 | 13.3 | 26.7 | 13.3 | 17.8 | 18.3 | 3.3 | 13.3 | 11.7 |
| Mean | 14.3 | 23.7 | 11.0 | 16.3 | 32.7 | 36.0 | 26.2 | 31.7 |
| $LSD_{(p\leq0.05)}$ storage materials | | | | 6.1 | | | | NS |
| $LSD_{(p\leq0.05)}$ moisture level | | | | 7.8 | | | | 11.5 |
| $LSD_{(p\leq0.05)}$ storage materials x moisture level | | | | 13.0 | | | | NS |
| CV(%) | | | | 65.2 | | | | 46.2 |

ML = moisture level, SM = storage materials, NS = not significant ($P\leq0.05$).

LSD = least significant difference.

Table 4.55: Percentage of kernels infected with *Aspergillus flavus* in maize sampled at 14, 35, 56, and 77 days after inoculation with the fungi and storage at varying moisture levels using different storage materials at Kabete and Machakos

| | Machakos | | | | | Kabete | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ML / SM | 14 | 35 | 56 | 77 | Mean | 14 | 35 | 56 | 77 | Mean |
| 10%synthetic | 15.0 | 10.0 | 16.7 | 6.7 | 12.1 | 28.3 | 20.0 | 18.3 | 25.0 | 22.9 |
| 10%Polythene | 3.3 | 18.3 | 10.0 | 10.0 | 10.4 | 18.3 | 13.3 | 15.0 | 21.7 | 17.1 |
| 10% Sisal | 3.3 | 11.7 | 13.3 | 11.7 | 10.0 | 8.3 | 16.7 | 11.7 | 23.3 | 15.0 |
| 13% synthetic | 8.3 | 15.0 | 10.0 | 21.7 | 13.8 | 40.0 | 15.0 | 20.0 | 38.3 | 28.3 |
| 13%Polythene | 6.7 | 23.3 | 13.3 | 30.0 | 18.3 | 16.7 | 20.0 | 30.0 | 38.3 | 26.3 |
| 13% Sisal | 13.3 | 18.3 | 10.0 | 10.0 | 12.9 | 25.0 | 28.3 | 21.7 | 23.3 | 24.6 |
| 15% synthetic | 18.3 | 23.3 | 21.7 | 10.0 | 18.3 | 26.7 | 26.7 | 13.3 | 35.0 | 25.4 |
| 15%Polythene | 8.3 | 50.0 | 36.7 | 26.7 | 30.4 | 31.7 | 23.3 | 33.3 | 56.7 | 36.3 |
| 15% Sisal | 11.7 | 15.0 | 6.7 | 3.3 | 9.2 | 6.7 | 18.3 | 15.0 | 30.0 | 17.5 |
| 18% synthetic | 3.3 | 26.7 | 13.3 | 20.0 | 15.8 | 35.0 | 15.0 | 18.3 | 46.7 | 28.8 |
| 18%Polythene | 1.7 | 35.0 | 21.7 | 5.0 | 15.9 | 28.3 | 48.3 | 26.7 | 60.0 | 40.8 |
| 18% Sisal | 1.7 | 35.0 | 36.7 | 6.7 | 20.0 | 25.0 | 40.0 | 21.7 | 41.7 | 32.1 |
| 20% synthetic | 5.0 | 15.0 | 6.7 | 13.3 | 10.0 | 18.3 | 13.3 | 6.7 | 18.3 | 14.2 |
| 20%Polythene | 1.7 | 41.7 | 36.7 | 6.7 | 21.7 | 13.3 | 16.7 | 5.0 | 3.3 | 9.6 |
| 20% Sisal | 0.0 | 3.3 | 10.0 | 3.3 | 4.2 | 8.3 | 31.7 | 11.7 | 13.3 | 16.3 |
| Mean | 6.8 | 22.8 | 17.6 | 12.7 | 15.0 | 22.0 | 23.1 | 17.9 | 31.7 | 23.7 |
| LSD $_{(p\leq0.05)}$ SM x ML | NS | NS | 25.5 | 13.0 | 6.9 | NS | NS | NS | NS | NS |
| CV (%) | 136.2 | 68.8 | 66.2 | 65.2 | 22.5 | 72.5 | 45.5 | 70.8 | 46.2 | 33.0 |

ML = moisture level, SM = storage materials, NS = not significant ($P\leq0.05$)

LSD = least significant difference.

# CHAPTER FIVE: DISCUSSION

## 5.1 Maize production and handling practices

The study found that most farmers in the North Rift region planted certified seeds but a large proportion of farmers from Eastern province planted seeds selected from previous harvests and neighbours. Majority of farmers in Eastern removed the cobs from the sheaths completely and heaped the maize cobs on the ground for drying, but farmers in the North Rift region practiced stooking of the maize for further drying and stripped later. Most farmers in both regions harvested maize before it was completely dry. Majority of farmers in Eastern region of Kenya stored their maize in their houses. The study found that most of the farmers and traders in both regions stored the maize in synthetic bags. The study also found that weevils (*Sitophilus zeamais*) were very common as storage pests in both regions. Larger grain borers (*Prostephanus truncatus*) were also encountered by some traders in both regions. Traders in Eastern and majority in the North Rift regions placed the storage containers in direct contact with concrete floors.

Use of uncertified seeds selected from previous harvests as a source of planting seeds by farmers in Eastern region could be a source of inoculum for *A. flavus*. Multiple *A. flavus* and strains are known to frequently infect individual locules and seed (Garber and Cotty, 1997). This could act as the primary source of inoculum for infection of maize by *Aspergillus flavus* while in the field.

Majority of the farmers in Kitui and Machakos harvested the maize before it was completely dry. The common harvesting practice by majority of farmers in Kitui and Makueni was complete removal of the sheaths and throwing the cobs onto the ground. The drying was in cobs by all farmers in Makueni and Kitui. A large proportion dried maize on bare earth surface. A survey conducted by the Ministry of Agriculture in Kenya (MOA, 2007) in eastern region of Kenya also found that the majority of the farmers dehusked and contaminated their maize by throwing it on the ground during harvesting. A study conducted in Benin by Udo *et al.* (1999) found that harvesting maize with husk in the southern zones was associated with reduction in aflatoxin contamination. Betran and Isakeit (2003) noted that lower contamination has been associated with the

expression of secondary traits such as husk coverage and tightness, insect resistance, kernel injury under environmental stress and drought resistance. In other regions of Africa, small-scale farmers harvest maize when the husks starts yellowing, an indication of physiological maturity. After dehusking, the ears are tied together and hung underneath a roof or other protective cover where they dry gradually (Ristanovic, 2001). Commercial farmers harvest maize when the grain moisture is at or below 15%. Harvesting is done with combine harvesters or by hand. When done by hand, the ears are picked, deposited directly into tractor-drawn trailers and transported to storage facilities on the farm or to mechanical shellers where they are directly shelled and the grain bagged for sale (Ristanovic, 2001).

The harvesting practices in the Eastern region expose the maize to contamination by *Aspergillus flavus*. The fungus is commonly found in soil and crop residue which act as the principal source of primary inoculum for infecting maize (Jaime-Garcia and Cotty, 2004; Kumar *et al.*, 2000; Horn, 2007; Atehnkeng *et al.*, 2008). The prevailing weather conditions and other factors may not allow the maize to be dried properly and this coupled with the fact that most farmers stored in poorly aerated rooms in their houses may create favourable conditions for aflatoxin production. According to Williams *et al.* (2004), drying is usually carried out very inefficiently and is dependent on the weather and adverse weather at harvest results in slow and inadequate drying with attendant risks of contamination.

Harvesting maize before it was completely dry and inadequate drying of maize before storage can promote aflatoxin contamination and aflatoxin development can be prevented by proper drying of the crop before storage (Stroisnider *et al.*, 2006; Hell *et al.*, 2000; Turner *et al.*, 2005). Hell *et al* (2000) Turner *et al* (2005) and Stroisnider *et al* (2006) also reported that timely harvest and rapid and adequate drying before storage are important in reduction of aflatoxin contamination. Lanyasunya *et al.* (2005) and Muthomi *et al.* (2008) also noted that rapid drying of agricultural products to low moisture level is critical as it creates less favourable conditions for fungal growth and proliferation, insect infestation and helps keep longer. Maize drying in Eastern region could pose a very serious

challenge particularly when the wet weather conditions prevail. To preserve quality in storage, it is necessary to prevent biological activity through drying to less than 10% moisture content.

Drying in cobs placed on bare ground surface was the major practice in eastern region but farmers in North Rift dried maize after shelling and placed it on tarpaulin, mats or polythene sheets to avoid contact with bare surface. This practice exposes the maize and the ground surface could be a source of contamination with *Aspergillus flavus.* The fungus typically lives as a saprophyte in the soil depending on organic matter to propagate and survive (Jaime-Garcia and Cotty, 2004). *Aspergillus flavus* is commonly found in soil and crop debris, which acts as the principal source of primary source of inoculum for infecting maize (Jaime-Garcia and Cotty., 2004; Horn, 2007; Atehnkeng *et al.*, 2008). Aflatoxin contamination is influenced by the population of toxin producing fungi that resides in soil, cob feeding lepidopteran insects, invading weevils and *other* beetles (Hell *et al.*, 2004). Kaaya and Warren (2005) reported that maize kernels dried at home on bare ground was more contaminated with aflatoxin than those dried on polythene sheets or mats.

Poor harvesting practices, improper storage and less than optimal conditions during transportation and marketing can also contribute to fungal growth and increase the risk of mycotoxin production (Bhat and Vasanthi, 2003; Muthomi *et al.*, 2009). *Aspergillus flavus* is ubiquitous in aflatoxin production. It can infest maize by air-borne spores in the field during crop development or after maturation when the crop is exposed to high temperature and moisture level either before harvest or in storage (Payne, 1992; Kumar *et al.*, 2000; Atehnkeng *et al.*, 2008). A study conducted in Thailand by Department of Agriculture also found low concentrations of *A. flavus* spores in the air and around stored maize . According to Sangare-Tigori *et al* (2006), after harvest, the conditions of storage and transportation of grains or foods may enhance aflatoxin $B_1$ suddenly. Maize transported for long distances risk exposure to contamination by the air-borne fungal spores. In case of maize with high moisture content, chances could be high that conditions may become favourable for fungal growth and aflatoxin production. It is also

likely that the trucks will traverse areas with high daily temperatures or they may encounter areas experiencing sudden showers posing the risk of wetting the maize and thus creating conducive conditions for fungal growth. Most of the maize sold by traders had been transported from far places like Kitale, Taveta, Loitokitok and Busia which are all more than 300 km away. The average moisture content of the maize samples from traders in Eastern was 11.1%, but 9.4% of the samples had moisture content of 13.5% and above with the highest being 14.7%.

A study conducted in 2004 after a serious aflatoxin poisoning outbreak in the Eastern region also found that although maize is traditionally stored in granaries, storage inside living houses occur during periods of food shortage and that may have facilitated contamination of maize with aflatoxin (Azziz-Baumgartner *et al.*, 2005; Lewis *et al.*, 2005). A survey conducted by the Ministry of Agriculture in Eastern region (MOA, 2007) revealed that most farmers kept their maize in the living houses. Most of the farmers in North Rift stored their maize in improved granaries. Traditional granaries are raised structures that are well ventilated, and they promote the drying of grain (Food and Agriculture Organisation (FAO), 2004; Azziz-Baumagartner *et al.*, 2005) the granaries' elevated platform isolates the maize from spores and insects on the ground (Azziz-Baumagartner *et al.*, 2005). This study found that farmers kept their maize in the living houses mostly when there is food shortage, away from potential thieves. The granaries had gaps through which maize could be seen exposing the farmer to the risk of theft or too much borrowing by neighbours and relatives. The warm environment inside these windowless homes and storage of maize on the dirt floor may have promoted fungal growth in wet kernels (Azziz-Baumgartner *et al.*, 2005). The Ministry of Agriculture in Kenya (MOA, 2008) discourages storage of maize on the flour and recommends that storage containers should be placed on wooden pallets to be at least 10 cm high.

The study also found that weevils (*Sitophilus zeamais*) were very common as storage pests in both regions. Larger grain borers (*Prostephanus truncatus*) were also encountered by some traders in both regions. Insects have been known to be associated with spread of the fungus *Aspergillus flavus* and grain contamination (Udo *et al.*, 1999).

Specifically the maize weevil has been reported to contribute significantly to *A. flavus* infection in maize and subsequent production of aflatoxins (McMillan *et al.*, 1981; Miller, 1995). According to Beti *et al* (1995), weevils facilitate the growth of *A. flavus* and aflatoxin production by increasing surface area susceptible to fungal infection and increasing moisture content as a result of weevil metabolic activity. In a mycological assessment of maize in Thailand by a team of scientists, it was found that *A. flavus* was higher in maize kernels infested by weevils than non-infested kernels. Weevils carried a significant amount of *A. flavus* and *F. moniliforme* spores in their bodies. Dead weevils in stored maize were found to be yellow-green with conidia of *Aspergillus flavus* .

Kernel infection by *Aspergillus flavus* leading to aflatoxin contamination can occur during crop development when the crop is damaged (for example by insects) or stressed by heat and drought and after maturation when the crop is exposed to high moisture and high temperature either before harvest or in storage (Payne, 1992; Atehnkeng *et al.*, 2008). Weevils and larger grain borers (*Prostephanus truncatus*) which were also encountered in both regions can attack maize in the field before harvest and in storage. They degraded the kernels through their excreta, loss of quality and marketability and ultimately made the maize unfit for human consumption thus compromising food security at the household level.

The study found that maize was consumed in one form or another in every household in both regions. It was consumed as whole grain boiled in a mixture with legumes ("Githeri"), semi-processed grains mixed with legumes ("Muthokoi"), and maize flour made into thick porridge ("Ugali") or thin porridge. In Eastern, most farmers ate "githeri", "ugali" and "Muthokoi" in that order, while in North Rift, "ugali" was the preferred meal followed by "githeri". In both regions, the major source of maize flour was own maize taken to the posho mills for grinding. Farmers in Eastern also relied on own maize for semi-processed grains either processed at home or taken to the posho mills for dehulling.

The heavy reliance on maize and maize based products as food poses a risk of chronic contamination if the maize contains low levels of mycotoxins. Shephard (2008) observed that diets consumed by the population in developing countries tend to be obtained from local markets with less emphasis on quality issues. The problem of excessive consumption of a single cereal can be seen in many African diets which rely on maize consumed at levels 400 to 500 g per person per day. Even moderate levels of mycotoxin contamination can result in exposure which exceeds the maximum daily tolerable daily intake (TDI) set by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA). Azziz-Baumgartner *et al.* (2005) and Lewis *et al.* (2005) in their studies reported that eating home grown contaminated maize was to blame for the aflatoxicosis outbreak in Kenya in 2004 that killed 125 people in eastern province.

Moisture is by far the most important factor affecting growth of microorganisms in stored maize (Stack and Carlsons, 2006) and aflatoxin levels may escalate within a few hours if grain moisture is not handled properly (Missouri State University (MSU), 2005). Before storage, proper drying of crops can prevent the development of aflatoxins (Strosnider *et al.*, 2006). After harvest, the conditions of storage and transportation of grains may enhance production of aflatoxin $B_1$ suddenly (Sangare-Tigori *et al.*, 2006). *Aspergillus flavus* grows best on maize at 18.0% – 18.5% moisture content and grows rapidly at 26.7°C to 37.8°C (Wrather and Sweet, 2008) but the growth approaches dormant levels when grain moisture drops below 15%. Drying harvested maize to 15.5% moisture or lower within 24 – 48 hours can reduce the risk of fungal growth and subsequent aflatoxin production (Hamilton, 2000; Stack and Carlson, 2006; Wagacha and Muthomi, 2008).

The study found that the long rains season maize and maize products from agro-ecological zone LM4 in Kitui and short rain season maize from agro-ecological zones LM5 and LM3 of Kitui and Makueni respectively had very high aflatoxin contents. The semi-processed maize from agro-ecological zone LM4 of Machakos had more than ten times the allowed limit of 10 µg/kg in Kenya. High levels of aflatoxin were found in semi-processed grains sampled in agro-ecological zone LM4 of Kitui and Machakos. Studies conducted by other scientists reported lower aflatoxin levels in semi-processed

grains. According to Mutungi *et al.* (2008), the traditional method of making semi-processed grains begins with selection of the grains to remove undersized, off-coloured, shriveled and damaged grains. The selected grains are then pounded in a mortar accompanied by intermittent wetting with water to soften and remove the pericarp (hull). The dehulled fraction is separated from the hulls by winnowing after sun drying. Fandohan *et al.* (2003) detected a significant level of aflatoxin (7.55ng/g) in the discarded hulls and embryo during production of ogi and akassa in West Africa while Mutungi *et al.* (2008) reported significant reduction of the mean total aflatoxin level by 57.3 ng/g through dehulling. Dehulling maize eliminates the pericarp (hull), underlying aleurone layer, hilum and a sizeable portion of the germ. These fractions are usually the more highly contaminated parts of the grain (Mutungi *et al.*, 2008).

The aflatoxin level in the semi-processed grains from agro-ecological zone LM4 Kitui and Machakos was high probably because of colonization of the grains and subsequent aflatoxin production by the fungus *A. flavus* after dehulling. The intermittent wetting during the dehulling process may have resulted in raised moisture content that could have supported fungal growth. Mutungi *et al.* (2008) noted that the traditional process of making semi-processed grains does not destroy moulds and the risk of recontamination remains high if the product is stored under conditions favouring proliferation of aflatoxigenic moulds. The pounding of grains during the process of dehulling using mortar and pestle may also result in mechanical damage of grains. This might have exposed the grains to infection by *Aspergillus flavus* and this agrees with Kumar *et al.* (2000) who observed that growth cracks, mechanical injury and damage by pests to the plant parts or seeds lead to infestation by fungi. Fandohan *et al.* (2005) and Mutungi *et al.* (2008) in their experiments dehulled maize using a mechanical mill and did not involve of intermittent wetting, a practice that is common with the farmers.

The agronomic, harvesting, storage and handling practices like throwing wet maize cobs on the ground, poor drying, storage in houses which are poorly aerated, and placing the storage containers on the ground may expose maize to the *A. flavus* inoculum and favourable conditions for aflatoxin production. The fungal inoculum was found in soils

under the stores, soil from outside, posho sweepings and in whole grains. If the stores are not properly cleaned, it implies that maize put there for storage may get contaminated. According to Riley and Norrad (1999), elimination of waste maize deposits will reduce the incidence of *A. flavus* in soils and kernels beneath the piles.

Maize samples from North Rift had no detectable levels of aflatoxins. Farmers in North Rift stooked maize and allowed it to dry further before stripping the cobs from the husks. While drying, they used mats and tarpaulins to avoid contact with the soil. Most of them also stored maize in improved stores which were well aerated and could allow maize to continue drying. North Rift is cooler compared to eastern region and the rainfall is usually adequate in terms of amount and distribution to support the maize crop. The maize does not suffer stress due to drought. The low temperatures might be unfavourable for the growth of aflatoxin-producing fungi and aflatoxin production. In Uasin Gishu, the average annual rainfall ranges from 900mm to 1200mm and falls during one long season from March to September with two peaks in May and August (DAO's annual report, 1994). The average temperature is 18°C during a wet season, with the maximum of 26.1°C during the dry season. The minimum temperatures experienced during the coldest month of July ranges from 8°C to 8.4°C (DAO's annual report, 1994). Kitui receives an average of 300mm to 800mm of rainfall which most of the times is very poorly distributed (DAO's annual report, 1992). On average, the probability of experiencing drought in every ten years is about 40%. The temperature ranges from 22°C to 33°C with February and September as the hottest months.

**5.2 Fungal and aflatoxin contamination of maize and maize products**

The major fungal genera isolated from soil, whole maize and maize products were *Fusarium*, *Aspergillus* and *Penicillium.* These fungi have species capable of producing a wide array of compounds shown to be toxic to man and animals (Marassas, 1988). The *Fusarium* species isolated from maize and maize products were *F. subglutinans, F. verticillioides, F. proliferatum*, *F. semitectum* and *F. graminearum*. In Eastern province, *F. subglutinans* (Basionym: *Fusarium moniliforme* Sheldon var. *subglutinans* Wollenw and Reinking), had the highest frequency of isolation during both seasons and in the

maize from traders. Other species with high frequencies of isolation were *F. verticillioides,* and *F. graminearum.*

The *Aspergillus* species isolated from soils, maize and maize products from eastern and whole maize from North Rift regions were *Aspergillus flavus, A. niger, A. fumigatus, A. clavatus, A. ochraceus, A. terreus* and *A. versicolor.* All aflatoxin producing fungi are contained within *Aspergillus* section Flavi. Fungal isolates within section Flavi vary widely in aflatoxin producing ability (Gonzalez et al., 2008). Interest in this variability has increased because of recent suggestions that atoxigenic strains of *A. flavus* and *A. parasiticus* might be applied to agricultural fields in order to reduce the risk of aflatoxin contamination. The species most commonly implicated in contamination is, *A. flavus* can be divided into two strains on the basis of morphological, physiological, and genetic criteria (Cardwell and Cotty, 2002). Typical or L strain isolates vary widely in aflatoxin production ability, and a significant percent of L strain isolates are atoxigenic (produce no aflatoxin). The S strain isolates have a tendency to produce greater quantities of smaller sclerotia than L strain isolates. The S strains isolates also produce more aflatoxin than L strains isolates, and atoxigenic S strain isolates are rare (Cardwell and Cotty, 2002; Lewis *et al* 2005; Yin *et al.*, 2008).

It is important to note that the presence of *Aspergillus flavus* on maize does not mean that aflatoxin is also present in that maize. Circumstances that favour mould growth may also favour aflatoxin production but mould growth may occur with little or no mycotoxin production (Shariff, 2004; Stack and Carlson, 2006). In addition to the production of aflatoxin, *Aspergillus* species of mold can affect humans or animals in two ways. Some people and animals are allergic to *Aspergillus* species and exhibit either acute or chronic reactions to the mould itself. *Aspergillus* moulds can infect animals, including humans, with inadequate immune system function causing a disease called Aspergillosis which is an invasion of the lungs, although colonization of other organs can occur (Stack and Carlson, 2006).

Several studies have found *Fusarium* as the most abundant fungal genus. Hell *et al.* (2004) and Morenoa *et al.* (2009) all reported *Fusarium* to be very abundant in the environment in their studies. Yarzar and Omurtag (2008) also noted that *Fusarium* species are probably the most prevalent mycotoxin-producing fungi and are commonly found in cereals. This genus comprises several toxigenic species including *F. verticillioides* and *F. proliferatum*, which are the most prolific producers of fumonisins (Riley and Norrad, 1999; Fandohan, 2003). Ramsey *et al.* (1986) and Fandohan *et al.* (2003) working on maize reported *F. verticillioides* as the most frequently isolated fungus. Maize samples from Western Kenya also had higher isolation frequencies of *F. verticillioides* (Kedera *et al.*, 1999). The predominant species isolated in this study were *F. subglutinans* in Eastern region and *F. proliferatum* in North Rift region.

The presence of these fungi could suggest presence of mycotoxins in the maize. The current study however did not analyse for mycotoxins produced by *Fusarium*. Cvetnić *et al.* (2004) and Yazar and Omurtag (2008) and Van-Egmond *et al.* (2007) reported that members of *Fusarium* genus are potent producers of trichothecenes like deoxynivalenol – DON, diacetoxyscirphenol – DAS, T-2 toxin, and HT-2toxin. They also produce mycotoxins zearalenone – ZEA, and fumonisin. Fumonisins, trichothecenes and zearalenone are hazardous for human and animal health (Yazar and Omurtag, 2008). According to Askun (2006), *F. verticillioides, F. proliferatum, F. subglutinans* and *F. anthopilum* belong to the *Liseola* section which has been referred to as a unique fumonisins producing section. *Fusarium* ear rot, the most common ear rot in maize is caused by *F. verticillioides*, *F. proliferatum*, or *F. subglutinans.*

*Aspergillus* species isolated from maize and maize products were *A. flavus, A. fumigatus, A. clavatus, A. terreus, A. niger, A. versicolor* and *A. ochraceus.* Maize and maize products from eastern region had higher isolation frequencies of *A. flavus* than the maize from North Rift but in both Eastern and North Rift, *A. flavus* had the highest frequency of isolation followed by *A. niger* in all cases. Although *Aspergillus* and *Penicillium* are classified as storage fungi based on studies carried out in temperate climates, under warm, humid subtropical and tropical climates, species of *Aspergillus* and *Penicillium*

can infect grains in the field (Wilson and Abraham, 1992; Morenoa *et al.*, 2009). *Aspergillus flavus* and *Aspergillus parasiticus* are the major producers of aflatoxins (Yin *et al*, 2008) while *Aspergillus ochraceus* produces ochratoxin A (Wagacha and Muthomi, 2008). In most of the isolations from soils and maize and maize products, *A. flavus* had the highest frequency of isolation and was significantly different among the agro-ecological zones. This implies that the fungus is available at higher concentrations in some agro-ecological zones.

Apart from *A. niger* which was significantly different in maize from farmers collected during the second season in Eastern region, *A. terreus* and *A. versicolor* from maize sampled from farmers during the first season, all the other *Aspergillus* species isolated were not significantly different among the agro-ecological zones. This implies that the fungus is widespread in all agro-ecological zones. Cardwell and Cotty (2002) in a study conducted in West Africa found that the colony forming unit counts of *A. flavus* did not differ from year to year significantly with cropping system within a zone, but differed significantly among zones.

The fungal genera comprising *Fusarium* and *Aspergillus* species were found to be abundant in the soils, maize and maize products as well as in the processed by-products. Good agricultural practices that would discourage fungal growth and mycotoxin production could reduce mycotoxin levels in the maize. Contact of the maize with the soil should be avoided during harvest and drying to avoid contamination with the fungal inoculum present in the soil. Drying of maize to low moisture levels and proper cleaning of stores before new produce should be done. Although it was not analyzed for aflatoxin content, posho mill dust should be disposed off safely and not fed to livestock as it could also be having high aflatoxin levels which may be converted into $M_1$. The dust contains spores of *Fusarium* and *Aspergillus* species and therefore it should not be scattered when discarding it to avoid spreading the fungal inoculum

Aflatoxins are produced by *A. flavus* and the fungus forms sclerotia which allows it to survive in soil and corn cobs for extended periods of time (Schedegger and Payne, 2003;

Jaime and Cotty, 2004, Wagacha and Muthomi, 2008). *A. flavus* typically lives as a saprophyte in the soil depending on organic matter to propagate and survive (Jaime and Cotty, 2004). It is commonly found in soil and crop debris, which acts as the primary source of inoculum for infecting maize (Jaime and Cotty, 2004; Horn, 2007; Atehnkeng *et al.*, 2008). According to Olanya *et al.* (1997) and Strosnider *et al.* (2006), elimination of inoculum sources such as infected debris from the previous harvest, may prevent infection of the crop. Aflatoxin contamination can occur during crop development when the crop is damaged (for example by insects) or stressed by heat and drought and after maturation when the crop is exposed to high moisture either before harvest or in storage (Payne, 1992; Atehnkeng *et al.*, 2008). The optimum conditions for growth and subsequent production of aflatoxins by A. *flavus* include moisture content above 14%, temperature of 28 to 30°C and water activity of 0.83 to 0.97 (Mutungi *et al.*, 2008). In a study conducted by Riley and Norrad (1999), they reported that soil-borne *A. flavus* was greatly increased when soil temperatures were 35 to 40°C. The optimal conditions for production of ochratoxin A (OTA) are temperature range of 20 to 25°C and crop moisture content of 16% or above (Bennett and Klich, 2003).

Awareness on mycotoxin poisoning and especially aflatoxicosis was very high. Awareness campaigns were held after the 2004 aflatoxin poisoning. Strosnider *et al* (2006) reported that individuals who received information on maize drying and storage through an awareness campaign run by the Food and Agricultural Organization and Kenya's Ministry of Agriculture had lower serum aflatoxin levels than those who did not receive the information. During the current study, farmers who had received the information were found drying their maize properly after harvesting. They were placing the maize on mats and polythene sheets to avoid contact with soil surface. The same farmers were storing their maize in cribs and improved stores which were properly aerated and allowed further drying instead of storing in their living houses. Those who stored in houses stored the maize in sisal or synthetic bags and placed them on wooden pallets to avoid contact with bare earth or cemented floors. Awareness campaigns should use systems that are in place for disseminating information to subsistence farmers (James, 2005).

### 5.3 Effect of storage material and moisture content on maize infection with storage fungi.

The study found that maize samples stored at 10% to 15% moisture content were not significantly different in the percentage of total kernel infection, infection by *Aspergillus* and *Fusarium* irrespective of storage materials. The study also found that the percentage kernel infection with *Aspergillus flavus* was significantly affected by storage materials and interaction between storage materials and moisture content. Maize stored in polythene bags at 13% to 20% moisture content had significantly higher infection than maize at 10% using the same storage materials. Moisture content and storage materials had no significant effect in maize stored at 10% to 15% moisture content in all storage materials. Generally, the samples stored at 18% and 20% had significantly higher proportion of discoloured and mouldy grains.

After the 2004 aflatoxicosis outbreak in eastern province, a study by Azziz-Baumgartner (2005) found no relationship between the storage materials (plastic burlap, plastic bucket, woven basket, clay pot, gourd or sisal) and the aflatoxin case status in Eastern province. Use of polypropylene bags in storage could promote aflatoxin contamination as studies conducted in Uganda suggest. Kaaya and Warren (2005) observed that woven polypropylene bags do not protect grains against aflatoxin contamination. The ministry of Agriculture in Kenya in their survey in 2007 (MOA, 2007) found majority of farmers in Eastern province using the synthetic bags and argued that these bags could promote aflatoxin contamination but according to the results of my storage experiment, there was no significant difference between sisal and synthetic (polypropylene) bags. Synthetic bags are porous and can allow loss of moisture and subsequent drying of maize in storage. At 10% moisture content, storage materials had no significant effect on percentage kernel infection by *Aspergillus flavus*. This implies that so long as the maize has moisture content below 13%, it can be stored using any type of storage container. Sisal bags are more expensive than the synthetic bags and majority of the farmers and traders could not afford to buy them.

Samples in sisal bags had lower infection than polythene but there was no difference between sisal and synthetic bags. This could imply that maize in polythene at moisture levels above 13% had favourable conditions for *A. flavus*. Polythene bags are not porous and limit aeration and this could lead to colonization by *A. flavus*. According to Abbas (2005), moisture levels in maize below 12 to 13% inhibit growth of the fungus at any temperature.

According to Stack and Carlson (2006), moisture content is by far the most important factor affecting growth of microorganisms in stored maize. After harvest, maize should be dried to moisture content of 15% within 24 hours and long storage grain should be dried to 13% moisture. Wrather and Sweets (2008) reported that moisture content below 13% prevents invasion by *A. flavus* and that fungal growth is best at 18% moisture. At 20% moisture and above, other fungi grow better and crowd out *A. flavus*. Lanyasunya *et al* (2005) and Wagacha and Muthomi (2008) observed that rapid drying of agricultural product to low moisture level is critical as it creates less favourable conditions for fungal growth and proliferation, insect infestation and helps keep produce longer. Drying harvested maize to 15.5% moisture content or lower would reduce the risk of fungal growth and subsequent aflatoxin production according to Hamilton (2000). The fungal growth approaches dormant levels when moisture drops below 15% (MSU, 2005). The findings of my study are consistent with other scientists' findings as infection was not found to be significant at 10% - 15% moisture level. Increasing moisture content beyond 15% caused a significant increase in the level of infection.

The optimum conditions for growth and aflatoxin production of aflatoxins by *A. flavus* include moisture content above 14%, optimal temperatures of 28°C to 30°C and water activity of 0.83 to 0.97 (Sauer, 1987; Kumar *et al.*, 2000; Mutungi *et al.*, 2008; Morenoa *et al.*, 2009). As the fungus grows, respiration occurs releasing heat and moisture and moisture into the surrounding areas in the grain mass. This results in an increase in the moisture content and temperature of the surrounding maize, causing a hotspot. If the moisture and temperature continue to rise, the environment becomes favourable for *A. flavus*. The sisal and synthetic bags had pores that possibly allowed rapid loss of moisture

and this may have lowered the water activity below the level necessary for fungal bioactivity.

The aflatoxin content in the maize stored at Kabete and Machakos was below detectable level. The *Aspergillus flavus* used for inoculation may have been an atoxigenic strain.

# CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

The current study revealed that farmers practice poor agronomic, harvesting, storage and handling practices that create favourable conditions for fungal growth and Aflatoxin production. A large proportion of the farmers from the Eastern region selected maize seeds from previous season crop and this could act as primary source of new infections. Harvesting maize with high moisture content, poor handling and storage practices such as, drying cobs on bare ground and storage in poorly aerated rooms exposes the maize to fungal and mycotoxin contamination. Farmers in North Rift allowed further drying of their maize after stooking and their handling was more hygienic than in eastern. Most of them dried their maize on mats, polythene sheets or tarpaulins but very few dried on bare ground.

Most farmers and traders in Eastern and North Rift regions were aware about aflatoxin poisoning. The information had been disseminated by extension officers and through radio programmes. The message that had been taken to achieve avoidance of mycotoxin production was proper drying of maize but farmers were not aware that the drying should be immediately after harvesting. The farmers and traders were also not aware that proper hygiene should be maintained when harvesting, drying and handling maize.

The study shows that fungal and aflatoxin contamination in maize and maize products in the greater Machakos, Makueni and Kitui district was due to *Aspergillus flavus*. *Aspergillus* was found in the soils outside the houses and under the stores. The fungus was also isolated from dust collected around the posho mills. There is also a high likelihood that the fungal inoculum in form of sclerotia and mycelia is present in the debris from previous crop. This inoculum acts as a constant primary source of *A. flavus* which thrives very well due to the favourable conditions created by the poor agronomic, harvesting, storage and handling methods. Besides *A. flavus*, the study also revealed the presence of other *Aspergillus* species like *A. niger, A. fumigatus, A. versicolor, A.*

*clavatus, A. terreus* and *A. ochraceus* some of which are known to produce various mycotoxins that have deleterious effects on the health of humans and animals. *Aspergillus flavus* was isolated at low frequencies in maize from North Rift and the aflatoxin content in grains from this region was too low for detection. The environmental conditions such as low temperatures, adequate and well distributed rainfall and good agricultural practices in North Rift do not promote fungal growth which leads to aflatoxin production.

Soils, maize and maize products were also found to be contaminated with *Fusarium* species. The mycotoxigenic fungi were found to be widespread in Eastern and North Rift regions. The presence of *Fusarium* species such as *Fusarium proliferatum, F. subglutinans, F. verticillioides*, and *F. graminearum* in the maize and maize products implies that the maize could be contaminated with *Fusarium* mycotoxins such as fumonisins, zearalenone and trichothecenes such as deoxynivalenol (DON), diacetoxyscirphenol, T-2 and HT-2 toxins. *Fusarium* and *Aspergillus* species were isolated in maize and maize products, soils and posho mill dust. This implies that there are fungal spores in the soils and contact of maize cobs with the soil during harvesting and drying predisposes maize to fungal growth and mycotoxin production. The fungi are also known to survive as saprophytes in crop debris and eliminating debris of the previous crop could reduce the primary fungal inoculum. The posho dust which was also found to contain *Aspergillus* and *Fusarium* fungal species should be discarded with utmost caution to avoid spreading it into the environment.

Maize and maize products from Eastern region were found to be contaminated with aflatoxin content above the allowed limit in Kenya of 10 μg/kg. Presence of mycotoxins in the maize poses a health risk to the local people who are continually exposed to low concentrations of mycotoxins in the food.

The study did not find any significant difference between synthetic and sisal bags. High moisture above 15% was found to encourage fungal growth and therefore, the grains stored in these bags should be at 15% moisture content or lower. Polythene bags were

however found to encourage fungal growth and deterioration of the grains in terms of discolouration and mouldiness at moisture content of 13% and above. Percentage kernel infection with *Aspergillus flavus* was significantly affected by storage materials and interaction between storage materials. Maize stored at 10% moisture content had no significant difference on percentage kernel infection by *Aspergillus flavus*. This implies that so long as the maize has moisture content below 13%, it can be stored using any type of storage container.

### 6.2 Recommendations

The following recommendations are proposed based on the findings of this study:-

1. Further research focusing on fumonisins, zearalenones and trichothecenes should be conducted to determine the types, levels and distribution in the country.

2. Further research should be conducted on the toxicity status of *Aspergillus flavus* strains in Eastern Province.

3. Constant monitoring of the presence of *A. flavus* and aflatoxin in maize sampled (from farm stores and markets) from time to time even when everything appears normal. Regional testing labs with trained personnel should be set up to facilitate this monitoring.

4. Consignments of imported maize and maize from local traders destined for milling should be tested for the presence of mycotoxins at source to avoid exposing the populace to chronic poisoning

5. Strict adherence to mycotoxin standards in the agricultural food products

6. Awareness campaigns and trainings should be conducted for farmers, traders and processors on good agronomic, harvesting, storage, handling, transportation and processing practices. Strict hygiene standards should be enforced by all stakeholders handling agricultural products meant for food.

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# APPENDICES

**Appendix 1: Questionnaire used to gather information on agronomic practices, harvesting, drying, storage materials, structures and duration, processing and form of consumption of maize and maize products in Eastern and North Rift regions during the long and short rainfall seasons of 2008.**

1. Name of the farmer.....

2. District.....Division.....

Location.....Sub-location.....

Village..... Agro-ecological Zone (AEZ).....

3. What is the acreage under maize?

4. Do you grow maize as a pure stand?

1. Yes ☐ 2. No ☐

If no what type of crops do you mix with maize in the same farm.....

.....

5. Where do you acquire your seeds from?

1. stockist ☐ 2. Market ☐

3. Select own seed ☐ 4. From a neighbour ☐

5. Others (specify....) ☐

6. When do you harvest your maize?

1. At physiological maturity ☐ 2. Completely dry maize ☐

7. Under what environmental conditions did your harvest your maize?

1. Dry conditions ☐ 2. Wet conditions ☐

8. How do you harvest your maize?

1. With sheath ☐ 2. Without sheath ☐

3. Stooking ☐ 4. Others (specify) ☐

9. How do you dry your maize?

1. In cobs ☐ 2. Shelled grains ☐

10. Where do you dry the maize?

1. Bare ground ☐ 2. On tarpaulin/ mats ☐

3. on tarmac road surface ☐ 4. others (specify) ☐

11. Where do you store the maize?

1. House ☐ 2. Crib ☐

3. improved granary ☐ 4.traditional Stores ☐

12. What type of walls does the store have?

1. Stone / brick ☐ 2. Timber ☐

3. Iron sheets ☐ 4. Mud ☐

13. What type of roof does the store have?

1. Iron sheet ☐ 2. Grass thatched ☐

3. Tiles ☐

14. What type of floor?

1. cemented ☐ 2. Earth bare ground ☐

15. How do you store your maize?

1. In paper bags ☐ 2. In sisal bags ☐

3. In synthetic bags ☐ 4. In containers ☐
(metallic / earthen/plastic / specify others)

16. Where do you place the maize?

1. On the floor ☐ 2. On wooden racks ☐

3. On raised platform in the store ☐

17. How long do you normally store the maize?

1. Less than 2 weeks ☐ 2. One month ☐

3. Between 1 and 4 months ☐ 4. More than 4 months ☐

18. In which form do you consume the maize?

1. Githeri ☐ 2. Muthokoi ☐

3. Ugali ☐ 4. Porridge ☐

5. Others (specify) ☐

19. Where do you get your maize meal?

1. Buy packed flour from shops ☐ 2. Grind at home ☐

3. Own maize taken to posho mill ☐ 4. Bought from open air market ☐

20. Where do you get your muthokoi from?

1. Processed at home using mortar and pestle ☐ 2. Bought from the shops ☐

3. Own maize processed by posho mill. ☐ 4. Bought from open air market ☐

21. Do you source maize from elsewhere?

1. Yes ☐ 2. No ☐

If yes, state the source

1. Market ☐ 2. Relief food ☐

3. Neighbours ☐ 4. Others (specify) ☐

22. Have you ever heard about aflatoxin poisoning?

1. Yes ☐ 2. No ☐

23. Do you know what measures can be taken to avoid aflatoxin poisoning?

1. Yes ☐ 2. No ☐

24. What measures need to be taken?

1. Drying maize properly. ☐ 2. Washing maize properly. ☐

2. Sorting maize to remove mouldy grains ☐ 4. Others (specify) ☐

25. What was the source of information?

1. Extension officer ☐ 2. Radio ☐

3. Newspaper ☐ 4. Neighbours ☐

5. Others ☐

26. Request for samples

27. Vote of thanks

**Appendix 2: Questionnaire used to gather information on sourcing, transportation, drying processing, handling and storage of maize and maize products from traders in Eastern and North Rift regions during short rainfall season of 2008.**

1. Name of the trader.....

2. Division.....Division.....

Location.....Sub-location.....

Shopping Centre.....

3. Where do you buy your maize from?.....

4. How is maize transported to your premises?

1. Lorry / pick-ups ☐ 2. Push-carts ☐

3. Bicycles ☐ 4. Passenger vehicles ☐

5. How do you determine that maize is properly dried?

1. Moisture meter ☐ 2. Personal experience ☐

3. Other methods (specify) ☐

6. Do you dry your maize further?

1. Yes ☐ 2. No ☐

If yes, where do you do the Drying?

1. On tarpaulin ☐ 2. On cemented floor ☐

3. Tarmac road surface ☐ 4. On bare earth ground ☐

5. Others (specify) ☐

7. How do you store your maize?

1. Sisal bags ☐ 2. Synthetic bags ☐

3. Metallic containers ☐ 4. Plastic containers ☐

5. Others (specify….) ☐

8. Where do you store the maize?

1. Cemented floor ☐ 2. Earth floor ☐

3. Wooden platform ☐

9. What are the roofing materials for the structure where the maize and maize products are stored?

1. Thatching grass ☐ 2. Iron sheets ☐

3. Tiles ☐ 4. Others (specify) ☐

10. What materials have been used for the walls?

1. Stones / bricks ☐ 2. Mud

3. Timber ☐ 4. Iron sheets

5. Others (specify. ...) ☐

11. How long do you store the maize?

1. Less than 7 days ☐ 2. Less than 4 weeks

3. Between 1 and 6 months ☐ 4. More than 6 months

12. Do you notice any pests on stored maize?

1. Yes ☐ 2. No

If yes, which pests?

1. Weevils ☐ 2. Larger grain borer

3. Rats ☐ 4. Others

13. How do you control them?

1. Trapping ☐ 2. Dusting

3. Spraying ☐ 4. Fumigating

5. Others (specify.....) ☐

14. What other maize products do you sell?

1. Muthokoi ☐ 2. Flour

3. Others (specify.....) ☐

15. What is your source of muthokoi?

1. Buy directly from posho mill ☐ 2. Middlemen

3. process own maize using posho mills ☐

16. What is the source of your maize flour?

1. Suppliers ☐ 2. Middlemen

3. Posho mills ☐ 4. Others

17. Have ever heard about aflatoxin poisoning?

1. Yes ☐ 2. No

18. What measures do you undertake to avoid it?

1. Proper drying of maize ☐ 2. Avoid selling mouldy maize

19. Request for maize, maize flour and muthokoi samples

20. Vote of thanks

## Appendix 3: Composition of agar media used in fungal isolation and identification

a) Spezieller Nahrstroffarmer Agar (SNA) Medium

| | |
|---|---|
| potassium dihydrogen phosphate ($KH_2PO_4$) | 1g |
| Potassium nitrate ($KNO_3$) | 1g |
| Magnesium sulphate ($MgSO_4.7H_2O$) | 0.5g |
| Potassium chloride (KCl) | 0.5g |
| glucose | 0.2g |
| sucrose | 0.2g |
| Oxoid Agar No. 3 | 20g |
| Distilled water | 1000mls |

Dissolve all the ingredients except agar in the distilled water and adjust $P^H$ to 6.0 to 6.5. Add agar and dissolve. Autoclave at 121°C, for 15 minutes.

b) Czapek Dox Agar medium

| | |
|---|---|
| Sucrose (analar) | 30g |
| Oxoid agar No.3 | 20g |
| Czapek stock solution A | 50mls |
| Czapek stock solution B | 50mls |
| Distilled water | 900mls |

czapek stock solution A

| | |
|---|---|
| Sodium nitrate ($NANO_3$) | 40g |
| Potassium chloride (KCL) | 10g |
| Magnesium sulphate ($MgSO_4.7H_2O$) | 10g |
| Ferrous sulphate ($FeSO_4.7H_2O$) | 0.2g |

czapek stock solution B

| | |
|---|---|
| Dipotassium hydrogen phosphate ($K_2HPO_4$) | 20g |
| Distilled water | 1000mls |

Dissolve agar in distilled water using a double saucepan, add sucrose and stock solutions prior to autoclaving at 121°C for 20 minutes