DECLARATION

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

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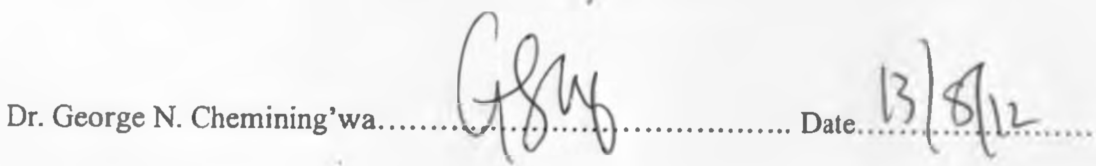

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DEDICATION

To my husband Samuel Muthoga and our daughter Sandra Wanjiku who was born during my studies.

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ABSTRACT

Maize is the staple food for the majority of households in East and Central Africa and the per capita consumption in Kenya is about 97 kg per year per person. However, the recurrent outbreaks of aflatoxin poisoning in Kenya as exemplified by outbreaks in 1981, 2004, 2006, 2007, 2009 and 2010 poses a major food safety concern. This study was therefore conducted to evaluate susceptibility of maize varieties to one of the main mycotoxin producing fungi, *Aspergillus flavus* and effectiveness of grain-inhabiting antagonistic micro-organism in the management of *Aspergillus flavus*.

Field trials were conducted over two growing cropping seasons in 2008 and 2009 at Mwea and Waruhiu. Cobs of 16 maize varieties: Duma 43, Dekalb 8031, Dry Highland 01, Dry Highland 02, Dry Highland 04, Panner 77, Panner 7–19M, Panner 4M, Panner 67/5243, Hybrid 513, Hybrid 515, Hybrid 516, Katumani, Hybrid 614, Pioneer 3253 and Katumani composite B were inoculated with *A. flavus* through the silk channel. Parameters assessed in the field included, number of days to 50% silking, days to maturity, cob husk coverage and *Aspergillus* rot on cobs. Clean grain samples (500g) were adjusted to 10, 13.5, 15, 17, 20% and *A. flavus* re-isolated after 7, 28 and 84 by plating on Czapek Dox Agar and aflatoxin B_1 content by enzyme linked- immunosorbent assay (ELISA). Growth inhibition of *A. flavus* by grain inhabiting antagonistic fungi in culture was evaluated by paired culture of *A. flavus* with *Fusarium oxysporum, Trichoderma* sp, *Alternaria* sp, *A. niger*, and *A. terreus*. Fungi showing growth inhibition of *A. flavus* were co-inoculated with *A. flavus* on to maize grain adjusted to 17% moisture content and incubated for 28 days. *Aspergillus flavus* was re-isolated by plating on Czapek Dox Agar and aflatoxin B_1 content determined by ELISA method.

Varieties differed significantly in days to 50% silking and maturity. Variety Katumani composite B silked and matured earliest at 60 and 87 days respectively, while H614 silked and matured at 87 and 137 days respectively. The varieties differed in *Aspergillus* rot rating, with Katumani having the highest rating of 17%, while Hybrid 614 and Duma 43 had the lowest *Aspergillus* infection of 2.5%. Kernels infection with *Aspergillus flavus* was significantly different among the varieties, with Panner 77m having a higher infection of 31.1% and Katumani composite B, which had infection of 16.4%. Higher moisture levels above 15% and longer grain incubation periods (7days to 28 days) resulted in increased *Aspergillus flavus* kernel infection. Varieties significantly ($P \leq 0.05$) differed in aflatoxin B_1 content with H516 having the highest aflatoxin content of 462 μg/kg compared to Katumani composite B which had the least aflatoxin content of 14 μg/kg. Fungal antagonists significantly differed in reducing *A. flavus* colony diameter with *Trichoderma* and *Alternaria* showing the highest (30.7%) and the least (12%) reduction respectively. The highest reduction of *A. flavus* was observed in different maize varieties co-inoculated with *Trichoderma* while the lowest reduction of *A. flavus* was observed in different maize varieties inoculated with *A. flavus* alone.

The results indicated that all the maize varieties were susceptible to *A. flavus* ear rot and aflatoxin contamination, although a few showed some tolerance. Grain inhabiting fungi showed promise in reducing growth of *A. flavus* and aflatoxin accumulation in grain. Further research on biological management of *A. flavus* and aflatoxin contamination is required. In addition, farmers in aflatoxin poisoning-prone areas should be encouraged to grow maize varieties that are less susceptible to *A. flavus* and associated aflatoxin contamination.

CHAPTER ONE: INTRODUCTION

1.1 Maize production and consumption in Kenya

Maize is by far the most important food crop in Kenya, being grown as both a subsistence and commercial crop (MOA, 2005). Per capital maize consumption in Kenya is estimated to be 97 kg per year per person (MOA, 2005). It is planted on 1.5 million ha, which is more than 30% of the arable land, and is widely distributed throughout the six major agro-ecological zones. The average annual production is estimated at 2.4 million tonnes (CIMMYT, 2008). There are six maize growing agro-ecological zones in Kenya, which are defined by elevation, total rainfall and length of the growing season and maturity period of the adapted maize cultivars (FAO, 2000).

Moving from east to west, are the humid coastal lowland tropics zone, at the coast are the dry mid-altitude and the dry transitional zones which are found between the mid-altitude and highlands tropics zones (CIMMYT, 2008). These zones are characterized by low grain yields below 1.5 ton/ha and although they cover 29% of the maize production areas in Kenya, they produce only 11% of the total maize production annually. In central and western Kenya, there are the highland tropics, bordered at the west and east by the mid-altitude moist and the mid –altitude transitional zones. These zones cover about 30% of the maize area, have average grain yield of more than 2.5 ton/ha, and produce about 80% of the maize annually (De Groote *et al.*, 2003). Productivity of maize as a national staple food item declined in 2008 to 14.7 bags per hectare from 20.1 bags per hectare in 2007 (MOA 2009). The trend is attributed to the high cost of farm inputs including fertilizers and diesel. Production fell by 19% from 32.5 million bags in 2007 to 26.3 million bags (CIMMYT, 2008). It is also almost 10 million bags from the highest level of 36.1 million bags recorded in 2006 (MOA, 2009).

Total maize production and maize yield per unit area has been affected by both biotic and abiotic factors. Abiotic stress factors include soil moisture stress, unreliable rainfall, and declining soil nutrients (Windham *et al.*, 1999). According to De Vries and Toenniessen (2001), drought and low soil fertility contribute greatly to the low yields realized and losses of up to 13% have been recorded in Eastern and Southern Africa. Among the biotic constraints, arthropod pests are a major contributing factor to low maize yields. Insect pests are more damaging in the tropical than in temperate environments, because the climatic conditions are conducive for accelerated insect development with multiple and overlapping generations leading to high infestation level (CIMMYT, 2008). The two most damaging insect species for maize are the maize weevil (*Sitophilus* sp) and the larger grain borer (*Prostephnus* sp) (Aish *et al.*, 2004). The maize weevil is ubiquitous and first colonizes maize ears in the field (Aish *et al.*, 2004). Weeds, especially the parasitic weeds, lack of improved seed alongside diseases, are other constraints of maize production (Odour, 2006).

Fungi are one of the most important factors contributing to deterioration of quality in stored maize (Fandohan *et al.*, 2003). It is reported that fungi can cause about 50-80% of damage on farmers 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 (Fandohan *et al.*, 2003). 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). The direct impact of mycotoxins on the staple product quality constitutes an important danger for human health (Fandohan *et al.*, 2003)

1.2 Problem statement and justification

Outbreaks of mycotoxicosis in Kenya received publicity in 1978 when large numbers of dogs and poultry died (Manwiller, 1986). The government Chemist department collected 336 samples of food and feed of which 52 had aflatoxin levels exceeding 150μg/kg which was much higher than Kenya legal limit of 10μg/kg. The highest levels of up to 300μg/kg were found in dog meal (Manwiller, 1986). The bumper harvest of 1977-78 exceeded proper storage facilities. In 1981, 12 people died in Eastern province after consuming contaminated grain (Manwiller, 1986).

In April 2004 in Kenya one of the largest and most severe outbreaks of acute aflatoxicosis documented worldwide was reported (Centre for Disease Control and Prevention, 2004). The outbreak resulted in 125 deaths and covered more than seven districts encompassing an area of 40,149 km^2 out of which 89% of the population resided in Makueni, Kitui, Machakos and Thika districts. Majority of the affected maize was locally grown (Lewis et al., 2005). In 2005, another outbreak claimed 16 lives in Eastern province (KEPHIS, 2006). Two fatal and one hospitalization incidences of aflatoxin poisoning were reported in Machakos in 2006 (MOA, 2008). In 2008, six people died in Eastern province and in 2010 an outbreak claimed three lives in Kitui and Makueni. Recurrent outbreaks of aflatoxin poisoning in Eastern province as exemplified by the outbreaks in 1981, 2004, 2006, 2007, 2008 and 2010 poses a major food safety concern.

Therefore, the overall objective of this study was to evaluate susceptibility of maize varieties to *Aspergillus flavus* and grain-inhabiting antagonistic micro-organism in management of *Aspergillus flavus.*

The specific objectives were

1) To determine the susceptibility of maize varieties to *Aspergillus flavus* infection and aflatoxin contamination.
2) To determine the effectiveness of maize grain-inhabiting antagonistic micro-organisms in managing *A. flavus* infection and aflatoxin accumulation.

CHAPTER TWO: LITERATURE REVIEW

2.1 Mycotoxins associated with maize

Mycotoxins are fungal metabolites that can contaminate agricultural products and threaten food safety. The Food and Agriculture Organization estimates that mycotoxins contaminate 25% of agricultural crops worldwide (Smith *et al.*, 1994). Mycotoxins associated with maize include aflatoxins, trichothecene, fumonisis, ochratoxins and zearalenone (Dorner et *al.*, 2004).

2.1.1 Aflatoxins

Aflatoxins refer to a group of four mycotoxins B1, B2, G1 and G2 produced primarily by two closely related fungi, *A. flavus* and *Aspergillus parasiticus*. Strains of *A. flavus* show a great variation in their ability to produce aflatoxins. Toxigenic strains of *A. flavus* typically produce two aflatoxins, B1 and B2, but most strains of *A. parasiticus* could produce all the four toxins (Dorner *et al.*, 2004). Since aflatoxins are potential carcinogens, their quantity in food and feed is closely monitored and regulated in most countries. For example, the European Union has a maximum level of 2µg/kg for B1 and 4µg/kg for total aflatoxins in crops (Edwards, 2004). The value of feed lost to aflatoxin is $225 million per year out of the $932 million lost due to all mycotoxins in the United States (Betran and Isakeit, 2004).

Aflatoxins can affect a wide range of commodities including cereals, oilseeds, spices, tree nuts, milk, meat, and dried fruit (Strosnider *et al.*, 2006). Maize and groundnuts are major sources of human exposure because of their greater susceptibility to contamination and frequent consumption through out the world (Bankole and Mabekoje, 2004). Aflatoxins are most prevalent in latitudes between 40°N and 40°S of the equator, but the greatest health risk

lies within developing countries in tropical regions, which rely on these commodities as their staple food source (Bankole and Mabekoje, 2004). Contamination can occur at any stage of food production, from pre-harvest to storage (De Groote *et al.*, 2003). Factors that affect aflatoxin contamination include the climate of the region, the genotype of the crop planted, soil type, minimum and maximum daily temperatures, and daily net evaporation (Brown *et al.*, 2001; Bankole and Mabekoje 2004, Fandohan *et al.*, 2005).

Aflatoxin contamination poses a risk to human health and has been identified as a major constraint to trade in Africa (Pingali, 2001). For instance, mean total aflatoxins in 45.0% and 38.8% of samples of maize collected from farmers in Nigeria and Benin had 200 and 105µg /kg, respectively (James, 2003). Total aflatoxins in 15 of 16 samples of Kenkey ranged from 6.15 to 196.1µg/kg. Also, a survey of aflatoxins in food supplies in Tanzania showed high contamination of these toxins in maize. In this survey, 11.2% of 472 samples of maize contained total aflatoxins at levels upto 69.3µg/kg (James, 2003).

2.1.2 Ochratoxins

Ochratoxins are produced by several fungal strains of the *Penicillium* and *Aspergillus* species. Ochratoxin A and its methyl esters are the toxic members of the group (Cole *et al.*, 1981). It is only Ochratoxin A that is widespread as a natural contaminant (Krogh, 1997). The main toxin in this group is found in wheat, corn, and oats having fungal infection and in cheese and meat products of animals consuming ochratoxin-contaminated grains (Aish *et al.*, 2004). *Aspergillus ochraceus* is found on dry foods such as dried and smoked fish, soybeans, garbanzo beans, nuts, and dried fruit. *Aspergillus carbonarius* is the major pathogen in grapes and grape products including, wines, and vinegars (Sage *et al.*, 2004). Although

reported to occur in foods around the world, the main regions of concern are Europe and Africa (Binder, 2007). The Joint Expert Committee on Food Additives of the Food and Agriculture Organization of the United Nations and the World Health Organization (JECFA , 2001) presented data indicating that cereals, wine, grape juice, coffee, and pork are the major sources of human ochratoxin exposure, at levels of 3%, 5%, 7%, 21% and 58% of total ochratoxin intake respectively. Levels reported range from 100 to 700μg/kg in cereals, 0.3 to 9μg /l in European wines, 0.17 to 1.3μg /kg in coffee and 0.15 to 2.9μg/kg in pork (Sage *et al.,* 2004). Ochratoxin presence in European wines is a relatively recent concern, with red wines typically containing higher ochratoxin levels than rosé or white wines (Binder, 2007).

2.1.3 Trichothecenes

Trichothecenes are a large group of mycotoxins produced by various species of fungal genera that include *Fusarium, Myrothecium, Phomopsis, Stachybotrys, Trichoderma* and *Trichothecium* (Binder, 2007). The most prevalent occurring mycotoxins of this group are the B-type trichothecenes as deoxynivalenol (DON), nivalenol (NIV), 3-or 15 acetyl-deoxynivalenol (Ac DON), and the A-type toxins T-2 toxin and HT-2 toxins (Binder, 2007).

Deoxynivalenol is generally found in cereals such as wheat, barley, oats, rice, rye and maize (Instanes and Hetland, 2004). DON is produced by *Fusarium crookwellense, Fusarium culmorom* and *Fusarium graminearum* (Binder, 2007). Deoxynivalenol is the most prevalent of the trichothecenes in human foods, although related mycotoxins such as 3-acetyl DON, T-2 toxin, and nivalenol also occur with some regularity. The Council for Agricultural Science and Technology (CAST, 2003) estimated that the annual US cost due to DON contamination of human food crops is $637 million. The CAST model included 727 samples of wheat and corn foods from data gathered from FDA and private surveys conducted during 1995 to

1997. Food losses were based on samples with >1μg/kg DON. DON contamination was greater than 0.001μg/kg in 6.9% of corn samples and 12.4% of wheat samples.

The DON toxins contaminate the produce before harvest or after harvest (CAST, 2003). Edwards (2004) reviewed the environmental conditions that favor DON accumulation in food crops. Minimal tillage, nitrogen fertilizers, moisture content of 22-25°C, application of azoxystrobin or glyphosate and production of grains where maize had been grown the previous year were the main risk factors associated with increased DON accumulation. Plant factors that affect trichothecenes biosynthesis include xantho-toxin and other furanocoumarins induced by a wide variety of plants (Desjardin *et al.*, 1993). Plant enzymes have also been suggested to metabolise DON (Merril *et al.*, 1996). Biological and insect control shows promise, in laboratory environments, for reducing DON contamination levels. Mycotoxin T-2 and HT-2 generally occur together in the infected cereal products (Eriksen and Pettersson, 2004). They are generally found in various cereal crops such as wheat, corn, barley, oats and rye (Desjardin *et al.*, 1993). They are usually produced by *Fusarium acuminaitum, Fusarium equiseti, Fusarium poae, Fusarium sporotrichioides* of the sporotrichioides section is the most producer of T-2 and has no known telemorph (Yarzar and Omurtag, 2008). It is basically a saprophyte species and is especially nassociated with cereals left in the fields after normal harvest. It grows at -2°C to 35°C and only at high water activities (Creppy, 2002).

Nivalenol is mainly produced by *Fusarium cerealis* and *F. poae*, but isolates of *F. culmorum* and *F. graminearum* are also able to produce nivalenol (Eriksen and Pettersson, 2004). It

occurs in various cereals such as wheat, corn, barley, oats and rye (Ito *et al.*, 1986). It occurs more often in years with dry and warm growing seasons (Yarzar and Omurtag, 2008).

2.1.4 Zearalenone

Zearalenone (ZEA) is an oestrogenic, white crystalline fungal metabolite produced by *Fusarium* species. It was isolated from a culture of *F. roseum* (Stob *et al.*, 1962). Other *Fusarium* species such as *F. gibbosum*, *F. lateritium*, *F. moniliformae*, *F. nivale* and *F. oxyporum* also produce this toxin (Cole and Cox, 1981). The highest incidence and levels of zearalenone in nature are seen in corn, oat, sorghum, sesame, hay and mixed animal feed (Scheidegger and Payne, 2003). ZEA production is favored by high humidity and low temperature conditions (Cole and Cox, 1981). It attracted recent attention due to concerns that environmental estrogens have the potential to disrupt sex steroid hormone functions (Mcerlean and Christensen, 1952). Occasional outbreaks of zearalenone mycotoxicosis in livestock are known to cause infertility. Alternatively, derivatives of zearalenone are used in some livestock feeds for growth promotion as alternatives to the more potent and controversial synthetic estrogen, diethylstilbestrol (Miller *et al.*, 1991).

The average human intake of zearalenone was estimated to be approximately 0.02µg/kg on the basis of limited data obtained in Canada, the United States, and Scandinavian countries, but it is likely that intakes are greater in countries from the regions of the world having less well-controlled grain storage systems (Pitet, 1998). This dose range is difficult to extrapolate to likely human exposure because no human bioavailability estimates are available (Miller *et al.*, 1991). With estimated human intake of approximately 1 to 2 µg per person, however,

occurrence of blood or tissue concentrations remotely close to 0.1 M approximately 30µg/L seems extremely unlikely (Miller *et al.*, 1991).

2.1.5 Fumonisins

Fumonisins are produced by the maize pathogens *Fusarium proliferatum* and *Fusarium verticillioides* and at very low levels by *Alternaria* in black end stem rot in tomatoes, asparagus, and garlic (Scheidegger and Payne, 2003). The fumonisins are highly water-soluble unlike all other food mycotoxins because they do not have an aromatic structure or a unique chromophore for easy analytical detection (Desjardins *et al.*, 1996). They are primary amines with 2 tricarballylic groups, which contribute to their water-solubility. Fumonisin synthesis is known to involve acetate precursors and alanine and several of the genes involved in fumonisin synthesis have been identified as a cluster on chromosome 1 in *F. verticillioides* (Desjardins *et al.*, 1996).

Bankole and Mabekoje, (2004) detected fumonisins in 92.5% of maize samples from eastern and southern Africa including nine samples from Tanzania suggesting a widespread occurrence of the toxins in that region. Fumonisin content in the US corn was relatively high between 1988 and 1991, but has been low <0.5 µg/kg in recent years. There are a few reports of high fumonisin levels up to 0.15µg/kg in homegrown corn consumed in China and South Africa. Most commercial foods contain 0.5µg/g or less due to low fumonisin levels in corn and ingredient quality control (Shephard, 1996). Merill *et al.* (1996) showed that water activities and temperature between 25 °C and 30 °C increased colonization and production of FB_1 and FB_2.

2.2 Aflatoxicosis in Kenya

In Kenya, outbreaks of acute aflatoxicosis from highly contaminated cereals have been documented (CAST, 2003). Outbreaks accompanied by high mortality in dogs and poultry were reported in 1978 (Manwiller, 1986). In surveys of aflatoxins in animal feed, Muraguri *et al.* (1981) found 34.8% contamination in 316 samples of animal feed while Gathumbi (2001) found a 35.6% contamination rate in 90 samples of poultry feed.

In 1981, 20 patients with hepatitis, 12 of whom died, were admitted to three hospitals in Machakos district of Kenya (Ngindu *et al.,* 1982, James 2003). Two families, from which 8 of 12 sick members died, were eating maize which contained aflatoxin as much as 12 000 µg/kg. Liver tissue at necropsy contained up to 89µg/kg of this mycotoxin. Probably most or all of the hepatitis cases were caused by acute aflatoxin poisoning (Ngindu *et al.,* 1982).

Western Kenya has repeatedly recorded high levels of stunting in children, an aspect often positively correlated with long-term ingestion of sub lethal doses of aflatoxin (Gong *et al.,* 2004). In 2004, a survey was carried out to obtain baseline data on levels of aflatoxin in peanuts from major production regions in western Kenya (Lewis *et al.,* 2005). A total of 384 and 385 samples from Busia and Homabay districts, respectively, were obtained and analyzed for aflatoxin content. Levels of aflatoxin ranged from 0 to 2688 and 752 µg/kg in samples from Busia and Homa Bay respectively. Of 769 samples, 87.01% contained 4µg/kg of aflatoxin, 5.45% were in the range ≥4 and 20µg/kg, while 7.54% exceeded Kenya's current legal limit of 10µg/kg (Mutegi *et al.,* 2009).

Aflatoxin poisoning has continued to be a major problem in various part of the country during the last decade (Table 2.1). The 2004 aflatoxin-poisoning outbreak in Kenya was the largest and most severe documented worldwide (Center for Disease Control and Prevention,

2004). Aflatoxin poisoning was associated with eating home grown maize stored under damp conditions (Lewis *et al.*, 2005). The outbreak covered more than seven districts and resulted in 317 case-patients and 125 deaths. In 2005, another outbreak in Makueni and Kitui districts affected 75 people with 32 deaths (KEPHIS, 2006).

Table 2.1: Cases of aflatoxin poisoning reported in Kenya from 1981 to 2011

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, 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
2011	-	-	North Eastern, Eastern and Coast

Source; Lewis *et al.*, 2005; KEPHIS, 2006, MOA, 2008, Daily nation 2010, Daily nation 2011

In 2006, another outbreak claimed 28 lives out of the 78 cases reported in Eastern province (MOA, 2008). The concentrations of aflatoxin B1 in maize was found to be as high as 4400 μg/kg, which is 440 times greater than the 10 μg/kg legal limit. Between 7th and 15th May 2007, 21 people died in Eastern province due to aflatoxin poisoning (MOA, 2008). Report by health personnel indicated that 450 kilograms of food contaminated with aflatoxin

was seized from households in Mutha and Ikutha divisions of Kitui district (MOA, 2008). In 2008, the Ministry of Agriculture reported two fatal cases out of the six reported (MOA, 2008).

In 2009, 31,781 bags of maize worth 65 million Kenya shillings held by the National Cereals and Produce Board at Ishiara Depot in Embu district were found to contain high levels of aflatoxins rendering maize unfit for human consumption (Daily Nation, 2010). In 2009, 117 MT of maize was rejected by World Food Programme due to aflatoxin contamination at Bura irrigation scheme. The maize was to be destroyed by burning but National Environmental Management Authority said that the steeping method which involves immersing the maize in acid after which it would be used for industrial purposes was the best method (Daily Nation, 2010).

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

In 2011, 60,000 school going children faced a health risk after consuming unimix contaminated with aflatoxins. The government recalled 362 metric tonnes of Unimix supplied as famine relief from schools in parts of North Eastern, Eastern and Coast region (Daily nation, October 2011).

2.3 Health Risks associated with mycotoxins

Mycotoxins encompass a wide spectrum of different chemicals and they may affect many target organs and systems, notably the liver, kidney, the nervous system, the endocrine system, and the immune system (Kuiper-Goodman *et al.*, 1996). Aflatoxins have been reported to be associated with exacerbation of the energy malnutrition syndrome kwashiorkor in children and vitamin A malnutrition in animals (CAST, 2003). In addition to being hepatotoxic, aflatoxin causes significant growth faltering and is strongly immune-suppressive at weaning.

Similar effects have been reported in human populations in a few African countries such as Ghana (CAST, 2003). It has been recently shown that 99% of all children weaned from mother's milk to maize-based diets in Benin and Togo had aflatoxin in their blood, indicating ingestion of aflatoxin-contaminated food (Eaton and Gallagher, 2004). Aflatoxin exposure in children was associated with stunting and being underweight (IARC, 1993).

Chronic exposure to aflatoxins is associated with impaired immunity and liver cancer which is the third most common cause of death in Africa (Williams *et al.*, 2004). Chronic toxicity results from long-term exposure to low aflatoxin concentrations. There is some observed carcinogenicity, mainly related to aflatoxins B1 (CAST, 2003). Liver damage is apparent due to the yellow colour that is characteristic of jaundice, and the gallbladder becomes swollen. Immuno-suppression is due to the reactivity of aflatoxins with T-cells, decrease in Vitamin K activities, and a decrease in phagocytic activity in macrophages (CAST, 2003). These immuno-suppressive effects of aflatoxins predispose the animals to many secondary infections due to other fungi, bacteria and viruses (McLean, 1995). A study by Azzi-

Baumgartner *et al.* (2005) demonstrated that males were more likely to die from aflatoxicosis, in spite of eating similar quantities of maize as females.

In addition, there may be an interaction between chronic mycotoxin exposure and malnutrition, impaired growth, and diseases such as malaria and HIV/AIDS (Gong *et al.*, 2003, 2004). In a recent study in Ghana, higher levels of aflatoxin B1-albumin adducts in plasma were associated with lower percentages of certain leukocyte immunophenotypes (Jones *et al.*, 1981). The most significant adverse effect of ochratoxins is their connection with human kidney disease known as Balkan endemic nephropathy (Cole *et al.*, 1981). It's now estimated that 20,000 people are affected with this disease and approximately half the sufferers die within two years (Miller, 1994).

Several thousand people were affected by gastrointestinal distress in an incident in the Kashmir valley of India in 1987 (Gong *et al.*, 2003). Ninety-seven reported feelings of fullness and mild to moderate abdominal pain within 15 min to 1 hour after consuming their breakfast or evening snack (Bhat and Miller, 1991; Gong *et al.*, 2003). Other reported symptoms including throat irritation, diarrhea, vomiting, blood in the stools, and facial rash (Bhat and Miller, 1991). Increased incidence of upper respiratory tract infections was reported in children who had consumed the wheat bread for more than a week.

Fumonisins have been shown to be hepatocarcinogenic in male rats and female mice and nephrocarcinogenic in male rats (Pingali, 2001). Fumonisins have also been associated with the high incidence of human oesophageal cancer in some regions of South Africa and China (Sydenham *et al.*, 1990). Recent findings suggest that they might increase the risk of neural

tube defects in populations consuming large amounts of fumonisin-contaminated maize (Missmer *et al.*, 2006).

Zearalenone has strong hyper-estrogenic effects, which result in impaired fertility, stillbirths in females and a reduced sperm quality in male animals (Cole *et al.*, 1995). Due to its structural similarity to estradiol it is able to bind to estrogen receptors in mammalian target cells, so that it is classified by some as non steroidal estrogen, myco-estrogen, or phyto-estrogen. Though the biological potency of zearalenone is high, actual toxicity is low (Shier, 1998).

The average human intake of zearalenone was estimated to be approximately 0.02µg/kg on the basis of limited data obtained in Canada, the United States, and Scandinavian countries, but it is likely that intakes are greater in countries from the regions of the world having less well-controlled grain storage systems (Wood, 1982). Genotoxicity is a reported concern with respect to zearalenone. Although this estrogenic compound showed no mutagenicity in Ames tests 1 to 500µg zearalenone/agar plate, the substance induced chromosomal anomalies in some lymphocyte, oocyte, and kidney cell cultures when present within a range of 0.1 to 20 µM (Stopper *et al.*, 2005). With estimated human intake of approximately 1 to 2µg per person, however, occurrence of blood or tissue concentrations remotely close to 0.1 M seems extremely unlikely.

2.4 Factors that influence mycotoxin contamination

Factors affecting fungal growth and mycotoxin contamination of feed and food include environmental, socio-economics and food production. Genotypes, agronomic practices,

drought, soil types, and insect activity are important in determining the likelihood of pre-harvest contamination (Cole *et al.,* 1995).

Environmental conditions especially humidity and temperatures favour proliferation resulting in contamination of food and feed (Chakrabarti, 1986). In warm climates, moulds occur and produce aflatoxins in drought stressed maize and groundnuts (Binder, 2007). Prolonged drought during the growth season favor the development of *A. flavus*, restrict development of competitors and inhibit normal pollination in maize plant, often leading to an increase in the extent and amount of aflatoxin contaminant (Stack and Carlson, 2006; Wrather and Sweets, 2007). The mould in residues increases as temperatures increase and release of spores may increase during and after rains thus distributing fungus over wide distances causing epidemics (Binder, 2007).

Infection in the field is also favored by high moisture conditions (Chakrabarti, 1986). At 18-19% moisture content *A. flavus* may grow quite rapidly in maize (Sauer, 1987). Fungal growth and aflatoxin production continue after harvest under high moisture and warm temperature and the process is enhanced if drying is delayed (Kumar *et al.,* 2006). Developing and maturing peanuts are not susceptible to colonization by *A. flavus* and *A. parasiticus* until water activity begins to decrease in response to late-season drought conditions with increased soil temperature (Dorner *et al.,* 1989). Maintaining high kernel water activity until the time of harvest maintains the natural defence mechanism of peanuts against growth by aflatoxigenic fungi, even if fungal invasion occurs. After peanuts are dug and harvested, contamination can be prevented by rapidly drying peanuts to or below a water activity of 0.83 that cannot support aflatoxin production (Diener *et al.,* 1987). For a given

substrate, the rate of mould growth decreases with decreasing temperature and the interaction between grain temperature and moisture content also affects the extent of mould colonization (Diener *et al.*, 1987). For a given moisture content, the water activity, and the propensity for mould growth, will increase with temperature (Scheidegger and Payne, 2003). Maize can be relatively safely stored for one year at a moisture level of 15 per cent and a temperature of 15°C. However, the same maize stored at 30°C will be substantially damaged by moulds within three months (Scheidegger and Payne, 2003).

Insects and anthropods make a significant contribution towards the biodeterioration of grain, through the physical damage and nutrient losses caused by their activity (Lillehoj and Wall, 1987). The metabolic activity of insects and mites cause an increase in both the moisture content and temperature of the infested grain (Windham *et al.*, 1999). When there is severe insect pressure in the field, extensive pod damage may give the fungi the opportunity to overwhelm the ability of kernels to wade off the fungal attack (Lillehoj and Wall, 1987). Arthropods also act as carriers of mould spores and their faecal material can be utilised as a food source by moulds (Windham *et al.*, 1999). European corn borer larvae carry spores of *Fusarium* species from the plant surface to the surfaces of damaged kernels or to the interior of stalks, where infection occurs (Greesel *et al.*, 2003). Viable spores can be found externally, internally, and in the frass of European corn borer larvae. Insects may form entry wounds for the fungi when larvae feed on stalks or kernels (Windham *et al.*, 1999). Even when the larvae do not directly carry the fungi into the stalks, spores subsequently deposited on the wounded tissue are very likely to germinate and infect the plant (Scheidegger and Payne, 2003). Additionally, root and stalk damaged by insects cause stress that predisposes the plants to stalk rot development (Scheidegger and Payne, 2003).

Maize cultivars differ in their susceptibility to growth of storage fungus (Campbell and White, 1994). Hybrids of short, Intermediate, and late maturities differ in aflatoxin accumulation with short-season maize escaping growth-limiting conditions of a hot, dry summer and the associated aflatoxin in contrast to the full-season maize (Payne, 1992). Late maize hybrids can have greater exposure to higher temperatures at flowering and post flowering stages, greater *A. flavus* inoculum, and increased insect activity compared with early hybrids. Secondary traits such as husk coverage, tightness, drought and heat tolerance are contributing factors to aflatoxin resistance (Betran *et al.*, 2002). Hybrids with loose husks are more vulnerable to loss of kernels integrity which can pre-dispose kernels to *A. flavus* infection (Oduody *et al.*, 1997).

Timeliness, clean up and drying of agricultural products is essential for preventing mycotoxin production 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 (Gressel *et al.*, 2003). Crops left in the field for longer periods of time may accumulate higher levels of toxin contamination (Lopez-Garcia *et al.*, 1999). Adequate drying also is essential to prevent fungal proliferation (Williams *et al.*, 2004). Improper storage 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 polythene sheets while others spread on bayer 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.

2.5 The disease cycle of *Aspergillus flavus*

Aspergillus flavus has been reported to occur in most agricultural soils and many types of organic material in various stages of decomposition including forages, cereal grains, food, and feed products. Fungal mycelium appears to be the predominant structure found in the soil, but sclerotia can be formed, thus contributing to the long-term survival of the fungus (Scheidegger and Payne, 2003). While still young and vigorous, the mycelium produces an abundance of conidiophores which are long, erect hyphae with phialides (Bankole and Adebanjo, 2003).

As the phialides reach maturity, they begin to form conidia at the tips which are the major source of primary inoculum in maize fields (Scheidegger and Payne, 2003). Conidia are carried by wind or insects to nearby healthy plants (Diener *et al.*, 1987). Invasion of maize by *A. flavus* occurs through silk (Marsh and Payne, 1984). Senescencing silk is a suitable media for microbial growth and provide entry for fungi into the ear (Horn *et al.*, 1996). The fungal mycelium spreads superficially among the kernels and penetrates the kernels mainly through the pericarp (Marsh and Payne, 1984). Later in the growing season, conidia produced on infected plants provide high levels of secondary inoculum when environmental conditions are conducive for disease development (Scheidegger and Payne, 2003).

Infected plant tissues such as corn kernels, cobs, and leaf tissue can remain in the soil and support the fungus until the following season when newly exposed mycelium or sclerotia can give rise to conidial structures, thus producing the primary inoculum for the next infection cycle (Payne and Brown, 1998).

2.6 Detection and Quantification of mycotoxin contamination in grains

Different types of samples and their vast number, as well as chemical diversity of mycotoxins and their simultaneous occurrence in samples present a need for rapid multi-analyte methods suited for various matrices (Zheng *et al.*, 2004). Chromatographic and immunological methods have been used widely for detection and quantification of mycotoxin in contaminated grains. The chromatographic methods include Thin-layer chromatography (TLC), High performance liquid chromatography (HPLC), or Gas chromatography (GS) while the immunological methods include the Enzyme linked–immunosorbent assay (ELISA) and immunoaffinity column based-analyses. Other methods include use of uv light and polymerase chain reaction.

Proper sampling procedures are pre-requisite for obtaining reliable results because of the heterogeneous distribution of mycotoxins in grains and other commodities (Zheng *et al.*, 2004). The European Commission has recommended a procedure for sampling according to the size of the batch (Richard *et al.*, 1993). This includes 10 to 100 incremental samples of one to two kilos, which have to be ground and thoroughly mixed. Sub samples of 50g are placed in 500ml glass stoppered Erlenmeyer flask with 25ml of water, 25g of diatomaceous earth and 250ml of acetone and shaken for 30 minutes. The extract (50ml) is placed on a column of silicagel and the lipids and pigments are eluted with 150ml of acetone (Zheng *et al.*, 2006). The fraction is collected and evaporated to dryness and quantitatively transferred to vial, the solvent is then evaporated and the vial is ready for analysis either by TLC, HPLC, or gas chromatography-mass spectrometry (Richard *et al.*, 1993).

Thin-layer chromatography is a low-cost, rapid analytical technique, yielding qualitative or semi-quantitative estimations by visual inspection with reliable quantitative results (Krska, 2007). It shows better repeatability and is less prone to overestimation of the concentration due to its better selectivity (González *et al.*, 2006). It has an important role, especially in developing countries, for surveillance purposes and control of regulatory limits (Gilbert and Anklam, 2002). It is used to screen for zearalenone, aflatoxin, and ochratoxin in agricultural commodities (Shotwell *et al.*, 1996). Detection limits for fumonisins is near 0.1 μg/kg and 100μg/kg for DON in wheat and 40μg/kg in corn (Shotwell *et al.*, 1996).

Thin-layer chromatography glass plates precoated with silica gel (20 cm x 20 cm) can be used for single-dimension development of the chromatograms. Two 5μl and two 20μl aliquots of sample are spotted on each plate along a line about 4cm from the bottom edge of the plate (González *et al.*, 2006). On the same plate, 2, 5, and 10μl of aflatoxins standard (0.5μg/ml) B_1, G_1, and M_1 (0.1μg/ml) B_2 and G_2) is spotted as well (Schoellenberg *et al.*, 1998). Five microliters of standard is spotted on top of one of the two 20μL sample spots as an internal standard (Shot well *et al.*, 1996). The plate is developed in an unlined but equilibrated tank; the developing solvent should be approximately 2 cm in depth. Quantification is accomplished by comparison to the reference standards on the TLC plate or by fluorescence densitometry (Shotwell *et al.*, 1996).

High performance liquid chromatography is a sensitive and selective quantitative method for the determination of regulated mycotoxins such as aflatoxins, fumonisins, zearalenone, trichothecenes and ochratoxin A. Detection limits for fumonisins is < 0.1 μg/kg and 50 μg/kg for zearalenone in corn (Shotwell *et al.*, 1996), in barley and malt, the detection range from 1 to 50 and 1 to 5 μg/l in beer. High performance liquid chromatography requires aflatoxins B1

and M1 be treated first with trifluoroacetic acid to maximize fluorescence of these compounds in the aqueous mobile phase (Shotwell *et al.*, 1996). The sample is finally dissolved in mobile phase solvent and injected. The mobile phase is isopropanol: acetonitrile: water (12.5:12.5:75) and a 25-cm, 5-µm C_{18} column and fluorescence detector (366 nm) are used. The flow rate is 1 ml per min. Deoxynivalenol can be determined in wheat and corn at 325µg/kg.

Gas chromatography (GC) combines superior separation on the capillary columns with a variety of general and specific detectors (González *et al.*, 2006). It is mostly used for the determination of type A trichothecenes, since they are not fluorescent and do not strongly adsorb in the UV-Vis range (Shotwell *et al.*, 1996). It is also widely employed for the determination of type B trichothecenes, detection of type A and type B trichothecenes (González *et al.*, 2006). The GC approach seems to give the best results for confirmatory analyses and also for obtaining structural information on several derivatives (Shotwell *et al.*, 1996).

A Gas chromatography method used for the analysis of DON and derivatives (3-ADON, 15-ADON, NIV, 7-ADON) in wheat and barley, described by Mirocha *et al.* (1998), is based on selected ion monitoring (SIM) detection of the mycotoxin astrimethylsilyl-ethers (TMS). The analysis involved electron impact ionization (EI) of the derivatives and monitoring of the diagnostic ions (Schoellenberg *et al.*, 2006). The sensitivity varied according to the column, the conditions of the filament, as well as the instrument tuning. Moreover, ionization turned out to be matrix dependent, so that internal calibration was often required, in particular with complex matrices such as feeds (Mirocha *et al*,. 1998). Quantification was performed by external calibration with good precision (5%) and good recovery (90%).

A Gas chromatography method for the determination of eight trichothecenes of type A and B developed by Schoellenberg *et al.* (1998) involved the use of an ion trap mass spectrometer operating in chemical ionization mode, using isobutane as reactant gas. Also in this case, it was necessary to apply different clean-up steps to remove the co-extracted matrix components (Scherma *et al.*, 2005). The critical point of the whole procedure, which was applied to complex matrices including heavily molded cereals, cereal foods, and feed, was the derivatization step (Schoellenberg *et al.*, 1998). Since the ion trap system allowed the typical fragmentation for each toxin, the quantification was based both on the main fragment ion and on a secondary characteristic ion, which allowed to correctly identify the analyte (Shapira *et al.*, 1996). The reproducibility of the fragmentation was good, with differences between the two selected ions below 10% and obtaining detection limits in cereals ranging from 5 to 10 (Schoellenberg *et al.* 1998)

The ultraviolet test is extremely convenient as a testing procedure because of its capability of screening large quantities of food material quickly with high sensitivity (Scherma *et al.*, 2005). The method is inexpensive to use, and can be easily be taught to nontechnical personnel. Corn kernels that are contaminated by aflatoxigenic fungi can be detected and level of aflatoxins in a corn sample estimated roughly (Munkvold *et al.* 2005). Exposure of infected corn kernels that contain aflatoxins to 365 nm UV light results in intense blue-green fluorescence of aflatoxin-containing kernels. More than four fluorescent kernels in a 5-pound sample of corn (approximately 6,000 kernels) indicate that the level of aflatoxins is at least 20µg/kg. The presence of less than four fluorescent kernels per 5-pound sample, though, does not mean that the sample is not contaminated with Aflatoxins (Munkvold *et al*,. 2005).

This assay should be followed by the identification of Aflatoxins and determination of their levels with portable kits, and by confirmation of their identity in the laboratory.

Polymerase chain reaction (PCR) involves use of primers to detect and identify aflatoxigenic strains of *A. flavus* and *A. parasiticus* among isolated colonies or in DNA extracts from foodstuffand feedstuff (Shapira *et al.*, 1996). DNA of *Aspergilli* is used as template for the amplification of genes involved in AF biosynthesis (Sweeney *et al.*, 2000). Sequencing of the amplified fragments confirms the identity of aflatoxins biosynthetic genes. However, the mere presence of the genes reflects only the potential of the fungus to produce aflatoxin (Scherma *et al.*, 2005). Aflatoxins production depends on temperature, humidity, composition of the growth medium, growth phase and age of the culture (Scherma *et al.*, 2005). The specificity and sensitivity of the PCR technology makes identification of microorganisms much easier and should therefore be used more in occupational exposure assessments (Sweeney *et al.*, 2000).

2.7 Management of mycotoxins

The most effective strategy for control of mycotoxin is to reduce or eliminate the initial toxin production processs (Lillehoj and Wall, 1987). This may be achieved through several approaches which include development of crop hybrids that are less susceptible to fungal growth and mycotoxin production, control of insect crop pests, reducing crop stress during growth, reducing physical damage to crops at harvest, drying crops below 0.68 water activity and use of suitable clean containers for storage (Robb, 1993). These preventive approaches may only be partially successful since some unanticipated environmental conditions such as

excessive drought and rainfall often result in wide spread occurrence of mycotoxins (Lillehoj and Wall, 1987).

2.7.1 Pre-harvest control

Prevention through pre-harvest management is the best method for controlling mycotoxin contamination 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 (Garber and Cotty, 1997). Ideally, the risks associated with mycotoxin hazards should be minimized at every phase of production. Control parameters for the processing of commodities that are susceptible to mycotoxin contamination would include time of harvesting, management of crop residues and crop rotation, irrigation and soil condition temperature, moisture during storage and transportation, selection of agricultural products prior to processing, processing/ decontamination conditions, addition of chemicals, and final product storage and transportation (Lopez-Garcia and Park, 1998).

Producers may reduce the likelihood of aflatoxin buildup in the field by harvesting maize before it reaches the industry standard of 15.5 percent moisture (Erick, 2002). This system reduces duration when ears may be exposed to unfavorable drying conditions that promote aflatoxin development in the field. Corn reaches physiological maturity at about 30 percent moisture and can be harvested any time thereafter (Murphy *et al.*, 2006). Research done at Mississippi university indicates maize will normally lose around 0.6 percent moisture per day during the dry-down period. This rate is not influenced much by hybrid maturity. Thus, field exposure can be reduced by at least one to two and a half weeks by harvesting corn at 20 to 25 percent moisture, compared to letting the corn dry in the field to 15 percent moisture

(Stack and Carlson, 2006; Wrather and Sweets, 2007). The disadvantage of early harvest is that wet, warm grain is an ideal environment for rapid aflatoxin escalation if it is not handled properly (Murphy *et al.,* 2006). High-moisture grain (16-30 percent) should be dried to below 15 percent moisture within 24 hours after harvest or immediately haul the grain to an elevator that will dry the grain (Murphy *et al.,* 2006). Inoculum potential is a prerequisite for *Aspergillus* infection and subsequent aflatoxin production (Lopez-Garcia and Park, 1998). Soil type and condition, as well as availability of viable spores, have been considered important factors in aflatoxin production (Bennett and Richard, 1996). 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 next crop. Proper management of crop residues would help avoid this problem (Bennett *et al.,* 1992).

Soil fertility and drought stress have been found to be contributing factors in pre-harvest aflatoxin contamination of maize (Richard and Payne, 2008). Moisture and temperature play the most important roles in the planning of any control strategy for fungal development (Garber and Cotty, 1997). High moisture and high relative humidity are essential for spore germination and fungal proliferation (Phillips *et al.,* 2002). Therefore, adequate efforts should be made to avoid extreme conditions of either drought or excessive moisture (González *et al.,* 2006).

Some studies have shown that drought stress followed by high-moisture conditions is ideal for *Fusarium moniliforme* proliferation and fumonisin production (Garber and Cotty, 1997). When this type of weather condition is present, it can be assumed that some degree of mycotoxin contamination will occur and other management strategies should be explored (Ramos *et al.,* 2006). Kernel damage is a pre-requisite for aflatoxin formation, the incidence

of *A. flavus* and *A. parasiticus* is usually higher in damaged kernels (Garber and Cotty, 1997). Insect-damaged kernels are routes for infection and are likely to dry to moisture levels that are more favorable for the growth of *A. flavus* and aflatoxin production than of other fungi (Lopez-Garcia and Park, 1998). Therefore, proper management of insect pests through any appropriate control strategy would reduce mycotoxins contamination problem.

2.7.2 Post-harvest practices

For post-harvest mycotoxin control, prevention of conditions that favor fungal growth and subsequent toxin production needs to be considered. These conditions include water activity of stored products, temperature, grain condition, gas composition of the intergranular air, microbial interactions, and presence of chemical or biological preservatives (Shapira *et al.*, 1996). Hamiton (2000) reported that drying harvested maize to 15.5% moisture content or lower within 24-48 hours would reduce the risk of fungal growth and consequent aflatoxin production.

Awuah and Ellis, (2002) demonstrated that when groundnuts were dried to 6.6% moisture level, they were free of fungi regardless of the local storage protectant used for 6 months, whereas at 12% moisture content, only jute bags with the plant Syzigumaromaticum effectively suppressed the cross infection of healthy kernels. However, when the moisture content was increased to 18.5%, the latter treatment was not as effective (Hamiton, 2000). A community-based intervention trial in Guinea, West Africa focused on thorough drying and proper storage of groundnuts in subsistence farm villages and achieved a 60% reduction in mean aflatoxin levels in intervention villages (Turner *et al.*, 2005).

Fungal infection is more likely in shriveled, cracked kernels and foreign material (MOA, 2008). Thus, grain quality may be significantly improved by reducing the combine ground speed, increasing fan speed and opening sieves, so that these sources of contamination are removed from the sample. A postharvest mechanical cleaner or gravity separator may also help reduce moderate aflatoxin levels of 50 to 100 μg/kg below the FDA standard 20 μg/kg (Cotty, 1994).

Fungal infection can occur during storage as well as in the field (Moreno-Martnez and Christensen, 1971). Thus, sanitation of handling and storage facilities is very important. Spores from fungi on infected grain readily disperse during handling and contaminate unaffected grain (Moreno-Martnez and Christensen, 1971). Removal corn and debris remaining in combines, trucks, grain carts, pits, augers, bins, elevators, on a daily basis is therefore recommended (Benford *et al.*, 2001).

2.7.3. Legislation

Legislation involves control methods that have been implemented for agricultural commodities entering international trade or located in countries with centralized or large buying and distribution systems (Bhat and Miller, 1991). Human foods are allowed 4–30μg/kg aflatoxin, depending on the country involved (Table 2.2) (FDA, 2004). In the US, 10μg/kg is the maximum aflatoxin residue limit allowed in food for human consumption, except for milk (Wu, 2006; FAO, 2000) while 4μg/kg total aflatoxin in food for human consumption are the maximum acceptable limits in the EU, the strictest in standard worldwide (Wu, 2006).

Table 2.2 Acceptable tolerance of aflatoxins in maize for food and feed uses as established by food and drug administration

Use	Levels (µg/ kg)
Food by humans	20
Feed by immature animals including immature Poultry	20
Feed by dairy animals or destination not known	20
Feed breeding beef cattle, breeding swine	100
Feed for finishing swine of 100 pounds or greater	200
Feed for finishing beef cattle	300

Source: Kenya Bureau of Standards (Kebs), 2005; Stack and Carlson, 2003; Wrather and Sweets, 2007.

2.7.4. Host resistance

Host resistance may present a promising strategy for the pre-harvest prevention of mycotoxin contamination (Lopez-Garcia *et al.,* 1999). Traditional grain-breeding strategies to select for preferred genetic traits have been conducted for many years but the methods are plagued by many hurdles (Munkvold, 2003). They are inconsistent, labor-intensive inoculation techniques, lack of single genes and resistant control genotypes, and the financial implications of evaluating results (Munkvold, 2003). Duvick (2001) pointed out that visible symptoms of plant mould can be selected for using traditional breeding techniques, but many of the mycotoxin-producing fungi have surface with no visible signs.

Genetic modification of mold-susceptible plants holds great promise for the development of host resistance through introducing a novel gene to express the target compound or

enhancing expression of such a compound by the existing gene, thereby capitalizing on the plant's own defense mechanisms (Duvick, 2001). Efforts are also under way to engineer plants to produce compounds that disrupt mycotoxin synthesis (DFID, 2001). Enhanced expression of an α-amylase inhibitor in *Aspergillus* sp could result in significantly reduced aflatoxin levels (Duvick, 2001 and Munkvold, 2003)

Maize breeding with aflatoxin resistance has yielded to development of resistance lines (Lopez-Garcia *et al.,* 1998). 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 priority. Breeding for ear mould tolerance and reduced mycotoxin levels is in practice for commercial and public programs (DFID, 2001). The control for aflatoxin contamination in maize can also be achieved by use of resistant hybrids (Naidoo *et al.,* 2002). Short-season maize could escape growth-limiting conditions of a hot, dry summer and the associated aflatoxin in contrast to the full-season maize (Betran and Isakeit, 2003). Late maize hybrids can have greater exposure to higher temperatures at flowering and postflowering stages, greater *A. flavus* inoculum, and increased insect activity compared with early hybrids.

2.7.5. Processing of food and feed

The most effective strategy for controlling aflatoxin contamination in food and feed is to reduce or eliminate the initial toxin production process (Lillehoj and Wall, 1987), but mycotoxins contamination will eventually occur (Lopez-Garcia *et al.,* 1999). Post harvest control and decontamination procedures represent an important hurdle to avoid consumer exposure.

Dry milling processes that are widely used for maize and cereals have been shown to result in reduced mycotoxin levels in several fractions such as soluble, gluten, fiber starch and germ (Lopez-Garcia *et al.,* 1999). Removing DON from feed barley by an abrasive dehulling process significantly reduces toxin levels (Murphy *et al.,* 2006). Grain sorting and sizing has shown to reduce toxin levels in corn (Murphy *et al.,* 1993). Physical separations of damaged, immature and mould-infested kernels result in reduction of aflatoxin and fumonisin (Lopez-Garcia *et al.,* 1999). Some food processing methods have been shown to reduce or eliminate aflatoxins. Prevention of aflatoxin intoxication in some animals is by dietary inclusion of aflatoxin clays that tightly bind these poisons in gastrointestinal tract, thus decreasing their bioavailability toxicities (Lopez-Garcia *et al.,* 1999).

Some phases of industrial processes can reduce specific mycotoxins once a contaminated product has reached a processing facility, clean-up and segregation are the first control options (Lovett *et al.,* 1975). These procedures are usually non-invasive and, except for milling, will not alter the product significantly (Lopez-Garcia *et al.,* 1999). In some cases, these are the best methods of reducing mycotoxin presence in final products. When peanuts are processed, a significant amount of aflatoxins can be removed by electronic sorting and hand-picking (Kirksey, 1989). Separation of mould-damaged maize can significantly reduce fumonisin and aflatoxin concentrations (Bennett *et al.,* 1992). In addition, the removal of rot from apples significantly reduces the patulin content in the final product (Lovett *et al.,* 1975). Although some contamination may persist, physical removal represents a good alternative for industry (Lopez-Garcia and Park, 1998).

2.7.6 Biological control

One of management strategies being developed for the 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). 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. For *A. flavus* competitive exclusion to be effective, the biological 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 registration as biopesticides to control aflatoxin contamination in cotton and peanuts in several states of America (Dorner *et al.*, 2004).

While soil application of a competitive non-toxigenic *A. flavus* strains is successful in reducing aflatoxin contamination in certain crops, direct application to aerial reproductive structures could be more effective for maize (Palumbo *et al.*, 2006). A spray based water dispensible granule formulation was developed to deliver non-toxigenic *A. flavus* strain K49 directly to maize ears (Lynt *et al.*, 2009). In field studies conducted to compare K49 colonization and effectiveness in reducing aflatoxin contamination when applied either as soil inoculants 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% (Lynt *et al.*, 2009).

Several bacterial species, such as *Bacillus subtilis*, *Lactobacilli* sp, *Pseudomonas* sp, *Ralstonia* sp and *Burkholderia* sp have shown the ability to inhibit fungal growth and production of aflatoxins by *Aspergillus* sp in laboratory experiments. Palumbo *et al.* (2006) reported that a number of *Bacillus*, *Pseudomonas*, *Ralstonia* and *Burkholderia* strains isolated from California almond samples could completely inhibit *A. flavus* growth. Several *Bacillus subtilis* and *P. solanacearum* isolated from the non-rhizophere of maize soil were also able to inhibit aflatoxin accumulation (Nesci *et al.*, 2005). In most cases, although these strains were highly effective against aflatoxin production and fungal growth under laboratory conditions, they do not give good efficacies in fields because it is difficult to bring the bacterial cells to the *Aspergillus* infection sites on commodities under field conditions (Dorner *et al.*, 2004).

Some saprophytic yeast species such as *Candida krusei* and *Pichia anomala* have shown promise as biocontrol agents against *A. flavus*. These yeast strains were able to inhibit *Aspergillus* growth greatly in laboratory conditions (*Hua et al.*, 1999; Masoud and Kaltoft, 2006). Although they were considered to be potential bio-control agents for management of aflatoxins, further field·experiments are necessary to test their efficacies in reducing aflatoxin contamination under field conditions.

2.7.7 Awareness about 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, as described by Turner *et al.* (2005). 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 aflatoxicosis.

Awareness of mycotoxins and the dangers that they pose to human and animal health could be done through government bodies, private organizations, non-governmental organizations, media networks such as radios, televisions, magazines and newspapers (Wagacha and Muthomi, 2008). These multiple means of spreading information have shown to significantly reduce level of aflatoxin. During the 2004 aflatoxin poisoning in Kenya, individuals who received information on maize drying and storage through awareness campaign run by the Food and Agriculture Organisation and Kenya's Ministry of Health had a lower serum aflatoxin than those who did not receive the information (Strosnider *et al.*, 2006).

The need for foreign exchange and the stringent mycotoxins control make exporting countries sell best of the commodities abroad leaving substandard or contaminated commodities for domestic use. A regional experts meeting held in 2005 on aflatoxins problem with particular reference to Africa made certain recommendations that could be instrumental in addressing mycotoxins in the continent (Wagacha and Muthomi, 2008). The meeting recommended continued mycotoxin awareness as public health issue, strengthened laboratory and surveillance capacities as well as establishing early warning systems.

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CHAPTER THREE: MATERIALS AND METHODS

3.1 Determination of susceptibility of maize varieties to *A. flavus* infection and aflatoxins contamination

3.1.1 Preparation of *A. flavus* inoculum

Aspergillus flavus was isolated from maize samples collected from farmers in Eastern province during long and short rain season of 2008. Grain samples were surface sterilized for 2-3 minutes in 2.5% sodium hypochlorite amended with 2-3 drops of Tween 80 and rinsed three times in sterile distilled water. The surface sterilized kernels were plated on Czapek Dox Agar media (sucrose 30g, agar 20g, $NaNo_3$ 2g, KCl 0.5g, $MgSO_4.7H_2O$ 0.5g, $FeSO_47H_2O$ 0.01g, K_2HPO_4 1g, distilled water 1000 ml) amended with 25µg/l chlorophenical, streptomycin, penicillin and fungicide pentachloronitrobenzene (PCNB). Five kernels were aseptically plated in each plate and the plates incubated at 25°C for 7-10 days. Colonies of *A. flavus* were identified on the basis of growth rate, color of colony and conidiophore, uni-biseriate phialides, round and radiate vesicles (Gilman, 1957: Larone, 1995).

A. flavus colonies were purified by sub culturing on Czapek Dox Agar and allowed to grow for 7-14 days. Inoculum was multiplied by streaking *A. flavus* conidia using a sterile rod on Czapek Dox Agar and incubating for 14 days. Conidia suspension was harvested by flooding the cultures with distilled water and scrapping off the fungal growth using a sterile glass slide. The conidial suspension was passed through two layers of cheese cloth and conidia concentration was standardized to 5 x 10^5 conidia/ml using a haemocytometer.

3.1.2 Field experimental design and crop husbandry

Field trials were conducted over two growing seasons in 2008 and 2009 at Waruhiu Agricultural Training Centre-Kiambu and Kenya Agriculture Research Institute-Mwea. Sixteen maize varieties commonly grown in drier areas of Eastern province Hybrid 513 (H513), Duma 43, Dekalb (DK8031), Dry highland 1 (DHO1), Dry highland 2 (DHO2), Dry highland 4 (DHO4), Panner 77 (P77), Panner 7-19M (P 7-19M), Panner 4M (P4M), Panner 67/5243 (P 67/5243), Hybrid 515 (H515), Hybrid 516 (H516), Katumani composite B (KCB), Hybrid 614 (H614), Pioneer Hybrid 3253 (PHB.3253) and Katumani composite (Table 3.1) were evaluated in a randomized complete block design with three replicates.

The land was ploughed and harrowed to moderate tilth. Two seeds per hole were planted in plots measuring 5 m x 6.75 m at spacing of 75 cm x 25 cm and N.P.K (17: 17: 0) fertilizer was applied at the rate of 200 kg/ha.. Top dressing was done with calcium ammonium nitrate (26% N) at the rate of 200 kg/ha three weeks after planting. Other agronomic practices like gapping, thinning, weeding and irrigation were done as required.

3.1.3 Cob inoculation and *Aspergillus* rot assessment

Inoculation of maize was done 10-14 days after 50% silking. Silk channel inoculation method (Betran *et al.*, 2002) was done by injecting 5 ml of the *A. flavus* spore suspension into each primary cob using a hypodermic syringe. Control cobs were injected with 5 ml sterile distilled water and fifteen cobs were inoculated for each variety. The inoculated cobs were tagged and covered with khaki papers to prevent desiccation of the inoculum.

Table 3.1: Characteristics of maize varieties used in the study

Variety	Year of release	Owner	Maintainer and seed source	Optimal production altitude range	Months to maturity	Grain yield ($t\ ha^{-1}$)
H513	1995	Kenya Seed Co.	Kenya Seed Co.	1200-1600	4-5	6-8
H515	2000	Kenya Seed Co.	Kenya Seed Co.	1200-1500	4-5	6-8
H516	2001	Kenya Seed Co.	Kenya Seed Co.	1200-1500	4-5	7-9
H614D	1986	Kenya Seed Co/ KARI	Kenya Seed Co. /K.A.R.I	1500-2100	6-9	8-10
Duma 43	2004	AgriSeed Co Ltd	SEEDCO Zambia	800-1800	4-5	6-7
DK 8031	2003	Monsanto	Monsanto	900-1700	4 - 4.7	6-8
DHO1	1995	Kenya Seed Co.	Kenya Seed Co.	900-1400	3-4	4-6
DHO2	1995	Kenya Seed Co.	Kenya Seed Co.	900-1400	3-4	4-6
DHO4	2001	Kenya Seed Co.	Kenya Seed Co.	900 - 1500	3-4	5-6
Panner 77	2008	Panner seed (PTY) Ltd	Panner Seed (K) Ltd SA	800 - 1600	3 -4	4-6
Panner 7M	2008	Panner Seed (PTY) Ltd	Panner Seed (K) Ltd SA	900-1500	3-4	4-6
Panner 4M	2008	Panner Seed (PTY) Ltd	Panner Seed (K) Ltd SA	900-1500	3-4	4-6
Panner 67	2001	Panner Seed Company	Panner Seed (K) Ltd SA	800 - 1600	4-5	5-6
PHB3253	1995	Pioneer Hybrid	Pioneer Hybrid, Zimbabwe	800-1800	4-5	7-9
KCB	1967	Kenya Seed Co/ KARI	Kenya Seed Co/KARI.	900-1350	3-4	3-5
Katumani	1987	KARI- Katumani	KARI-Katumani	1000-1800	2.5	1.2-1.5

Source: Kenya Plant Health Inspectorate Service, 2009; National Crop Variety List

Key: KARI= Kenya Agricultural Research Institute; SA= South Africa; Co=Company

KCB= Katumani composite B, DH01=Dry highland 1, DH02= Dry highland 02, DH04= Dry highland 04, P.77= Panner 77, H516=Hybrid 516, H515=Hybrid 515, H614= Hybrid 614, PHB 3253=Pioneer hybrid, DK8031=Dekalb 8031

The data collected included days to 50% silking, days to physiological maturity, meteorological data and cob husk coverage. Cob husk coverage was visually rated using a scale of 1-5 where: 1 = husk leaves extended more than 2.54 cm from the tip of the ear; 2 = husk leaves covering the tip of the ear between 0 and 2.54 cm; 3 = husk leaves of the same length as the ear, and no grain is exposed; 4 = husk leaves are shorter than the ear, and tip kernels are exposed; and 5 = few husk leaves with more than few kernels exposed (SAS, 1997).

The cobs were evaluated for *Aspergillus* and other fungal rots at harvest based on the percentage kernel with visible rot symptoms based on disease severity rating scale of 1-7 where: 1= no infection, 2 = 1-3%, 3 = 4-10%, 4 = 11-25%, 5 = 26-50%, 6 = 51-75%, 7 = 76-100% infection (Reid *et al.,* 1996). Mature cobs were harvested at physiological maturity and the 15 inoculated cobs per variety were dehusked and shelled separately. The kernels from cobs of the same variety in each plot were thoroughly mixed and 0.5 kg sub samples were stored at 4°C until aflatoxin analysis and fungal re-isolation. The remaining grain was dried to safe moisture content of less than 13.5%.

3.1.4 Post harvest incubation and re-isolation of *A. flavus*

Maize samples weighing 500 g with initial moisture content of 13.5% were adjusted to 10, 13.5, 15, 17 and 20% moisture content by soaking in water for a specified period of time and the moisture content measured using a moisture meter (Acott and Labuza, 1975). Sub-samples weighing about 200g were incubated in khaki papers at 25°C placed on laboratory bench and arranged in a randomized complete block design with a split plot layout. The moisture content served as the main plot and varieties as the sub-plots. Moisture content and re-isolation of *A. flavus* was done at 7, 28, and 84 days. *A. flavus* was isolated from the

inoculated grain by plating surface sterilized kernels on Czapek Dox Agar and the number of kernels showing infection by *A. flavus* infection and other fungi were determined after 5-7 days.

3.1.5 Determination of aflatoxin content

Levels of aflatoxin B_1 in the samples were determined by the enzyme linked-immunosorbent assay (ELISA) as described by Gathumbi (2001). The maize grains were ground into fine powder. Five grams of the powder was weighed and extracted with 25ml methanol: water 50:50 (v/v). The extract was defatted with 10ml hexane and the mixture centrifuged at 1500g for 10 minutes. The methanolic extract was diluted 1:5 in Phosphate Buffered Saline (PBS) and again 1:4 in methanol PBS. Each micro-plate of the Elisa plates was coated by adding 100µl of coating solutions containing antibody dissolved in the bicarbonate buffer and incubated overnight in a moist chamber. The plates were then emptied and blocking of the free protein binding sites was done by adding 200µl of 3% bovine serum albumin in PBS for 20 min. The plates were then washed three times with Tween 20 solutions and semi dried with bloating paper.

Four standard concentration levels 0 parts per million (ppm), 111 ppm, 333 ppm and 1000 ppm were prepared by diluting the calibrated aflotoxins standard. Five tubes were kept in a rack and marked N (neat), S1, S2, S3 and S4. Then 50 µl of the sample extract and 50 µl calibrated aflatoxin standards were incubated simultaneously with an aflatoxin- enzyme conjugate solution in wells of the coated microtitre plate. Each sample was replicated twice. The plates were covered with aluminum foil and incubated for two hours at room temperature. The wells were then emptied and washed three times with Tween solution (Sodium chloride 8.5g, Tween 20 200µl, distilled water 1000ml) and semi dried with blotting

paper. The amount of aflatoxin-enzyme conjugate bound to the antibody was determined by incubation with 100µl of enzyme substrate solution and color was allowed to develop for 20 minutes. Enzyme reaction was stopped by adding 100µl of 1M sulphuric acid (H_2SO_4).

The resultant colour intensity was determined with a spectrophotometer ELISA reader (Model uniskan 11 type 364 labystems, Finland) at A450 nm. The percentage inhibition for each standard and sample was calculated as B/Bo % where B is mean absorbance reading of the blank standard curve. A calibration curve for the standards for each toxin dilutions was plotted using $\log_{10}$ of standards concentration on x-axis against the % inhibition of the standards and a best fit curve drawn each from which the concentration of samples were read.

3.2 Determination of the effectiveness of maize grain-inhabiting micro-organism in managing *A. flavus* infection and aflatoxins accumulation

3.2.1 Isolation of fungal antagonists

The antagonistic fungi tested were *Fusarium oxysporum*, *Trichoderma* sp, *Alternaria* sp, *Aspergillus niger*, *Aspergillus terreus*. They were isolated from maize samples that were harvested in Mwea and Waruhiu during an earlier experiment. The grain samples were surface sterilized for 2-3 minutes in 2.5% sodium hypochlorite amended with 2-3 drops of Tween 80 and rinsed three times in sterile distilled water. The surface sterilized kernels were plated on Czapek Dox Agar media and the plates incubated for 7-14 days. The fungal colonies were identified on basis of macroscopic features like color of the colony and microscopic structures like conidiophores, phialides, vesicles and sclerotia (Gilman, 1957).

3.2.2 Screening the fungi for inhibition of *A. flavus* growth in culture

For each fungal antagonist, mycelia fragments were spot inoculated 2 cm from the edge of the plate at four equidistant points and an agar plug cut from *A. flavus* culture using a 6 mm diameter cork borer was placed at the centre of the plate. The plates were labeled, incubated for 21 days at 28°C and arranged in a randomized complete block design on laboratory bench. Control plates consisted of *A. flavus* agar discs alone placed at the centre of plate. *Aspergillus flavus* growth inhibition was determined by measuring the colony diameter and clear zones between the *A. flavus* and antagonistic fungal colonies. The size of the colony reduction was calculated by subtracting the mean pathogen colony diameter in the absence of the antagonist (P_O) from the mean pathogen diameter in the presence of antagonist (P_1)

$$\frac{Po - P_1}{Po}$$

The fungi showing the highest growth inhibition of *A. flavus* were selected and grown on Czapek dox agar and allowed to grow for 7-14 days. The Inoculum was multiplied by streaking conidia on fresh Czapek Dox agar media and spores were harvested by flooding the cultures with sterile distilled water. The suspension of each fungus was passed through a double layer of cheese cloth and the inoculum was adjusted to 10^5 spores/ml using a haemocytometer. Clean maize grains weighing 500g of varieties that were more susceptible to *A. flavus* contamination (DUMA 43 and Hybrid 516), and less susceptible to *A. flavus* Katumani and KCB were used. Grain weighing 150g were adjusted to 17% moisture content and inoculated together with the fungi showing the highest inhibition of *A. flavus* (*Fusarium oxysporum, Trichoderma* and *A. niger).* This was accomplished by placing grain in fungal

spore suspension (10^5spore/ml) for a specified period. The inoculated grain samples were then allowed to surface dry at room temperature for 45 minutes. The grain was then incubated in polythene bags, wrapped in khaki paper bags to maintain constant moisture content of 17% and placed on a laboratory bench for 28 days at 25°C. The experiment was arranged in a randomized complete block design with split plot layout replicated three times. The main plot was the varieties and the sub-plot consisted of the fungal antagonists.

Aspergillus flavus was re-isolated after 5-7 days on Czapek Dox agar by plating a total of 100 surface sterilized kernels per variety and determining the number of kernels showing *A. flavus* infection. Grain sub samples weighing 200g were ground to fine powder and aflatoxin content determined by ELISA method as described in section 3.1.5.

3.3 Data analysis.

Data collected during the fungal isolation, reduction of *A. flavus* growth and mycotoxin analysis was subjected to analysis of variance using Genstat (Lawes Agricultural Trust, Rotham Experimental station 2006, version 9). Differences among the means were compared using Fisher's protected least significance difference (LSD) test at 5% probability level (Steel and Torie, 1987; Clewer and Scarisbrick, 1991).

CHAPTER FOUR: RESULTS

4.1 Susceptibility of maize varieties to *A. flavus* infection and aflatoxins B1 accumulation

4.1.1 Days to 50% silking, maturity and cob husk coverage

There were significant differences ($P \leq 0.05$) between locations and seasons and among varieties in the number of days to 50% silking at Waruhiu and Mwea (Table 4.1). At Mwea during long rain season, Katumani variety attained 50% silking earlier than all the other varieties. However, during the short season, KCB took the shortest time to reach 50% silking than all the other varieties. At Waruhiu during long and short rain seasons, KCB and Katumani attained 50% silking earlier than all the other varieties while H614, P4M and H515 took longer to attain 50% silking than all other varieties. Overall, all the varieties in Mwea attained 50% silking earlier than those from Waruhiu.

There were significant differences ($P \leq 0.05$) between the locations and seasons and among varieties in number of days to maturity (Table 4.2). Variety KCB took a shorter period to attain maturity than all the other varieties while H614 took a longer time to maturity than all the other varieties at Mwea and Waruhiu during both short and long rain seasons. The varieties in Mwea attained maturity earlier than those from Waruhiu.

Location and season had no significant effect on mean cob husk coverage (Table 4.3). Only the variety had a significant effect on mean cob husk coverage .Variety P.77 had a few husk leaves with more than few kernels exposed while the other varieties had leaves extended more than 2.54 cm from the tip of the ear.

Table 4.1 Days to 50% silking of 16 maize varieties grown at Waruhiu Agriculture Training Centre and KARI – Mwea during short and long rain season of 2008 and 2009

	Mwea			Waruhiu		
Variety (V)	LR	SR	Mean	LR	SR	Mean
DHO 1	63.0	49.0	56.0	70.3	65.0	67.7
DHO 2	58.0	47.7	52.8	67.3	60.3	63.8
DHO 4	66.3	57.3	61.8	85.3	80.3	82.8
DK 8031	70.0	55.0	62.5	81.7	69.7	75.7
DUMA 43	70.7	55.0	62.8	76.3	73.0	74.7
H513	62.0	59.0	60.5	85.0	80.3	82.7
H515	68.3	59.0	63.7	86.7	74.0	80.3
H516	69.7	61.3	65.5	80.7	77.3	79.0
H614D	74.3	68.3	71.3	87.0	86.0	86.5
Katumani	54.0	40.0	47.0	65.7	57.3	61.5
KCB	56.3	37.0	46.7	62.7	56.7	59.7
P. 4M	68.3	57.7	63.0	86.0	73.0	79.5
P.67	71.7	59.0	65.3	84.7	73.0	78.8
P. 77	67.7	60.7	64.2	79.3	77.7	78.5
P.7M	64.7	60.0	62.3	73.7	80.3	77.0
PHB3253	61.7	58.7	60.2	78.0	76.7	77.3
Mean	65.4	55.3	60.4	78.1	72.5	75.3

$LSD_{(p \leq 0.05)}$ S = 2.4, $LSD_{(p \leq 0.05)}$ L= 1.0, $LSD_{(p \leq 0.05)}$V= 1.7, $LSD_{(p \leq 0.05)}$S x Lx V=3.6, CV% = 0.2

S= season, L= location, V = variety, LR=Long rain season, SR=Short rain season,
L.S.D = least significance difference, CV(%) = coefficient of variation,
DHO 1= Dry highland 1, DHO 2= Dry highland 2, DHO 4= Dry highland 4, DK 8031= Dekalb 8031
H513=Hybrid 513, H515=Hybrid 515, H516=Hybrid 516, H614=Hybrid 614D,
KCB= Katumani composite B, P 4M= Panner 4M, P 67= Panner 67,P 77M= Panner 77M, ,
P7M= Panner 7M , PHB 3253=Pioneer Hybrid 3253

Table 4.2 Days to maturity of 16 maize varieties grown at Waruhiu Agriculture Training centre and KARI – Mwea during short and long rain season of 2008 and 2009

Variety (V)	Mwea			Waruhiu		
	SR	LR	Mean	SR	LR	Mean
DHO 1	84.0	102.0	93.0	93.7	121.7	107.7
DHO 2	92.0	99.7	95.8	97.3	120.7	109.0
DHO 4	105.0	129.3	117.2	113.0	142.7	127.8
DK 8031	99.0	113.0	106.0	110.3	124.3	117.3
DUMA 43	97.0	123.0	110.0	108.7	128.3	118.5
H513	101.3	125.3	113.3	117.0	140.0	128.5
H515	107.0	127.7	117.3	116.7	136.0	126.3
H516	106.3	124.0	115.2	117.7	136.7	127.2
H614D	111.7	136.3	124.0	123.3	151.3	137.3
Katumani	89.0	96.0	92.5	92.7	116.7	104.7
KCB	82.0	93.3	87.7	90.3	114.3	102.3
P. 4M	104.3	120.7	112.5	113.3	127.0	120.2
P. 67	103.3	115.7	109.5	113.0	128.0	120.5
P. 77	104.3	124.3	114.3	117.0	129.0	123.0
P. 7M	103.3	120.0	111.7	114.7	138.0	126.3
PHB.3253	95.3	123.3	109.3	114.0	136.7	125.3
Mean	99.1	117.1	108.1	109.5	130.7	120.1

$LSD_{(p \leq 0.05)}$ S = 0.9, $LSD_{(p \leq 0.05)}$ L= 0.3, $LSD_{(p \leq 0.05)}$ V= 2.7, $LSD_{(p \leq 0.05)}$S x Lx V=5.26, CV% = 0.2

S= season, L= location, V = variety, LR=Long rain season, SR=Short rain season, L.S.D = least significance difference, LR=long rain, SR=short rain, CV (%) = coefficient of variation.

Table 4.3 Cob husk coverage of 16 maize varieties planted at two diverse agro-ecological zones during short and long rain season of 2008 and 2009

	Mwea			Waruhiu		
Varieties (V)	SR	LR	Mean	SR	LR	Mean
DHO1	2.0	1.7	1.8	1. 3	2.3	1.8
DHO2	1.3	1.0	1.2	1.0	1.3	1.2
DHO4	1.0	1.0	1.0	1.0	1.0	1.0
DK 8031	1.0	1.7	1.3	1.7	1.0	1.3
DUMA 43	1.7	1.3	1.5	1.3	1.3	1.3
H 513	1.0	1.0	1.0	1.0	1.0	1.0
H 515	1.0	1.0	1.0	1.3	1.0	1.2
H 516	1.0	1.0	1.0	1.0	1.0	1.0
H614	1.0	1.0	1.0	1.0	1.0	1.0
Katumani	1.0	1.0	1.0	1.0	1.0	1.0
KCB	1.0	1.0	1.0	1.0	1.0	1.0
P. 7M-10	1.0	1.0	1.0	1.0	1.0	1.0
PHB 3253	1.0	1.0	1.0	1.0	1.0	1.0
P.4M	1.0	1.0	1.0	1.0	1.0	1.0
P.67/5243	1.7	1.0	1.3	2.3	1.7	2.0
P.77	5.0	5.0	5.0	5.0	4.7	4.8
Mean	1.4	1.4	1.4	1.4	1.4	1.4

$LSD_{(p\leq0.05)}$ S=NS, $LSD_{(p\leq0.05)}$ L=NS, $LSD_{(p\leq0.05)}$V = 0.2, $LSD_{(p\leq0.05}$ LXSXV = NS, CV% = 5.2

LSD = Least significant difference; CV (%) = coefficient of variation, V=variety, NS = not significant, S=season, L= location, LR=long rain, SR=short rain,

Key to husk coverage

1 = husk leaves extended more than 2.54 cm from the tip of the ear

2 = husk leaves covering the tip of the ear between 0 and 2.54 cm

3 = husk leaves of the same length as the ear, and no grain is exposed

4 = husk leaves are shorter than the ear, and tip kernels are exposed

5 = few husk leaves with more than few kernels exposed

4.1.2 Visual ear rot rating on 16 maize varieties inoculated with *A. flavus*

Aspergillus and *Fusarium* rots were observed on 16 maize varieties inoculated with *A. flavus*. The symptoms of *Aspergillus* rot observed were a yellow green or gray-green powdery mold growth on and between the corn kernels. *Fusarium* rots were observed as pink, yellow and white kernel discoloration. The visual *Aspergillus* rots were found to be significantly different between the locations and among the varieties during short and long rain season (Table 4.4). At Mwea during the long rain season, PHB 3253 had a higher *Aspergillus* rot than all varieties while during the short rain season, H515 had a higher *Aspergillus* rot than all the other varieties.

At Waruhiu during long rain season, Katumani had a higher *Aspergillus* rot than all the other varieties. During the short rain season, Katumani and DHO1 had a higher *Aspergillus* rot than all the other varieties. Overall, DHO1, Katumani, H515, and P77, had a higher *Aspergillus* rot than all the other varieties at both Mwea and Waruhiu. Waruhiu had a higher *Aspergillus* rots than Mwea.

The *Fusarium* rots were significantly affected by only the varieties during both short and long rain season at Waruhiu and Mwea (Table 4.5). At Mwea during long rain season, P.7M, had a higher *Fusarium* rots than all the other varieties while during the short rain season, H515 had the highest *Fusarium* rots. At Waruhiu during the long rain season, KCB and P.77 had higher *Fusarium* ear rots than all the other varieties while during the short rain season, only KCB had higher *Fusarium* ear rots than all the other varieties. Overall, H515, P.67 had higher *Fusarium* ear rots than all the other varieties at Mwea. Variety KCB had the highest *Fusarium* ear rots at Waruhiu (Table 4.5).

Table 4.4 Percentage *Aspergillus* visual ear rot on 16 maize varieties inoculated with *A. flavus* at Waruhiu and KARI-Mwea during short and long rain seasons of 2008 and 2009

	Mwea			Waruhiu			
Variety (V)	LR	SR	Mean	LR	SR	Mean	Overall mean
DHO1	11.3	10.5	10.9	14.2	14.6	14.4	12.3
DHO2	3.2	5.0	4.1	4.9	5.3	5.1	4.5
DHO4	5.0	1.3	3.2	4.6	5.8	5.2	4.0
DK8031	8.5	5.0	6.7	9.3	4.4	6.9	6.7
DUMA 43	2.9	0.0	1.4	4.2	4.4	4.3	2.5
H513	12.5	6.3	9.4	4.2	3.8	4.0	7.2
H515	6.7	30.0	18.5	4.6	3.8	4.2	12.6
H516	5.8	6.3	6.0	7.5	6.4	6.9	6.4
H614	2.1	0.0	1.0	4.2	5.0	4.6	2.5
Katumani	10.8	17.5	14.2	23.4	19.4	21.4	17.0
KCB	12.1	6.3	9.2	17.9	10.7	14.3	11.2
PHB 3253	13.8	7.5	10.6	7.1	5.6	6.3	8.9
P4M	10.0	5.0	7.5	1.0	1.7	1.3	5.0
P67/5243	6.6	1.0	3.8	5.8	6.3	6.0	4.7
P77	5.8	22.5	14.1	13.5	9.3	11.4	13.0
P7M-19M	7.1	3.8	5.4	14.6	13.8	14.2	8.9
Mean	7.7	8.0	7.8	8.8	7.5	8.1	7.9

$LSD_{(p\leq0.05)}$ S = NS, $LSD_{(p\leq0.05)}$ L=1.6, $LSD_{(p\leq0.05)}$ V=5.1, $LSD_{(p\leq0.05)}$ LXVXS=NS, CV%=34

LSD = Least significant difference; V=variety, LR=Long rain season, SR=Short rain season, NS = not significant, S=season, L= location ,CV (%) = coefficient of variation,

Table 4.5 Percentage *Fusarium* visual ear rot on 16 maize varieties inoculated with *A. flavus* at KARI – Mwea during short and long rain seasons of 2008 and 2009

Variety (V)	Mwea LR	Mwea SR	Mwea Mean	Waruhiu LR	Waruhiu SR	Waruhiu Mean	Overall mean
DHOI	1.7	0.0	0.8	1.7	0.0	0.8	0.8
DHO2	0.8	0.0	0.4	0.8	0.8	0.8	0.7
DHO4	3.3	0.5	1.9	0.8	0.2	0.5	0.8
DK8031	1.8	0.0	0.9	0.0	0.0	0.0	0.2
DUMA 43	0.8	3.8	2.3	1.4	1.3	1.3	1.5
H513	2.1	2.5	2.3	1.4	2.1	1.7	1.8
H515	0.6	25.0	12.8	1.7	2.2	1.9	4.6
H516	1.0	0.0	0.5	0.3	0.0	0.2	0.2
H614	0.8	0.0	0.4	1.0	0.8	0.9	0.7
Katumani	0.0	0.0	0.0	0.0	0.0	0.0	0.0
KCB	0.0	0.0	0.0	8.8	8.8	8.8	6.6
PHB3253	4.6	0.0	2.3	0.4	0.4	0.4	0.8
P4M	1.7	0.5	1.1	0.6	0.6	0.6	0.7
P67/5243	0.8	25.0	12.9	0.2	8.5	4.3	6.4
P77	2.5	0.0	1.3	0.6	0.6	0.6	0.7
P7M-19M	9.6	0.0	4.8	0.4	0.0	0.2	1.3
Mean	2.0	3.6	2.8	1.3	1.6	1.5	1.8

$LSD_{(p\leq0.05)}$ L = NS, $LSD_{(p\leq0.05)}$ V=3, $LSD_{(p\leq0.05)}$ S=NS, $LSD_{(p\leq0.05)}$ LXV=NS, $LSD_{(p\leq0.05)}$ LXVXS=NS, CV%=34

LSD = Least significant difference; V=variety, NS = not significant, S=season, L= location, CV (%) = coefficient of variation, LR=Long rain season, SR=Short rain season

4.1.3 Kernels infection on 16 maize varieties inoculated with *A. flavus* and stored at varying moisture levels

The percentage of kernels infected with *A. flavus* was significantly affected by moisture content, variety and the interaction between moisture level and variety in Mwea during the long rain season (Table 4.6). Variety DHO4 had a higher *A. flavus* infection than all the other varieties Increase in moisture levels did not significantly affect *A. flavus* infection in all varieties except in DHO4, P7M-10M and P77M. Increase of moisture levels to 17% and above significantly increased *A. flavus* infection in DHO4 and P7M-10M. There were no significant differences in maize stored at 10% to 15% moisture levels.

During the short rain season at Mwea, moisture level and variety significantly affected *A. flavus* infection but interaction between moisture level and variety did not have any effect (Table 4.6). Variety DHO4 had a higher infection than all the other varieties and DHO 1had the least infection. Maize stored at 20% moisture levels had the highest infection while those stored at 10% had the least infection.

Variety, moisture content and interaction between moisture level and variety had a significant effect on the mean percentage of kernels infected with *A. flavus* at Waruhiu during both short and long rains (Table 4.7). During short rain season, variety H513 had higher *A. flavus* infection in all varieties. Increase in moisture levels significantly affected *A. flavus* infection in all the varieties. Increase of moisture levels to 15% and above significantly increased *A. flavus* infection in DHO1, H515, KCB and P67M (Table 4.7).

During long rain season, variety H614 had a higher *A. flavus* infection than all the other varieties. Increase in moisture levels significantly affected *A. flavus* infection in all the varieties. Increase of moisture levels to 15% and above significantly increased *A. flavus* infection in DHO1, Duma 43, H513, H515 and Katumani. However, increase of moisture levels to 15% and 20% increased infection (Table 4.7).

Variety, season, site, and their interaction had a significant effect on percentage of kernels infected with *A. flavus* at both Mwea and Waruhiu (Table 4.8). At Mwea during the short rain season, H614 and H515 had higher *A. flavus* infection than the other varieties while during the long season DHO4, H516, and P77 had higher infection than the other varieties. In both seasons, DHO4 had the highest infection and Katumani the lowest infection. The short season had a higher infection than the long season (Table 4.8).

At Waruhiu during the short rain season, H614 and DUMA 43 had a higher *A. flavus* infection than the other varieties while during long season Katumani, H516, DUMA and DHO4 had lower infection than the other varieties. Overall, in both seasons H614 had the highest infection and Katumani the lowest infection while the short season had a higher infection than the long season (Table 4.8).

Table 4.6 Percentage *A. flavus* infection of kernels harvested from cobs of 16 maize varieties gr own at KARI and inoculated with *A. flavus* and stored at varying moisture levels

	Long rain season					
	Moisture content (%)					
Variety (V)	10.0	13.5	15.0	17.0	20.0	Mean
DHO1	26.7	28.3	33.3	31.7	25.0	29.0
DHO2	26.7	28.3	30.0	28.3	20.0	26.7
DHO4	35.0	45.0	50.0	80.0	78.3	57.7
DK 8031	15.0	25.0	21.7	21.7	21.7	21.0
Duma 43	11.7	15.0	20.0	31.7	30.0	21.7
H 513	10.0	16.7	23.3	21.7	21.7	18.7
H 515	38.3	46.7	55.0	50.0	48.3	47.7
H 516	40.0	46.7	43.3	43.3	46.7	44.0
H 614	46.7	48.3	61.7	60.0	65.0	56.3
Katumani	13.3	11.7	11.7	6.7	10.0	10.7
KCB	23.3	21.7	18.3	16.7	16.7	19.3
P. 7M-10	30.0	35.0	48.3	58.3	56.7	45.7
PHB3253	26.7	26.7	31.7	36.7	41.7	32.7
P.4M	46.7	38.3	30.0	36.7	33.3	37.0
P.67/5243	30.0	25.0	26.7	36.7	40.0	31.7
P.77	36.7	41.7	33.3	28.3	21.7	32.3
Mean	28.5	31.3	33.6	36.8	36.0	33.3

$LSD_{(p\leq0.05)}$ MC = 5.5, $LSD_{(p\leq0.05)}$ V= 8.7, $LSD_{(p\leq0.05)}$ (M xV) = 19.4, CV % = 33.8

	Short rain season					
	Moisture content (%)					
Variety (V)	10.0	13.5	15.0	17.0	20.0	Mean
DHO1	21.7	16.7	13.3	16.7	38.3	21.3
DHO2	28.3	31.7	30.0	25.0	45.0	32.0
DHO4	36.7	30.0	56.7	48.3	46.7	43.7
DK 8031	18.3	20.0	21.7	28.3	38.3	25.3
Duma	15.0	30.0	30.0	13.3	33.3	24.3
H 513	26.7	36.7	23.3	26.7	40.0	30.7
H 515	26.7	35.0	30.0	21.7	41.7	31.0
H 516	18:3	26.7	38.3	43.3	45.0	34.3
H 614	23.3	33.3	35.0	38.3	35.0	33.0
Katumani	16.7	28.3	21.7	23.3	31.7	24.3
KCB	13.3	20.0	30.0	21.7	50.0	27.0
P. 7M-10	21.7	26.7	50.0	28.3	63.3	38.0
PHB3253	26.7	36.7	36.7	41.7	53.3	39.0
P.4M	16.7	26.7	33.3	30.0	36.7	28.7
P.67/5243	20.0	35.0	30.0	36.7	41.7	32.7
P.77	26.7	38.3	41.7	38.3	55.0	40.0
Mean	22.3	29.5	32.6	30.1	43.4	31.6

$LSD_{(p\leq0.05)}$ MC = 8.1, $LSD_{(p\leq0.05)}$ V= 9, $LSD_{(p\leq0.05)}$ (M C xV) = NS, CV % = 33.8

M C= moisture content, V = variety, MCXV=moisture content xvariety, NS = not significant,.

Table 4.7 Percentage *A. flavus* infection of kernels harvested from cobs of 16 maize varieties grown at Waruhiu and inoculated with *A. flavus* and stored at varying moisture levels

	Short rain season					
	Moisture content (%)					
Variety (V)	10.0	13.5	15.0	17.0	20.0	Mean
DHO1	26.7	5.0	15.0	25.0	30.0	30.0
H 515	0.0	15.0	18.3	20.0	20.0	20.0
Duma 43	1.7	13.3	13.3	13.3	25.0	25.0
H 513	15,0	26.7	16.7	3.3	46.7	46.7
H 515	18.3	36.7	0.0	10.0	20.0	20.0
H 516	8.3	23.3	8.3	0.0	20.0	20.0
H 614	8.3	11.7	20.0	25.0	28.3	28.3
Katumani	13.3	23.3	15.0	3.3	18.3	18.3
KCB	3.3	28.3	23.3	35.0	36.7	36.7
P. 7M-10	15.0	25.0	15.0	23.3	21.7	21.7
P.3253	15.0	35.0	30.0	0.0	35.0	35.0
P.4M	26.7	33.3	18.3	13.3	25.0	25.0
P.67/5243	1.7	40.0	6.7	25.0	31.7	31.7
P.77	30.0	30.0	3.3	16.7	15.0	15.0
Mean	12.4	23.4	14.1	14.5	26.6	26.6

$LSD_{(p<0.05)}$ MC = 3.5, $LSD_{(p<0.05)}$ V= 6.7, $LSD_{(p<0.05)}$ (M xV) = 15, CV % = 56.8

	Long rain season					
	Moisture content (%)					
Variety (V)	10.0	13.5	15.0	17.0	20.0	Mean
DH O1	23.3	21.7	8.3	10.0	35.0	19.7
DHO2	1.7	23.3	23.3	18.3	56.7	24.7
DHO4	21.7	21.7	36.7	8.3	56.7	29.0
DK 8031	11.7	20.0	45.0	15.0	23.3	23.0
Duma	25.0	35.0	23.3	35.0	41.7	32.0
H 513	13.3	0.0	16.7	36.7	43.3	22.0
H 515	26.7	8.3	10.0	15.0	28.3	17.7
H 516	28.3	26.7	21.7	0.0	31.7	21.7
H 614	20.0	53.3	50.0	38.3	43.3	41.0
Katumani	25.0	0.0	8.3	10.0	30.0	14.7
KCB	23.3	0.0	23.3	28.3	21.7	19.3
P. 7M-10	11.7	11.7	6.7	0.0	41.7	14.3
PHB 3253	11.7	5.0	28.3	20.0	55.0	24.0
P.4M	21.7	0.0	16.7	20.0	5.0	12.7
P.67/5243	10.0	3.3	30.0	6.7	26.7	15.3
P.77	41.7	11.7	31.7	10.0	18.3	22.7
Mean	19.8	15.1	23.8	17.0	34.9	22.1

$LSD_{(p\leq0.05)}$ MC = 4, $LSD_{(p<0.05)}$ V=7.9, $LSD_{(p\leq0.05)}$ (M C xV) = 17.5, CV % = 44.8

LSD= least significant difference, M C= moisture content, V = variety, MCXV=moisture content xvariety.

Table 4.8 Percentage of maize kernels infected with *A. flavus* from Mwea and Waruhiu during short and long rain season of 2008 and 2009.

	Mwea			Waruhiu			
Variety (V)	SR	LR	Mean	SR	LR	Mean	Overall mean
DH O1	29.0	21.3	25.2	19.7	20.3	20.0	23.1
DHO2	26.7	32.0	29.3	24.7	14.7	19.7	25.5
DHO4	57.7	43.7	50.7	29.0	12.3	20.7	38.7
DK 8031	21.0	25.3	23.2	23.0	14.7	18.8	21.4
Duma	21.7	24.3	23.0	32.0	13.3	22.7	22.9
H 513	18.7	30.7	24.7	22.0	21.7	21.8	23.5
H 515	47.7	31.0	39.3	17.7	17.0	17.3	30.5
H 516	44.0	34.3	39.2	21.7	12.0	16.8	30.2
H 614	56.3	33.0	44.7	41.0	18.7	29.8	38.7
Katumani	10.7	24.3	17.5	14.7	14.7	14.7	16.4
KCB	19.3	27.0	23.2	19.3	25.3	22.3	22.8
P. 7M-10	45.7	38.0	41.8	22.7	19.0	20.8	33.4
PHB 3253	32.7	39.0	35.8	14.3	20.0	17.2	28.4
P.4M	37.0	28.7	32.8	12.7	23.3	18.0	26.9
P.67/5243	31.7	32.7	32.2	15.3	21.0	18.2	26.6
P.77	32.3	40.0	36.2	24.0	23.0	23.5	31.1
Mean	33.3	31.6	32.4	22.1	18.2	20.1	26.3

$LSD_{(p\leq0.05)}$ (Season)= 1.6, $LSD_{(p\geq0.05)}$ Site = 4.2, $LSD_{(p\geq0.05)}$ V = 4.5,

$LSD_{(p\leq0.05)}$ Site x Season X V= 9.5, $LSD_{(p\leq0.05)}$ SitexV= 7.1, $LSD_{(p\geq0.05)}$ site x season= NS,

$LSD_{(p\geq0.05)}$ Vx season =6.3, CV % = 15.7

V=variety, SR = short season, LR= long season, NS = not significant ($P\leq0.05$), LSD = least significant difference, CV=coefficient of variation.

4.1.4 Infection of maize varieties inoculated with *A. flavus* spore suspension in maize sampled at different times

The mean percentage of kernels infected with *A. flavus* was significantly affected by the varieties and sampling time, but the interaction between varieties and sampling time had no effect on *A. flavus* infection at Mwea during the short season (Table 4.9). Variety DHO4 had higher *A. flavus* infection than all the other varieties. Increase of sampling time from 7 to 84 days decreased infection (Table 4.9).

During the long rain season, mean percentage of kernels infected with *A. flavus* was significantly affected by the varieties, sampling time and interaction between varieties and sampling time. Variety DHO4 had a higher *A. flavus* infection than all the other varieties Increase in sampling time significantly affected *A. flavus* infection in DHO4, H614, KCB, P7M, P77 and P7M. As the sampling time increased from 7 to 28 days the infection rate increased only in P77, DHO1 and PHB 3253.. However, decrease of sampling time to 7 and 28 days increased infection relative to 84 days (Table 4.9).

The mean percentage of kernels infected with *A. flavus* was significantly affected by the varieties, sampling time, and the interaction between varieties and sampling time at Waruhiu during short season (Table 4.10). Variety KCB had significantly higher *A. flavus* infection than all the other varieties except DHO1, H513, P3253, P4M, P77 and P67. There were significant differences noted in maize sampled after 7, 28 and 84 days, As time increased from 28 to 84 days the infection rate decreased except for varieties DHO2, DK8031, H515, P77, P4M and P7M (Table 4.10).

During the long rain, mean percentage of kernels infected with *A. flavus* was significantly affected by the varieties, sampling time and interaction between varieties and sampling time. Variety H614 had a higher *A. flavus* infection than all the other varieties (Table 4.10).

Increase in sampling time significantly affected *A. flavus* infection in all varieties except DHO1, KCB, and P67M. There was a significant difference noted as the sampling time increased from 7 to 84 days. However there was a decrease of *A. flavus* infection at 84 relative to 28 days except for varieties DK8031 and H515 (Table 4.10). Variety, site, and the interaction among variety, site and season had a significant effect on percentage of kernels infected with *A. flavus* at both Mwea and Waruhiu (Table 4.11. During both seasons at Mwea and Waruhiu, DHO4 had the highest infection and Katumani the lowest infection (Table 4.11).

At Waruhiu during the short season, all the varieties had similar *Aspergillus* infection while during long season, DHO1, H513, KCB, P7M, P.3253, P67, P77 had higher *Aspergillus* infection. During both seasons at Mwea, P77, P67, P4M, P3253, P7M, H515, H516, H614 had higher infection than the other varieties while Katumani had the lowest infection (Table 4.11).

Table 4.9 Percentage *A. flavus* infection on kernels of 16 maize varieties grown at Mwea-KARI and inoculated with *A. flavus* and sampled at different times

Short rain season				
	Sampling time (days)			
Variety (V)	7	28	84	Mean
DHO1	30.0	23.0	11.0	21.3
DHO2	53.0	16.0	27.0	32.0
DHO4	56.0	39.0	36.0	43.7
DK 8031	42.0	23.0	11.0	25.3
Duma	33.0	32.0	8.0	24.3
H 513	41.0	41.0	10.0	30.7
H 515	47.0	34.0	12.0	31.0
H 516	42.0	43.0	18.0	34.3
H 614	44.0	35.0	20.0	33.0
Katumani	27.0	27.0	19.0	24.3
KCB	39.0	27.0	15.0	27.0
P. 7M-10	48.0	34.0	32.0	38.0
PHB 3253	44.0	44.0	29.0	39.0
P.4M	44.0	28.0	14.0	28.7
P.67/5243	43.0	34.0	21.0	32.7
P.77	53.0	30.0	37.0	40.0
Mean	42.9	31.9	20.0	31.6

$LSD_{(p\leq0.05)}$ T= 31.6, $LSD_{(p\leq0.05)}$ V=7.6, $LSD_{(p\leq0.05)}$ VXT= NS, CV% = 2.9

Long rain season				
	Sampling time (days)			
Variety (V)	7	28	84	Mean
DH O1	27.0	34.0	26.0	29.0
DHO2	39.0	33.0	8.0	26.7
DHO4	80.0	51.0	42.0	57.7
DK 8031	32.0	21.0	10.0	21.0
Duma	32.0	19.0	14.0	21.7
H 513	27.0	20.0	9.0	18.7
H 515	45.0	40.0	58.0	47.7
H 516	44.0	39.0	49.0	44.0
H 614	47.0	42.0	80.0	56.3
Katumani	14.0	10.0	10.0	11.3
KCB	39.0	16.0	3.0	19.3
P. 7M-10	64.0	50.0	23.0	45.7
PHB 3253	39.0	40.0	19.0	32.7
P.4M	55.0	36.0	20.0	37.0
P.67/5243	37.0	37.0	21.0	31.7
P.77	44.0	46.0	7.0	32.3
Mean	41.6	33.4	24.9	33.3

$LSD_{(p\leq0.05)}$ T= 7.9, $LSD_{(p\leq0.05)}$ V=13.5, $LSD_{(p\leq0.05)}$ VXT= 23.6, CV% = 23.2

V=variety, NS = not significant ($P\leq0.05$), LSD = least significant difference

Table 4.10 Percentage *A. flavus* infection on kernels of 16 varieties grown at Waruhiu and Inoculated with *A. flavus* and sampled at different times

	Short rain season			
	Sampling time (days)			
Variety (V)	7	28	84	Mean
DHO1	11.0	33.0	17.0	20.3
DHO2	9.0	16.0	19.0	14.7
DHO4	6.0	19.0	12.0	12.3
DK8031	7.0	17.0	20.0	14.7
DUMA	2.0	27.0	11.0	13.3
H513	3.0	31.0	31.0	21.7
H515	4.0	22.0	25.0	17.0
H516	5.0	18.0	13.0	12.0
H614	8.0	24.0	24.0	18.7
Katumani	0.0	24.0	20.0	14.7
KCB	8.0	34.0	34.0	25.3
PHB3253	3.0	32.0	25.0	20.0
P.77	7.0	30.0	32.0	23.0
P4M	0.0	34.0	36.0	23.3
P67	6.0	40.0	17.0	21.0
P7M	9.0	20.0	28.0	19.0
Mean	5.5	26.3	22.8	18.2

$LSD_{(p\leq0.05)}$ T=7.1, $LSD_{(p\leq0.05)}$ V= 5.7, $LSD_{(p\leq0.05)}$ TXV=11.5, CV % = 24.7

	Long rain season			
	Sampling time (days)			
Variety (V)	7	28	84	Mean
DHO1	14.0	19.0	26.0	19.7
DHO2	16.0	39.0	19.0	24.7
DHO4	12.0	40.0	35.0	29.0
DK8031	14.0	21.0	34.0	23.0
DUMA	13.0	42.0	41.0	32.0
H513	9.0	41.0	16.0	22.0
H515	7.0	15.0	31.0	17.7
H516	12.0	29.0	24.0	21.7
H614	25.0	61.0	37.0	41.0
Katumani	4.0	34.0	6.0	14.7
KCB	10.0	24.0	24.0	19.3
PHB3253	4.0	20.0	19.0	14.3
P.77	15.0	37.0	20.0	24.0
P4M	5.0	25.0	8.0	12.7
P67	11.0	22.0	13.0	15.3
P7M	11.0	35.0	22.0	22.7
Mean	11.4	31.5	23.4	22.1

$LSD_{(p\leq0.05)}$ T= 8.5, $LSD_{(p\leq0.05)}$ V= 7.9, $LSD_{(p\leq0.05)}$ TXV= 15.2 CV % = 24.1

Table 4.11 Percentage *A. flavus* infection of maize kernels infected with *A. flavus* from Mwea and Waruhiu during short and long rain season of 2008 and 2009.

Variety (V)	Mwea			Waruhiu			Overall mean
	SR	LR	Mean	SR	LR	Mean	
DH O1	29.0	21.3	25.2	15.0	20.3	17.7	22.2
DHO2	26.7	32.0	29.3	15.0	14.7	14.8	23.5
DHO4	57.7	43.7	50.7	15.0	12.3	13.7	35.9
DK 8031	21.0	25.3	23.2	15.0	14.7	14.8	19.8
Duma	21.7	24.3	23.0	15.0	13.3	14.2	19.5
H 513	18.7	30.7	24.7	15.0	21.7	18.3	22.1
H 515	47.7	31.0	39.3	15.0	17.0	16.0	30.0
H 516	44.0	34.3	39.2	15.0	12.0	13.5	28.9
H 614	56.3	33.0	44.7	15.0	18.7	16.8	33.5
Katumani	11.3	24.3	17.8	15.0	14.7	14.8	16.6
KCB	19.3	27.0	23.2	15.0	25.3	20.2	22.0
P. 7M-10	45.7	38.0	41.8	15.0	19.0	17.0	31.9
PHB 3253	32.7	39.0	35.8	15.0	20.0	17.5	28.5
P.4M	37.0	28.7	32.8	15.0	23.3	19.2	27.4
P.67/5243	31.7	32.7	32.2	15.0	21.0	18.0	26.5
P.77	32.3	40.0	36.2	15.0	23.0	19.0	29.3
Mean	33.3	31.0	32.4	15.0	18.2	16.6	26.1

$LSD_{(p\leq0.05)}$ Season =NS, $LSD_{(p\leq0.05)}$Site =2.7, $LSD_{(p\leq0.05)}$ V =NS, $LSD_{(p\leq0.05)}$ (Season x site) =4.6 $LSD_{(p\leq0.05)}$ Season x v =6.1, $LSD_{(p\leq0.05)}$ Sitex V=5.6, , $LSD_{(p\leq0.05)}$ Seasonx site X V = 8.3

SR = short season, LR= long season, NS = not significant ($P\leq0.05$), LSD = least significant difference, CV=coefficient of variation V=variety, S=season,

4.1.5 *Fusarium* infection of maize varieties inoculated with *A. flavus* and stored at varying moisture levels

The mean percentage of kernels infected with *Fusarium* sp was not significantly affected by moisture content, variety and their interaction at Mwea during the short rain season. The *Fusarium* infection ranged from 55.0 to 90% (Table 4.12).

During the long season at Mwea, the percentage of kernels infected with *Fusarium* sp was significantly affected by moisture content, variety and the interaction between moisture content and variety. Variety DUMA 43, and P77 had a higher *Fusarium* infection than all the other varieties. Increase in moisture levels significantly affected *Fusarium* infection in all varieties except in DHO1, Katumani, P7M, P4M and P67M. Increase of moisture levels to 15% and above significantly increased *Fusarium* infection in DHO2, DHO4, DUMA 43, H513, P3253 and P77. There were no significant differences in maize stored at 10% to 13.5% moisture levels (Table 4.12).

Moisture levels, variety and their interaction had a significant effect on kernels infected with *Fusarium* sp at Waruhiu during the short and long season. During the short season, Katumani, DK8031 and P77 had a higher *Fusarium* infection than all the other varieties. Increase in moisture levels significantly affected *Fusarium* infection in all varieties except in DHO1, DHO2, DK8031, H516, Katumani, P77, and P67M. Increase of moisture levels from 10 to 17% significantly increased *Fusarium* infection in DHO4, DUMA 43, H614, P4M and P7M. Maize stored at 20% had a relative higher *Fusarium* infection relative to those stored at 17% (Table 4.13).

During the long season, Katumani had a higher *Fusarium* infection than all the other varieties. Increase in moisture levels significantly affected *Fusarium* infection in all varieties

except in DHO2, DK8031, H515, Katumani and P3253 (Table 4.13). Maize stored at 10, 13.5 and 15% moisture levels had no significant difference while those stored at 17% and 20% were significant. Maize stored at 17% had a high *Fusarium* infection relative to those stored at 10 and 13.5% moisture level (Table 4.13).

Variety, site, and the interaction among variety, site and season had significant effects on the percentage of kernels infected with *Fusarium* at both Mwea and Waruhiu (Table 4.14). There was no significant difference between the seasons. At Mwea DUMA, P.77 had a higher *Fusarium* infection than the other varieties.

At Waruhiu DK8031, DUMA 43, Katumani had higher infection than the other varieties. Overall, P77, DUMA 43, KCB and DK8031 had higher *Fusarium* infection. In adddition, more *Fusarium* was isolated at Waruhiu than in Mwea (Table 4.14).

Table 4.12 Percentage *Fusarium* infection on kernels of 16 maize varieties grown at Mwea- KARI and inoculated with *A. flavus* and stored at varying moisture levels

	Short rain season					
	Moisture content (%)					
Variety (V)	10.0	13.5	15.0	17.0	20.0	Mean
DH O1	75.0	73.3	66.7	75.0	75.0	73.0
DHO2	80.0	85.0	80.0	68.3	65.0	75.7
DHO4	66.7	70.0	88.3	61.7	65.0	70.3
DK 8031	75.0	75.0	78.3	68.3	58.3	71.0
Duma	63.3	88.3	81.7	73.3	68.3	75.0
H 513	73.3	73.3	70.0	78.3	71.7	73.3
H 515	73.3	75.0	73.3	70.0	68.3	72.0
H 516	76.7	78.3	76.7	61.7	60.0	70.7
H 614	76.7	70.0	70.0	65.0	66.7	69.7
Katumani	70.0	73.3	83.3	63.3	66.7	71.3
KCB	71.7	85.0	70.0	80.0	60.0	73.3
P. 7M-10	80.0	80.0	78.3	58.3	68.3	73.0
PHB 3253	71.7	78.3	76.7	70.0	55.0	70.3
P.4M	85.0	78.3	90.0	63.3	75.0	78.3
P.67/5243	81.7	71.7	66.7	61.7	73.3	71.0
P.77	63.3	66.7	90.0	63.3	63.3	69.3
Mean	74.0	76.4	77.5	67.6	66.3	72.3

$LSD_{(p\leq0.05)}$ MC= NS, $LSD_{(p\leq0.05)}$ V= NS, $LSD_{(p\leq0.05)}$ MCXV= NS, CV % = 13.9

	Long rain season					
	Moisture content (%)					
Variety (V)	10	13.5	15	17	20	Mean
DH O1	76.7	83.3	75.0	73.3	76.7	77.0
DHO2	50.0	51.7	58.3	61.7	78.3	60.0
DHO4	45.0	46.7	71.7	90.0	93.3	69.3
DK 8031	80.0	83.3	78.3	66.7	71.7	76.0
Duma	76.7	80.0	86.7	95.0	100.3	87.7
H 513	43.3	65.0	51.7	78.3	76.7	63.0
H 515	43.3	38.3	56.7	33.3	23.3	39.0
H 516	40.0	33.3	30.0	16.7	5.0	25.0
H 614	53.3	53.3	80.0	75.0	95.0	71.3
Katumani	61.7	66.7	68.3	78.3	80.0	71.0
KCB	60.0	73.3	88.3	81.7	86.7	78.0
P. 7M-10	55.0	46.7	45.0	45.0	53.3	49.0
PHB 3253	46.7	46.7	46.7	60.0	73.3	54.7
P.4M	48.3	43.3	36.7	30.0	30.0	37.7
P.67/5243	68.3	65.0	71.7	78.3	68.3	70.3
P.77	71.7	78.3	80.0	91.7	98.3	84.0
Mean	57.5	59.7	64.1	65.9	69.4	63.3

$LSD_{(p\leq0.05)}$ MC= 4.5, $LSD_{(p\leq0.05)}$ V = 10.4, $LSD_{(p\leq0.05)}$ MCXV = 23.1, CV % = 11

V=variety,MC=moisture content,LSD=least significant difference

Table 4.13 Percentage *Fusarium* infection on kernels of 16 maize grown at Waruhiu and inoculated with *A. flavus* and stored at varying moisture levels

	Short rain season					
	Moisture content (%)					
Variety (V)	10.0	13.5	15.0	17.0	20.0	Mean
DH O1	70.0	78.3	63.3	70.0	70.0	70.3
DHO2	63.3	71.7	71.7	68.3	75.0	70.0
DHO4	41.7	46.7	80.0	91.7	75.0	67.0
DK 8031	81.7	81.7	83.3	88.3	75.0	82.0
Duma	50.0	76.7	85.0	95.0	73.3	76.0
H 513	55.0	76.7	61.7	90.0	60.0	68.7
H 515	48.3	70.0	85.0	60.0	26.7	58.0
H 516	68.3	66.7	70.0	56.7	50.0	62.3
H 614	58.3	68.3	75.0	81.7	93.3	75.3
Katumani	80.0	88.3	86.7	91.7	85.0	86.3
KCB	63.3	66.7	88.3	80.0	85.0	76.7
P. 7M-10	53.3	41.7	66.7	66.7	66.7	59.0
PHB3253	76.7	73.3	86.7	93.3	83.3	82.7
P.4M	81.7	78.3	78.3	83.3	60.0	76.3
P.67/5243	63.3	78.3	83.3	85.0	88.3	79.7
P.77	48.3	65.0	76.7	85.0	83.3	71.7
Mean	62.7	70.5	77.6	80.4	71.9	72.6

$LSD_{(p\leq0.05)}$ MC= 7.6, $LSD_{(p\leq0.05)}$ V= 9, $LSD_{(p\leq0.05)}$ MCXV= 20.8, CV % = 10.9

	Long rain season					
	Moisture content (%)					
Variety (V)	10	13.5	15	17	20	Mean
DH O1	46.7	75.0	61.7	85.0	78.3	69.3
DHO2	71.7	86.7	86.7	81.7	83.3	82.0
DHO4	35.0	63.3	86.7	86.7	75.0	69.3
DK8031	83.3	66.7	80.0	81.7	70.0	76.3
DUMA	88.3	70.0	90.0	98.3	66.7	82.7
H513	75.0	81.7	61.7	78.3	81.7	75.7
H515	80.0	80.0	80.0	66.7	80.0	77.3
H516	73.3	83.3	80.0	75.0	65.0	75.3
H614	93.3	73.3	85.0	73.3	81.7	81.3
Katumani	95.0	83.3	96.7	90.0	85.0	90.0
KCB	83.3	85.0	76.7	91.7	71.7	81.7
PHB 3253	76.7	65.0	76.7	80.0	75.0	74.7
P.77	83.3	61:7	65.0	88.3	88.3	77.3
P4M	76.7	70.0	76.7	95.0	85.0	80.7
P67	43.3	75.0	71.7	70.0	73.3	66.7
P7M	35.0	61.7	66.7	81.7	83.3	65.7
Mean	71.3	73.9	77.6	82.7	77.7	76.6

$LSD_{(p\leq0.05)}$ MC= 7.5, $LSD_{(p\leq0.05)}$ V = 5.8, $LSD_{(p\leq0.05)}$ MCXV = 17.3, CV % = 10.2

MC = moisture content, NS = not significant ($P\leq0.05$), LSD = least significant difference

Table 4.14 Percentage *Fusarium* infection on kernels of 16 maize varieties inoculated with *A. flavus* and stored at varying moisture levels at Mwea and Waruhiu during short and long rain season of 2008 and 2009

	Mwea			Waruhiu			
Variety (V)	SR	LR	Mean	SR	LR	Mean	Overall mean
DH O1	77.0	73.0	75.0	77.0	70.3	73.7	74.3
DHO2	60.0	75.7	67.8	60.0	70.0	65.0	66.4
DHO4	69.3	70.3	69.8	69.3	67.0	68.2	69.0
DK 8031	76.0	71.0	73.5	76.0	82.0	79.0	76.3
Duma	87.7	75.0	81.3	87.7	76.0	81.8	81.6
H 513	63.0	73.3	68.2	63.0	68.7	65.8	67.0
H 515	39.0	72.0	55.5	39.0	58.0	48.5	52.0
H 516	25.0	70.7	47.8	25.0	62.3	43.7	45.8
H 614	71.3	69.7	70.5	71.3	75.3	73.3	71.9
Katumani	71.0	71.3	71.2	71.0	86.3	78.7	74.9
KCB	78.0	73.3	75.7	78.0	76.7	77.3	76.5
P. 7M-10	49.0	73.0	61.0	49.0	71.7	60.3	60.7
PHB 3253	54.7	70.3	62.5	54.7	59.0	56.8	59.7
P.4M	37.7	78.3	58.0	37.7	76.3	57.0	57.5
P.67/5243	70.3	71.0	70.7	70.3	79.7	75.0	72.8
P.77	84.0	69.3	76.7	84.0	82.7	83.3	80.0
Mean	63.3	72.3	67.8	63.3	72.6	68.0	67.9

$LSD_{(p\leq0.05)}$ Site= 1.6, $LSD_{(p\leq0.05)}$ Season=NS, $LSD_{(p\leq0.05)}$ V= 5.5,
$LSD_{(p\leq0.05)}$ seasonxsite=4.6,
$LSD_{(p\leq0.05)}$ SitexSeasonXV=10.8, $LSD_{(p\leq0.05)}$ Sitex Variety = 8.5 ,
$LSD_{(p\leq0.05)}$ Vx Season= 7.2 CV % = 15.7

S=site , SR= Short rain, LR=long rain, NS = not significant
LSD=least significant difference, CV = coefficient of variation

4.1.6 *Fusarium* infection of 16 maize varieties inoculated with *A. flavus* in maize sampled at different time

Only the varieties significantly affected the percentage of kernels infected with *Fusarium* at Mwea during the short rain season (Table 4.15). During the short rain season, variety Duma, DHO1, KCB and P.77M had a higher *Fusarium* infection than all the other varieties. During the long rain season, variety DHO2, DUMA and P4M had higher infection than all the other varieties. As sampling time increased from 28 to 84 days, *Fusarium* infection significantly increased except in Katumani, KCB, P4M and P4M (Table 4.15).

At waruhiu during the short rain season, the variety, sampling time and their interaction significantly affected the percentage of kernels infected with *Fusarium* (Table 4.16). Variety DK8031, P77, Katumani had a higher *Fusarium* infection than all the other varieties. Increase in sampling times significantly affected *Fusarium* infection in all varieties except in DK8031, DUMA, H614, KCB, P.3253, P77, P67 and Katumani. As the sampling time increased from 28 to 84 days, *Fusarium* infection increased in DHO1, DK8031, H513, KCB and P77. There was a significant difference between 7 and 28 sampling times in *Fusarium* infection while no difference was noted between 28 and 84 sampling times. Maize sampled after 28 days had a higher *Fusarium* infection than those sampled at 7 and 84 days (Table 4.16).

Variety and sampling time significantly affected the percentage of kernels infected with *Fusarium* at Waruhiu during long rain season (Table 4.16). Variety DUMA, KCB and Katumani had a higher *Fusarium* infection than all the other varieties. Increase in sampling times significantly affected *Fusarium* infection in all varieties except in KCB and Katumani. As the sampling time increased from 28 to 84 days, *Fusarium* infection increased. There was

a significant difference between 7 and 28 days sampling times while no difference was noted between 28 and 84 days sampling times. Maize sampled after 84 days had a higher *Fusarium* infection than those sampled at 7 and 28 days.

Variety, site, and their interaction had significant effect on percentage of kernels infected with *Fusarium* (Table 4.17). At Waruhiu H614, P77 and DUMA 43 had a higher *Fusarium* infection than the other varieties. The short rain season had a higher infection than long rain season at both Waruhiu and Mwea (Table 4.17).

At Mwea, KCB, DUMA, H614, KCB, DK8031, P77 and DHO1 had a higher infection than all the other varieties. Overall, Mwea had a higher *Fusarium* infection than Waruhiu (Table 4.17).

Table 4.15 Percentage *Fusarium* sp infection on kernels of 16 maize varieties grown at Mwea-KARI and inoculated with *A. flavus* and sampled at different times

	Sampling time (days) Short rain season			
Variety (V)	7	28	84	Mean
DH O1	76.0	77.0	78.0	77.0
DHO2	76.0	43.0	61.0	60.0
DHO4	68.0	72.0	68.0	69.3
DK 8031	71.0	77.0	80.0	76.0
Duma	73.0	96.0	94.0	87.7
H 513	71.0	64.0	54.0	63.0
H 515	29.0	31.0	57.0	39.0
H 516	27.0	10.0	38.0	25.0
H 614	67.0	68.0	79.0	71.3
Katumani	70.0	63.0	77.0	70.0
KCB	78.0	87.0	69.0	78.0
P. 7M-10	68.0	40.0	39.0	49.0
PHB 3253	50.0	67.0	47.0	54.7
P.4M	29.0	33.0	51.0	37.7
P.67/5243	64.0	63.0	84.0	70.3
P.77	87.0	87.0	78.0	84.0
Mean	62.8	61.1	65.9	63.3
LSD$_{(p\leq0.05)}$T = NS, LSD$_{(p\leq0.05)}$ V= 13.5, LSD$_{(p\leq0.05)}$ TXV= NS, CV % = 23.2				
	Sampling time (day) Long rain season			
Variety (V)	7	28	84	Mean
DH O1	68.0	67.0	84.0	73.0
DHO2	67.0	75.0	85.0	75.7
DHO4	67.0	61.0	83.0	70.3
DK 8031	55.0	67.0	91.0	71.0
Duma	65.0	71.0	89.0	75:0
H 513	68.0	69.0	83.0	73.3
H 515	61.0	74.0	81.0	72.0
H 614	58.0	65.0	86.0	69.7
Katumani	56.0	79.0	79.0	71.3
KCB	59.0	78.0	83.0	73.3
P. 7M-10	61.0	76.0	82.0	73.0
PHB 3253	.63.0	62.0	86.0	70.3
P.4M	72.0	80.0	83.0	78.3
P.67/5243	62.0	64.0	87.0	71.0
P.77	65.0	59.0	84.0	69.3
Mean	62.8	69.9	84.4	72.3

LSD$_{(p\leq0.05)}$T=6.6, LSD $_{(p\leq0.05)}$V= NS, LSD $_{(p\leq0.05)}$TXV =NS, CV=3.5

LSD=Least significant difference,V=variety,T=sampling time

Table 4.16 Percentage *Fusarium* sp infection of kernels harvested from 16 maize varieties grown at Waruhiu and sampled at different times

	Short rain season			
	Sampling time (days)			
Variety (V)	7	28	84	Mean
DHO1	57.0	75.0	79.0	70.3
DHO2	60.0	75.0	75.0	70.0
DHO4	54.0	81.0	66.0	67.0
DK8031	79.0	82.0	85.0	82.0
DUMA	74.0	82.0	72.0	76.0
H513	48.0	76.0	82.0	68.7
H515	46.0	66.0	62.0	58.0
H516	35.0	77.0	75.0	62.3
H614	69.0	78.0	79.0	75.3
Katumani	82.0	90.0	87.0	86.3
KCB	74.0	77.0	79.0	76.7
P.3253	53.0	63.0	61.0	59.0
P.77	86.0	76.0	86.0	82.7
P4M	62.0	85.0	82.0	76.3
P67	78.0	80.0	81.0	79.7
P7M	60.0	80.0	75.0	71.7
Mean	63.6	77.7	76.6	72.6

L.S.D (t) = 7.1, L.S.D (Variety) = 11.7, L.S.D (t x variety) = NS, CV % = 6.1

	Long rain season			
	Sampling time (days)			
Variety (V)	7	28	84	Mean
DHO1	59.0	67.0	82.0	63.0
DHO2	77.0	76.0	93.0	76.5
DHO4	67.0	77.0	64.0	72.0
DK8031	66.0	79.0	84.0	72.5
DUMA	79.0	81.0	88.0	80.0
H513	65.0	78.0	84.0	71.5
H515	74.0	79.0	79.0	76.5
H516	59.0	80.0	87.0	69.5
H614	75.0	73.0	96.0	74.0
Katumani	85.0	90.0	95.0	87.5
KCB	81.0	79.0	85.0	80.0
P.3253	60.0	75.0	89.0	67.5
P.77	72.0	70.0	90.0	71.0
P4M	67.0	89.0	86.0	78.0
P67	48.0	69.0	83.0	58.5
P7M	46.0	81.0	70.0	63.5
Mean	67.5	77.7	84.7	72.6

$LSD_{(p<0.05)}$ T= 7.1, $LSD_{(p<0.05)}$ V= 11.7, $LSD_{(p<0.05)}$ VXT= NS, CV % = 6.1

LSD=Least significant difference, V=variety, T=sampling time

Table 4.17 Percentage *Fusarium* sp infection of kernels harvested from16 maize varieties inoculated with *A. flavus* at Mwea and Waruhiu

Variety	Waruhiu SR	Waruhiu LR	Waruhiu Mean	Mwea SR	Mwea LR	Mwea Mean	Overall mean
DH 01	70.3	19.6	45.0	73.0	77.0	75.0	67.5
DHO2	70.0	24.6	47.3	75.6	60.0	67.8	62.7
DHO4	67.0	29.0	48.0	70.3	69.3	69.8	64.3
DK 8031	82.0	23.0	52.5	71.0	76.0	73.5	68.2
Duma	76.0	32.0	54.0	75.0	87.6	81.3	74.5
H 513	68.6	22.0	45.3	73.3	63.0	68.2	62.4
H 515	58	17.6	37.8	72.0	39.0	55.5	51.0
H 516	62.3	21.6	42.0	70.6	25.0	47.8	46.3
H 614	75.3	41.0	58.2	69.6	71.3	70.5	67.4
Katumani	86.3	14.6	50.5	71.3	70.0	70.7	65.6
KCB	76.6	19.3	48.0	73.3	78.0	75.7	68.7
P. 7M-10	71.6	22.6	47.2	73.0	49.0	61.0	57.5
PHB3253	59	14.3	36.7	70.3	54.6	62.5	56.0
P.4M	76.3	12.6	44.5	78.3	37.6	58.0	54.6
P.67/5243	79.6	15.3	47.5	71.0	70.3	70.7	64.8
P.77	82.6	24.0	53.3	69.3	84.0	76.7	70.8
Mean	75.5	22.1	47.3	72.3	63.3	67.7	62.6

$LSD_{(p\leq0.05)}$ Season=3.1, $LSD_{(p\leq0.05)}$ Site =3.3, $LSD_{(p\leq0.05)}$ v=4.8 $LSD_{(p\leq0.05)}$ (Season x site) = 4.2, $LSD_{(p\leq0.05)}$ Season x v =7.1

$LSD_{(p\leq0.05)}$ Site x V=7.2, $LSD_{(p\leq0.05)}$ Season x site xV=10.0

SR = short season, LR= long season, NS = not significant ($P\leq0.05$)

V=variety, S=season, LSD = least significant difference, CV=coefficient of variation

4.1.7 Aflatoxins content (μg/kg) on 16 maize varieties inoculated with *A. flavus* and stored at varying moisture content at Mwea and Waruhiu

At Mwea, there was significant difference in aflatoxins content among the moisture content and varieties (Table 4.18). Variety KCB had the lowest aflatoxin content of 14.2 μg/kg while variety H516 had the highest aflatoxin content of 462 μg/kg . At Waruhiu, variety Katumani had the lowest aflatoxin content of 16.9 μg/kg while variety P.7M had the highest aflatoxin content of 264.4 μg/kg (Table 4.18). Overall, Mwea had higher aflatoxin content than Waruhiu.

Table 4.18 Aflatoxin content (µg/kg) in maize kernels of 16 varieties inoculated with *A. flavus* and stored at varying moisture content at Mwea and Waruhiu

	Mwea					
	Moisture content (%)					
Variety (V)	10	13.5	15	17	20	Mean
DHO1	422.8	62.1	72.7	9.4	53.7	124.1
DHO2	23.7	14.3	28.4	39.1	552.8	131.7
DHO4	165.4	21.9	123.1	22.2	25.3	71.6
DK8031	37.5	295.7	735.0	124.1	224.0	283.3
DUMA 43	349.0	221.5	200.0	29.9	33.7	166.8
H513	22.2	362.0	448.0	87.5	73.5	198.6
H515	528.5	63.8	584.0	22.2	636.6	367.0
H516	613.5	618.1	613.9	360.8	107.8	462.0
H614	591.0	560.0	574.0	22.4	92.0	367.9
Katumani	11.7	11.9	11.0	21.9	19.8	15.3
KCB	17.6	14.0	11.7	11.2	16.7	14.2
PHB3253	5.2	399.0	31.5	16.2	11.4	92.7
P4M	29.6	616.0	62.2	70.8	620.0	279.7
P67/5243	36.0	606.9	3.5	62.3	422.0	226.1
P77	19.6	19.8	54.3	60.0	54.6	41.7
P7M-19M	554.7	574.5	46.1	53.5	34.8	252.7
Mean	214.3	278.8	225.0	63.3	186.2	193.5

L.S.D$_{(p<0.05)}$ (MC) = 22.0.5, L.S.D$_{(p\leq0.05)}$ (V) = 32.5, L.S.D $_{(p<0.05)}$ (MCXV) = NS

	Waruhiu					
	Moisture content (%)					
Variety (V)	10	13.5	15	17	20	Mean
DHO1	21.7	30.1	2.5	53.9	27.2	27.1
DHO2	60.4	133.9	263.4	60.7	28.7	109.4
DHO4	32.7	44.6	67.0	77.7	52.8	55.0
DK8031	20.8	34.6	79.9	107.2	192.4	87.0
DUMA 43	30.6	59.5	470.0	14.0	14.6	117.7
H513	349	251.7	63.0	17.9	54.4	147.2
H515	50.1	24.9	71.1	308.3	52.1	101.3
H516	17.0	156.9	207.5	216.2	601.6	239.8
H614	146.1	12.9	42.2	155.9	85.4	88.5
Katumani	24.7	10.0	21.0	15.3	13.5	16.9
KCB	14.8	97.0	6.5	12.6	462	129.4
PHB 3253	13.3	18.1	297.3	16.8	15.4	72.2
P4M	340.7	73.9	242.8	269.8	18.4	189.1
P67/5243	43.1	35.0	163.2	27.2	21.3	58.0
P77	508.5	64.9	320.0	52.0	38.6	196.8
P7M-19M	297.8	92.1	574.0	248.3	109.7	264.4
Mean	123.2	71.3	184.1	103.4	111.8	118.7

L.S.D $_{(p\leq0.05)}$ (MC) =20 , L.S.D $_{(p\leq0.05)}$ (V) = 34, L.S.D $_{(p\leq0.05)}$ (MC x V) = NS,

V=variety, S=season, MC= moisture content, LSD = least significant difference, CV=coefficient of variation

4.2 Management *of A. flavus* and aflatoxins contamination by different fungal antagonists

4.2.1 Reduction of *A. flavus* growth *in vitro*

There was a significant difference in pathogen colony diameter for the cultures with different antagonists (Table 4.19). The highest colony diameter reduction among the antagonists was obtained in the treatment with *Trichoderma* (30.7%) while the lowest reduction was observed with *Aternaria*. The antagonist's colonies did not merge with those of the pathogens. *Bacillus* had a larger clear zone while *Trichoderma* had a smaller clear zone (Table 4.19).

Table 4.19 Percentage reductions in colony diameter and the clear zone of *A. flavus* inoculated together with effective antagonists

Treatments	Colony diameter	% reduction	Clear zones
Trichoderma+A. flavus	2.2	30.7	0.5
F. oxysporum+A. flavus	2.3	28.0	1.2
Alternaria+A. flavus	2.8	12.0	1.0
A. terreus+A. flavus	2.6	17.9	1.8
A. niger+A. flavus	2.4	23.3	1.7
Bacillus+A. flavus	2.5	20.8	2.4
A. flavus (Control)	3.2	0.0	0.0
LSD $_{(p \leq 0.05)}$ antagonists	0.4	2.9	0.3
CV	2.5	2.3	3.2

LSD=Least significant difference, CV= Coefficient of variation

4.2.2 Reduction of *A. flavus* and other fungal contaminants growth in *vivo*

The percentage of maize kernels infected with *Aspergillus* was significantly affected by variety, antagonists and their interaction. Variety DUMA and H516 had higher *A. flavus* infection than the other varieties (Table 4.20).

The percentage of maize kernels infected with *Penicillium* and *A. niger* was significantly affected by antagonists but not significantly affected by variety and the interaction between variety and different antagonists (Table 4.20).

When different maize kernels of different varieties were co-inoculated with *A. flavus*, the *A. flavus* infection increased significantly in all the varieties. In contrast, when maize kernels of different varieties were co- inoculated with *A. flavus* and different fungi antagonists, *A. flavus* infection significantly decreased. A great reduction was observed when maize kernels of different varieties were co–inoculated with *Trichoderma* and this was followed by *F. oxysporum* and lastly *A .niger*. The mean percentage of maize kernels infected with *F.oxysporum* was higher than *Aspergillus*, *Penicillium* and *A. niger* infection while the lowest infection was observed in *A. niger* (Table 4.20).

Table 4.20 Percentage infection of maize kernels of different maize varieties inoculated with *A. flavus* and different fungal antagonists.

Treatments	*A. flavus*	*Fusarium*	*Penicillium*	*A. Niger*
DUMA 43 + *A.flavus*	90.0	85.0	15.0	5.0
DUMA 43 + *F.oxysporum* + *A. flavus*	60.0	100.0	25.0	15.0
DUMA 43 + *Trichoderma* + *A. flavus*	0.0	70.0	0.0	0.0
DUMA 43 alone	70.0	100.0	20.0	20.0
H516 + *A. flavus*	80.0	100.0	0.0	0.0
H516 + *F.oxysporum* + *A. flavus*	15.0	95.0	10.0	0.0
H516 + *A.niger* + *A. flavus*	0.0	100.0	0.0	0.0
H516 + *Trichoderma* + *A. flavus*	0.0	75.0	0.0	0.0
H516 alone	10.0	100.0	5.0	25.0
Katumani + *A. flavus*	15.0	70.0	0.0	0.0
Katumani + *F.oxysporum*+ *A. flavus*	50.0	90.0	5.0	10.0
Katumani alone	20.0	100.0	45.0	0.0
Katumani + *A. niger* + *A. flavus*	15.0	100.0	30.0	35.0
Katumani + *Trichoderma* + *A. flavus*	10.0	90.0	0.0	0.0
KCB + *A. flavus*	70.0	60.0	35.0	0.0
KCB + *F. oxysporum* + *A. flavus*	15.0	85.0	0.0	0.0
KCB + *A. niger* + *A. flavus*	0.0	75.0	0.0	5.0
KCB + *Trichoderma* + *A. flavus*	10.0	75.0	5.0	0.0
KCB alone	40.0	90.0	50.0	20.0
Mean	29.0	86.5	15.0	7.8
L.S.D$_{(p\leq0.05)}$ (variety)	18.6	NS	NS	NS
L.S.D$_{(p\leq0.05)}$ (antagonists)	14.7	NS	15.6	10.5
L.S.D $_{(p\leq0.05)}$ (variety x antagonists)	31.1	NS	NS	NS
C.V	9.0	9.1	54.8	20.3

LSD=least significant difference, CV=coefficient of variation

4.2.3 Aflatoxin content (µg/kg) of maize kernels inoculated with *A. flavus* and different antagonists

There was a significant difference in aflatoxin content among the different varieties and antagonists (Table 4.21). Variety Katumani had a higher aflatoxin content than all the other varieties (61.6 µg/kg) while KCB had the lowest aflatoxin content (27.6). The aflatoxin content ranged between 11.2 to > 100 µg/kg. The highest reduction of *A. flavus* was observed in different maize varieties inoculated with *Trichoderma* while the lowest reduction of *A. flavus* was observed in different maize varieties inoculated with *A. flavus* alone.

Table 4.21 Aflatoxin content (µg/kg) of maize kernels of four different maize varieties inoculated with *A. flavus* and different antagonists

	Maize varieties			
	KCB	Katumani	Duma	H516
Control	43.4	41.9	32.4	67.9
A. flavus alone	41.9	70.0	20.0	47.6
A. flavus + *Trichoderma*	26.4	19.2	48.0	16.8
A. flavus + *A. niger*	11.2	76.8	48.4	31.8
A. flavus + *F. oxysporum*	15.3	100.0	38.4	14.8
Mean	27.6	61.6	37.4	35.8

LSD $_{(p\leq0.05)}$ variety= 33
LSD $_{(p\leq0.05)}$ antagonist=38.2
LSD $_{(p\leq0.05)}$ variety x antagonist= NS
CV =9

LSD=least significant difference, CV=coefficient of variation

CHAPTER FIVE: DISCUSSION

5.1 Evaluation of the susceptibility of different maize varieties to *Aspergillus flavus* and aflatoxin contamination

5.1.1 Kernel rot in maize varieties inoculated with *Aspergillus flavus*

The study found that the major fungi found in the maize varieties inoculated with *A. flavus* were *A. flavus* and *Fusarium* sp. The *A. flavus* rot were more than the *Fusarium* rots implying that all the varieties were more susceptible to *A. flavus* rot than the *Fusarium* rots. It also confirmed the Koch postrate that the pathogen introduced was the same one re-isolated.

The significant difference in *Aspergillus* rot among different varieties at both Mwea and Waruhiu suggested that the varieties differ in susceptibility to *A. flavus* infection. Katumani, DHO1, H515, P77 had higher *Aspergillus* rot than all the other varieties suggesting that they were more susceptible to *Aspergillus* rot. Host resistance may present a promising strategy for the pre-harvest prevention of mycotoxin contamination (Lopez-Garcia *et al.,* 1999).

This study also found out that Katumani, DH01, H515 were early maturing and more susceptible to *A. flavus* infection. Similar findings were reported by Betran and Isakeit, (2003) who evaluated maize of early, late, and full maturity at Texas and found out that maize of early hybrids had higher *A.flavus* infection than late-maturing hybrids. Lower aflatoxin contamination has been associated with the expression of secondary traits such as husk coverage and tightness, insect resistance, kernel integrity under environmental stress, and drought tolerance (Lillehoj *et al.,* 1975; Odvody *et al.,* 1997). This study presents a quantification of this relationship in that Panner 77 which had loose husk coverage had a high *A. flavus* infection. Similar studies were carried out at Texas with white and yellow

grained standards and Quality Protein Maize (Betrán *et al.*, 2002; Bhatnagar *et al.*, 2003). Hybrids with loose husks are more vulnerable to loss of kernel integrity (Odvody *et al.*, 1997), which can predispose kernels to *A. flavus* infection

The presence of *Fusarium* in the cobs inoculated with *A. flavus* suggested the presence of these fungi in the soil or air and that they attack maize much earlier than *Aspergillus*. Morenoa *et al.* (2009) reported *Fusarium* to be very abundant in the environment. Similar findings have shown that *Fusarium* species are probably the most prevalent mycotoxin-producing fungi and are commonly found in cereals (Yarzar and Omurtag 2008; Wang *et al.*, 2008). The presence of *Fusarium* could also suggest presence of mycotoxins in the maize. The most important *Fusarium* mycotoxins are fumonisins which are wide spread, acute toxicity to certain livestock and their potential carcinogenicity (Duvick, 2001).

5.1.2 Effect of moisture content on maize infection with storage fungi

This study found that varieties, moisture content and interaction between moisture content and varieties significantly affected the percentage of kernels infected with. At Mwea during long rain season, samples stored at 10% to 15% were not significantly different in percentage of kernel infection and increasing the moisture levels to 17% and 20% increased the *A. flavus* infection. This suggested that there were more fungal contaminants at 17 to 20% moisture than at 10 to 15%. Similar findings were reported by Chakrabarti (1986) who reported that infection in the field is favored by high moisture conditions. At 18-19% moisture *A. flavus* may grow quite rapidly in maize at favorable temperatures (Sauer, 1987) and temperatures of 20- 35°C are suitable for aflatoxins production. After harvest, maize should be dried to moisture content of 15% within 24 hours and long storage grain should be dried to 13%

moisture levels. Wrather and Sweets, (2008) reported that moisture content below 13% prevents invasion by *A. flavus* and fungal growth is best at 18% moisture levels. Awuah and Ellis, (2002) demonstrated that when groundnuts were dried to 6.6% moisture level, they were free of fungi regardless of the local storage protectant used for 6 months, whereas at 12% moisture content, only jute bags with the plant Syzigumaromaticum effectively suppressed the cross infection of healthy kernels.

The study also showed that different varieties respond differently to *A. flavus* infection with Katumani composite B showing good resistance to *A. flavus* infection while DH04 and H614 being susceptible to *A. flavus*. Higher *A. flavus* isolation in H614 could be attributed to its late maturity which resulted in prolonged exposure to high temperatures at flowering and post flowering which is favorable to fungal growth. Similar findings were reported by Betran and Isakeit, 2004 who reported in late maize hybrids had a greater exposure to higher temperatures at flowering and post flowering stages, greater *A. flavus* inoculum, and increased insect activity as .compared with early maturing. Although varieties of short, intermediate, and late maturities differed in aflatoxin accumulation, the differences seemed to be affected by other factors such as planting date, location, and climatic conditions (Betran and Isakeit, 2004). Short-season maize could escape growth-limiting conditions of a hot, dry summer and the associated aflatoxins in contrast to the full-season maize (Betran and Isakeit, 2004). Similar findings were reported by Njenga *et al,* (2008) who reported that Katumani which is an early maturing had low *A. flavus* isolation frequency and H614 which is a late maturing had a higher *A. flavus* infection.

5.1.3 Effect of sampling time on maize infection with storage fungi

Aspergillus flavus infection was isolated at higher frequency from maize samples stored at 7 and 28 days than those stored at 84 days which suggested that there were more fungal contamination at 7 and 28 days than at 84 days. Similar findings are reported by Garraway and Evans, (1984) who reported an increase in population of *A. flavus* at 1 and 2 months of storage, from 48.5 colonies/g to 586.8 colonies/g, However, at 3 months of storage, population of *A. flavus* decreased to 482 colonies/g. This was as a result of a decrease in moisture content, relative humidity and nutrition contents and there were other competitive fungi to *A. flavus*. This could explain the findings of my study that 28 days after incubating maize in khaki papers the *Aspergillus* infection decreased due to a decrease in moisture levels since the storage material could not maintain constant moisture levels. The decrease may also have occurred due to a decrease of viable spores (Wagacha and Muthomi, 2008). Inoculum potential is a pre-requisite for *Aspergillus* infection and subsequent aflatoxins production (Wagacha and Muthomi, 2008). Availability of viable spores has been considered important factors in aflatoxins production (Wagacha and Muthomi, 2008).

There were no significant differences on *Aspergillus* infection among the varieties at both Mwea and Waruhiu. This indicates that all varieties were equally susceptible to *A. flavus* infection. The high isolation of *A. flavus* in Mwea could be attributed to variation in temperature and rainfall (appendix 2).

5.1.4 Aflatoxins content in kernels from cobs inoculated with *Aspergillus flavus*

During this study, it was found out that there was a significant difference in aflatoxin content among the varieties. Variety H516, H614, H515 which were more susceptible to *A. flavus* had a higher aflatoxin content while Katumani (14.2μg/kg) which was less susceptible had

the lowest aflatoxin content. The study showed that all the varieties had more than the maximum 10μg /kg aflatoxin B1 allowed in Kenya suggesting chronic poisoning to the consumers (Fandohan *et al.*, 2005). Betran *et al.* (2002) and Berardo *et al.* (2007) found that susceptible maize varieties accumulated upto 80g/μkg compared to 0 in tolerant varieties. Betran and Isakeit, (2004) reported that early and intermediate hybrids have loose husk coverage than full season hybrids, a characteristic that was positively correlated with aflatoxin levels. Maturing time affected aflatoxin contamination with early maturing hybrids escaping aflatoxin contamination that result from environmetntal stress due to drought while late maturing get contaminated. Secondary traits such as husk coverage, heat stress tolerance and physical properties of pericarp are other factors contributing to aflatoxin resistance (Betran *et al.*, 2002).

5.2 Management of *A. flavus* and aflatoxin contamination by microbial antagonists

The study found significant differences in pathogen diameter for the culture with different antagonists. *Trichoderma* was observed to have a highest colony diameter reduction (30%) while *Alternaria* had the lowest colony reduction suggesting that *Trichoderma* inhibited fungal mycelial growth and development. Elad et al. (1983) reported that *Trichoderma* sp have ability to inhibit fungal pathogen growth and development thus used as a potential biocontrol agents. *Trichoderma* control pathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, antibiosis, or directly by mechanisms such as mycoparasitism (Benítez et al., 2004).

Aspergillus niger reduced the mycelim of *A. flavus* by 23% suggesting that it inhibited fungal mycelial growth and development. Several recent studies have promoted the antagonistic activity of *A. niger* toward different plant pathogens. Rai and Upadhyay, (2002) reported that

colonization of pigeon-pea substrate by *Fusarium udum* was highly suppressed by antagonism from *A. niger*, and *Trichoderma* viride when these were used in inoculums mixtures with F. udum or when substrates had already been colonized by them.

Aspergillus flavus infection decreased significantly when different maize varieties were co-inoculated with different antagonists. A great reduction of *Aspergillus* infection observed when different maize varieties were co-innoculated with *Trichoderma*. This could be attributed to highest reduction of colony diameter among the antagonists implying that *Trichoderma* is an effective bio control agent.

It was also found that *F. oxysporum* also reduced *A. flavus* infection. Luongo et al. (2005) reported the use of biological agents to suppress growth of fumonisin-producing fungi has been reported. Desjardins et al. (1998) observed inhibition of fumonisin formation by atoxigenic *F. verticillioides* strains although these caused higher disease incidence when applied through the silk channel. The observation implied that the ability to produce fumonisins is not required to produce ear rot and that effective colonization of plant with atoxigenic strains could competitively exclude fumonisin-producing strains or prevent them from producing fumonisins. Luongo et al. (2005) also reported suppression of saprophytic colonization and sporulation of toxigenic *F. verticillioides* and *F. proliferatum* in maize residue by nonpathogenic *Fusarium* species.

The study found out .that there was significant difference on aflatoxin content among varieties co-inoculated with different antagonists and *Trichoderma* was more effective in reducing aflatoxin content impling that it can effectively control aflation contamination in maize..

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The significant difference among varieties to *Aspergillus* ear rot suggested that varieties differed in levels of susceptibility. Maturity of different maize varieties was noted to influence ear rot infection. Short-season maize could escape growth-limiting conditions of a hot, dry weather and the associated aflatoxin in contrast to the full-season maize. Late maize hybrids can have greater exposure to higher temperatures at flowering and post flowering stages, greater *A. flavus* inoculum, and increased insect activity compared with early hybrids. Husk coverage also influenced *A. flavus* infection; loose husk coverage is normally associated with high *A. flavus* infection. It is also vunerable to loss of kernel integrity which disposes kernels to *A. flavus* infection.

Fusarium was predominantly isolated from the maize samples suggesting the presence of these fungi in soil, soil debris or airborne and possibility of contamination with fusarium mycotoxins such as fumonisins and deoxynivalenol.

The study found that moisture content significantly affected the percentage of kernels infected with *A. flavus*. Samples stored at 10% to 15% were not significantly different in percentage of kernel infection and increasing the moisture levels to 17% and above increased the *A. flavus* infection. This implies that drying harvested maize to 15% moisture content or lower can reduce the risk of fungal growth and subsequent aflatoxins production.

There was a significant difference in maize sampled 7 and 28 days after storage. Maize sampled 84 days after incubation in khaki papers decreased *Aspergillus flavus* infection due to a decrease in moisture content since the storage material encouraged rapid loss of moisture

and this may have lowered the water activity below the level necessary for fungal activity thus a decline in number of viable spores.

It was also found out that all the varieties were susceptible to *A. flavus* and aflatoxin contamination and they accumulated more than the maximum 10µg/kg aflatoxin B_1 allowed in Kenya. Variety H516 accumulating the highest aflatoxin content of 462µg/kg while KCB accumulated the lowest aflatoxins levels of up to 14.2µg/kg. *Trichoderma* was found to significantly reduce *A. flavus* infection and aflatoxin accumulation assuring the efficiency of *Trichoderma* as biocontrol agents against *A. flavus* and also indicate the need of production and development of *Trichoderma* based biocontrol agents to serve as a model for environmental friendly biocontrol agents.

6.2 Recommendations

1. More research to be done on susceptibility of *A. flavus* and aflatoxin on different maize varieties grown in Kenya under diverse environments .
2. Aflatoxins standards based on local conditions should be developed.
3. More research to be done on biological management of *A. flavus* and aflatoxin contamination.
4. Farmers in aflatoxin poisoning-prone areas should be encouraged to grow maize varieties that are less susceptible to *A. flavus* and and associated aflatoxin contamination.

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APPENDICES

Appendix 1. The temperatures and rainfall data at Waruhiu Agricultural Training Institute during the study period.

Month	Rainfall (mm)	Mean daily temperature (°C)
October	230.4	19.0
November	197.4	18.0
December	0.0	15.0
January	45.3	20.0
February	41.8	22.0
March	87.2	20.0
April	182.4	20.0
May	144.6	21.0
June	38.4	22.0
July	11.0	22.0
August	6.4	20.0
Total	984.9	219.0
Mean	89.5	19.9

Appendix 2. The temperatures and rainfall data at KARI-Mwea during the study period.

Month	Rainfall (mm)	Mean daily temperature (°C)
October	28.9	23.0
November	28.0	24.0
December	29.5	22.0
January	29.0	23.0
February	27.0	22.0
March	28.0	21.0
April	205.1	24.2
May	87.9	23.0
June	0.0	22.0
July	0.0	21.1
August	0.0	222.0
Total	463.4	247.3
Mean	42.1	22.5

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

Sucrose	30g
Oxoid agar no. 3	20g
Czapek stock solution A	50ml
Czapek stock solution B	50ml
Distilled water	900ml
Czapek stock solution A	
Sodium nitrate (NaNo3)	40g
Potassium chloride (Kcl)	10g
Magnesium Sulphate (MgSo4.7H20)	10g
Ferrous Sulphate (FeSO4.7H2O)	0.2g
Distilled water	100ml
Czapek stock solution B	
Dipotassium hydrogen phosphate (K2HPO4)	2g
Distilled water	100ml