

**MYCOTOXINS CONTAMINATION IN MAIZE AND GROUNDNUT:
IMPLICATIONS ON HOUSEHOLD FOOD SAFETY IN KILOSA DISTRICT,**

TANZANIA



**FOR REFERENCE
ONLY**

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**A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR
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ABSTRACT

Mycotoxins have become a major concern for health and economic problems all over the world particularly in the tropical countries. These countries, in particular are experiencing hazard exposure to mycotoxins. The aim of the study was to assess the effects of mycotoxin incidences in maize and groundnuts on household food safety in Kilosa District. Storage practices and weather variables (temperature, rainfall and relative humidity) were examined on their influence on mycotoxin contaminations. Eighteen samples of maize and groundnuts per household were collected from farmers in four villages. Weather variables were collected using data loggers. Aflatoxins and fumonisins analyses were done by the high performance liquid chromatography (HPLC) method. Log-linear model was used in estimating effect of weather variables. Ordinal logistic regression analyses were performed to predict the contribution of socio-demographic and socio-economic factors on the knowledge level of mouldy infections among respondents. Furthermore, multiple regression models were used in the analysis of factors contributing to mycotoxins production. The t-test, ANOVA and Chi-square tests were used to test the significance of relationships across variables. The levels of aflatoxins and fumonisins in all samples collected were $> 20 \mu\text{g}/\text{kg}$, which is the US Food and Drugs Authority regulatory level destined for use in human food. A significant difference ($p < 0.05$) existed between samples which were collected at different agroecological zones. Fumonisin concentration was significantly higher when maize had been stored in terms of heaps on a floor in a house ($179.54 \mu\text{g}/\text{kg}$) and lower levels of fumonisins were recorded when maize was stored in the crib ($135.91 \mu\text{g}/\text{kg}$). Fumonisin B₁ and aflatoxin B₁ were found to increase over the storage period and were significant ($p < 0.05$). There were highly significant ($p < 0.001$) effects of temperature, relative humidity and rainfall on the production of mycotoxins in stored maize. Roughly, one-fifth of maize produced for human consumption in the study area is lost through fungal spoilage and this loss

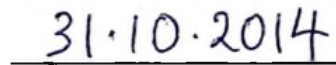
amounted to about 4331 tons per year. Most of the respondents had low level of knowledge regarding mouldy infection in stored crop products. Strategic interventions such as sorting, proper drying, proper storage and insect management to curtail mycotoxin contamination should be directed towards improved postharvest practices of maize and groundnuts to avoid food spoilage.

DECLARATION

I, KIJA STEVEN MAGEMBE, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.



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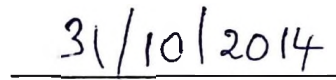


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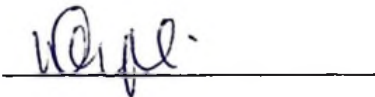
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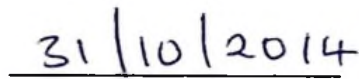
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“TO GOD BE THE GLORY”

DEDICATION

This work is dedicated to the memory of my late parents, Mr Godfrey Kija and Mrs Kwandu Hewa who laid down the foundation of my education, with a lot of sacrifices and efforts. Also, this work is dedicated to my loving wife, daughters and sons whom I love. May the Almighty God bless you all, AMEN!

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ABBREVIATIONS, SYMBOLS AND ACRONYMS

ACDI	Agricultural Cooperative Development International
AF	Aflatoxin
AFB ₁	Aflatoxin B ₁
AFBO	AFB ₁ -8,9-epoxide
AFM ₁	Aflatoxin M ₁
AIDS	Acquired Immune Defficiency Syndrome
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ARI	Agriculture Research Institute
a _w	Water activity
B ₁ , B ₂	Blue 1 and 2
CAST	Council for Agricultural Science and Technology
CDC	Centers for Disease Control and Prevention
Codex	Codex Alimentarius Commission
CV	Coefficient of variation
df	Degrees of Freedom
DON	Deoxynivalenol
ECFFC	European Commission Factsheet on Food Contaminants
EFSA	European Food Safety Authority
ELEM	Equineleukoncephalomalacia
ELISA	Enzyme- linked immunosorbant assay
EU	European Union
exp(x)	e ^x
ln(x)	Natural logarithm

FAO	Food and Agriculture Organization of the United Nations
FB ₁	Fumonisin B ₁
FDA	Food and Drug Administration
FFS	Farmer Field School
GAP	Good Agricultural Practices
GNP	Gross Net Profit
HACCP	Hazard Analysis and Critical Control Point
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HIV	Human Immune Virus
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
IFPRI	International Food Policy Research Institute
IITA	International Institute for Tropical Agriculture
LEM	Leukoencephalomalacia
LSD	Least Significant Difference
m.a.s.l	meters above sea level
NAFDAC	Nigeria Agency for Food and Drug Control
NDA	Naphthalene-2, 3-dicarboxaldehyde
Ng	Nanogram
NGOs	Non-Governmental Organizations
PHDR	Population Human Development Report
PLWHA	People Living With HIV/AIDS
ppb	Parts per billion
ppm	Parts per million

p-values	Standard normal probability
RH	Relative Humidity
SE	Standard Error of the mean
SED	Standard Error of the Difference
SD	Standard Deviation
T-2 toxin	Type-A Trichocethenes
TBS	Tanzania Bureau of Standards
TDRI	Tropical Development and Research Institute
TFDA	Tanzania Food and Drugs Authority
TMV ₁	Tanzania Maize Variety 1
µg/kg	Microgramme per kilogramme
UN	United Nations
URT	United Republic of Tanzania
US	United States
USD	United States Dollar
USAID	United States Agency for International Development
VIF	Variance Inflation Factor
VOCA	Volunteers in Overseas Cooperative Assistance
WHO	World Health Organization
ZEA	Zearalenone

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Mycotoxins are natural chemical substances produced by fungi (moulds) which grow as contaminants on some food items obtained from crops (in the field and in the storage), in particular cereals, nuts and fruits (ECFFC, 2007; Zinedine and Mañes, 2009). Their presence in crops, humans, and animal feeds is undesirable (Peraica *et al.*, 2014). They are toxic and can affect the immune system, nervous system, liver, kidney, and blood (Wild and Gong, 2010). In addition, some mycotoxins are known to be carcinogenic (Rahimi *et al.*, 2010; Pedro *et al.*, 2014). The fungi produce mycotoxins from conidia which allow them to survive in substrates and soil for the extended periods of time (Scheidegger and Payne, 2003; Probst *et al.*, 2010). The conidia are the principal source of primary inocula. The most important mycotoxins are aflatoxins, ochratoxins, deoxynivalenol (DON), zearalenone (ZEA), fumonisins, and T-2 like toxins (Wagacha and Muthoni, 2008; Codex Alimentarius, 2011). Food borne mycotoxins which are likely to be of greatest significance in the tropical developing countries are the fumonisins and aflatoxins (Kumar *et al.*, 2008; Kriska *et al.*, 2008; Khlangwiset *et al.*, 2011). Aflatoxin B₁, the most toxic of the aflatoxins, is a potent liver carcinogen, causing hepatocellular carcinoma (HCC) in humans and a variety of animal species and may lead to stunted growth in children (Khlangwiset *et al.*, 2011).

It has been estimated that 5 to 10% of the world's food production is lost as a result of fungal spoilage (Pitt and Hocking, 2009). Also it has been estimated that more than five billion people in developing countries are at risk of exposure to aflatoxins through contaminated foods (Strosnider *et al.*, 2006). Much of Sub-Saharan Africa is at risk of

unsafe levels of aflatoxin exposure that can negatively affect human health, food security and economic trade (Williams *et al.*, 2004). Aflatoxins also impact international trade. Globally, about USD 1.2 billion in commerce is lost annually due to aflatoxin contamination, with African economies losing USD 450 million each year due to lost trade (Bandyopadhyay and Lopez, 2013).

Aflatoxins are potent hepatotoxic and hepatocarcinogenic compounds which are produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* which are found particularly in hot and humid climates (Matić *et al.*, 2009; Peiwu *et al.*, 2012). The most important species which produce aflatoxins is *A. flavus* which mostly contaminates a variety of staple foods including maize and groundnuts (Kumar *et al.*, 2008). Similarly, the most important species which produces fumonisins is *Fusarium moniliforme* which is a common fungal contaminant of maize and maize-derived products worldwide (Wang and Zhu, 2006; Wild and Gong, 2010). In Asia and Africa, human hepatic cancers and acute fatal diseases, including hepatic lesions, have been associated with consumption of foods heavily contaminated with these toxins (Muture and Ogana, 2005; Wild and Gong, 2010). In 2011, the famine victims from parts of Pokot and Marakwet in West districts of Kenya rejected maize supplied to them by the government because of heavy infection with *Aspergillus* spp. (Ndanyi, 2011).

Countries such as Tanzania, those are located between 40°N and 40°S latitude, offer suitable growing conditions for the fungi (Strosnider *et al.*, 2006; Hussaini *et al.*, 2012). The available data show the presence of fumonisins in the stored maize in Tanzania. Doko *et al.* (1996) reported fumonisin levels of up to 225 µg/kg in maize from Tanzania. In the late 2000s, Kimanya *et al.* (2008a) reported that 52% of samples of maize which was collected from the year 2005 harvests in four maize producing regions of Tanzania,

namely Tabora, Iringa, Ruvuma and Kilimanjaro, contained levels of up to 11 048 $\mu\text{g}/\text{kg}$. In the same study, aflatoxins were detected in 18% of 120 samples at levels up to 158 $\mu\text{g}/\text{kg}$ with 12% of all samples exceeding the Tanzania maximum limit (ML) of 10 $\mu\text{g}/\text{kg}$, for total aflatoxins. Similarly, high levels of aflatoxins contaminations of maize and cassava in Tanzania had been reported (Manjula *et al.*, 2009). The rural populations are therefore exposed to high levels of mycotoxin throughout their lifespans.

The occurrence of mycotoxins in crops is strongly influenced by weather during and after the growing season including accumulation during storage. Conditions such as high temperatures and moisture during harvest, unseasonal rains during harvest and flash floods can lead to fungal proliferation and production of mycotoxins (Bhat and Vasanthi, 2003). Similarly, poor harvesting practices, improper storage and less than optimal conditions during transportation, marketing and processing can also contribute to fungal growth and increase the risk of mycotoxins production (Wild and Gong, 2010; World Bank *et al.*, 2011).

In a country like Tanzania whose economy depends on agriculture, mycotoxin problems can pose a serious threat not only to individual producers but also to the welfare of the entire nation in terms of reduced income and widespread food shortage. However, no study has been conducted to relate mycotoxin problems in maize and groundnuts on household food safety in Kilosa. Therefore, the aim of this study was to assess the implications of the incidence of mycotoxins in maize and groundnuts on household food safety in Kilosa District, Tanzania.

1.2 Problem Statement and Justification

Mycotoxin contamination is a serious problem in tropical and sub-tropical regions of the world (Narayan, 2014). Areas situated between 40°N and 40°S of the equator, which include all of Africa, are the most at risk potentially exposing up to 5 billion people in the developing world (Williams *et al.*, 2004). Commodities stored under these conditions get easily deteriorated and susceptible to fungal infections (Sulaiman *et al.*, 2007). The main fungal species of (*Aspergillus flavus*) and (*Fusarium moniliforme*) which produce these mycotoxins thrive under favourable conditions on a wide range of foods and feed such as maize and groundnuts, and are a world-wide problem (Wu and Khlangwiset, 2010). Mycotoxin contamination can occur before harvest when the crop undergoes drought stress due to elevated temperatures at the grain filling stages and when wet conditions occur at harvest periods (Wild and Gong, 2010; Proietti *et al.*, 2014). Contamination also occurs when there is insect damage, early and delayed harvesting, improper drying, high relative humidity and high temperature, farmers' storage practices, poorly constructed storage structures and high moisture levels during storage and transportation (Miraglia *et al.*, 2009; Hell and Mutegi, 2011).

The direct economic damage of mycotoxins are: losses that affect the entire chain of food and feed production by the reduction of marketable grain, price discounts, increased inability to obtain loans on stored grain, and disposal of useless crops (burning) (Zinedine and Mañes, 2009). Mycotoxin contaminations are also known to have health hazards on both humans and animals (Godet and Munaut, 2010), but also they reduce non-ruminant productivity thereby reducing profitability of animal industries (CAST, 1989). During seasons of extensive mycotoxin contamination, grain shortages may occur leading to the rise of prices and costs for keeping livestock and poultry. Furthermore, consumers of grain products have to pay high prices due to demand created by shortages.

Mycotoxin contamination of stored maize and groundnuts in Kilosa District is ill-quantified although visual observation of the said stored commodities show severe contamination by storage fungi. So far only few surveys on the natural occurrence of mycotoxin contamination have been conducted in the rural areas of Tanzania, and very little information has been generated. Inadequate technical, human and economic resources may be a reason for this paucity of information. Kimanya *et al.* (2008a) did a study on the fumonisin contaminations in maize in Tanzania; however this study was only limited to a review of toxicological exposure of fumonisins and its consequences to human health. Similarly, Muzanila *et al.* (2000) did a research on residual cyanogens, chemical composition and aflatoxins in cassava flour from Tanzanian villages and reported no aflatoxin in eighteen (18) samples of cassava processed by smallholder farmers using sun drying and solid state fermentation in Tanzania.

Similarly, Manjula *et al.* (2009) did a research on aflatoxin and fumonisin contamination of cassava products from markets in Tanzania. Low levels of fumonisins ranging from 0 to 0.07 ppm were found in cassava chips and flour with mean values ranging from 0.001 to 0.006 ppm. In view of the information from the cited studies, there is need of getting additional data on incidences of mycotoxin contamination in stored maize and groundnuts and the implication (of this contamination) on household food safety in Kilosa District where maize and groundnuts production is predominant. Specifically, there is lack of information on the incidence and levels of mycotoxin across agroecological areas and seasons, the association between storage practices and mycotoxin contamination, the effect of weather on mycotoxin contamination, the effects of food spoilage and fungi induced loss to household food security and factors contributing to mycotoxin contaminations in maize and groundnuts. Such knowledge would help in raising awareness and increase understanding among farmers on how to manage levels of

mycotoxins in grains and other food items at a household level. Similarly, knowledge would provide vital information to policy makers and other stakeholders such as Tanzania Bureau of Standards (TBS) geared towards enforcement of existing regulations on mycotoxins policy formulation on agriculture and setting standards on minimum aflatoxin and fumonisin levels in maize and groundnuts in Tanzania.

1.3 Objectives of the Research and Hypothesis

1.3.1 General objective

The study aimed at assessing the effects of mycotoxin incidence in maize and groundnuts on household food security and safety in Kilosa District.

1.3.2 Specific objectives

Specifically, the study intended to:

- i. determine the incidences of mycotoxins in household stored maize and groundnuts;
- ii. assess the effects of storage practices on mycotoxin incidences in maize and groundnuts;
- iii. assess the effect of weather on mycotoxin incidence in maize and groundnuts;
- iv. analyze the effects of mycotoxin contamination and loss due to food spoilage on household food security and safety in the study area; and
- v. examine farmers' practices contributing to mycotoxins contaminations in stored maize and groundnuts.

1.3.3 Null operational hypotheses

Five operational null hypotheses in this study were clearly stated as follows:

- H0₁:** Mycotoxin contamination in stored maize and groundnuts has no effect on household food safety.
- H0₂:** Weather conditions have no effect on mycotoxin contamination in household stored maize and groundnuts.
- H0₃:** Storage practices have no impact on mycotoxin contamination in household stored maize and groundnuts.
- H0₄:** Storage time has no effect on the level of mycotoxin contamination in household stored maize and groundnuts.
- H0₅:** Sociodemographic and socio-economic factors have no effect on determining the awareness of mouldy infections in stored maize and groundnuts.

1.4 Conceptual Framework

The potential interactions among variables which were taken into account are shown in Fig.1 and include: interaction between relative humidity and temperature during storage, harvesting, and drying period; and interaction between temperature and rainfall during storage; interaction between geographical location and weather; interaction between cultural practices and storage practices and fungal productions; and finally, between the amount of food spoiled and household food safety.

High temperature and high relative humidity tend to enhance mould growth and toxins production. It has been observed that in most cereal grains, every 10°C rise in temperature cause an increase of about 3% in relative humidity (ACDI/VOCA 2003). In most instances, mycotoxins are formed after harvest, particularly when harvesting is done during floods, or unseasonal rains or when there is improper storage of insufficiently dried agricultural commodities. If commodities are incorrectly stored, that is, in an improperly dried state or under high humidity with inadequate protection, fungi will

inevitably grow. Direct losses are related to the reduced crop yields for growers and due to the reduced animal performance and increased losses due to disease for livestock producers. The reduced yields and decreased animal performance may be so widespread as to pass unnoticed in many instances. This is especially true when the level of infection and/or contamination is low. Disease symptoms are more readily apparent and losses can be catastrophic in individual cases. Direct consequences of consumption of mycotoxin-contaminated animal feed include: reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence (due to immune-suppression), and reduced reproductive capacities (Morgavi and Riley, 2007) which leads to economic losses (Wu, 2004; Wu, 2006). The consumption of mycotoxin contaminated commodities is related to several acute and chronic diseases in human and animals (Bhat and Miller, 2010).

For the grower, mycotoxin contamination will restrict markets, reduce the marketable value of the crop, and may render the crops unmarketable. There would also be increased costs associated with fungicide or pest control. For the livestock producer, increased costs are associated with both searching for uncontaminated feed supply as well as secondary costs associated with feeding the animals with contaminated feed. The latter include veterinary bills, decontamination or dilution of contaminated feedstuffs, reproductive failures and loss of markets. There is also a considerable cost to the industry as a whole, in terms of research, monitoring, and extension. There are also extra handling and distribution costs, increased processing costs, legal suits, and loss of consumer confidence in the safety of food products. In order to capture the essential highlights of these relationships, a schematic representation of interactions among major variables is depicted in Fig. 1.

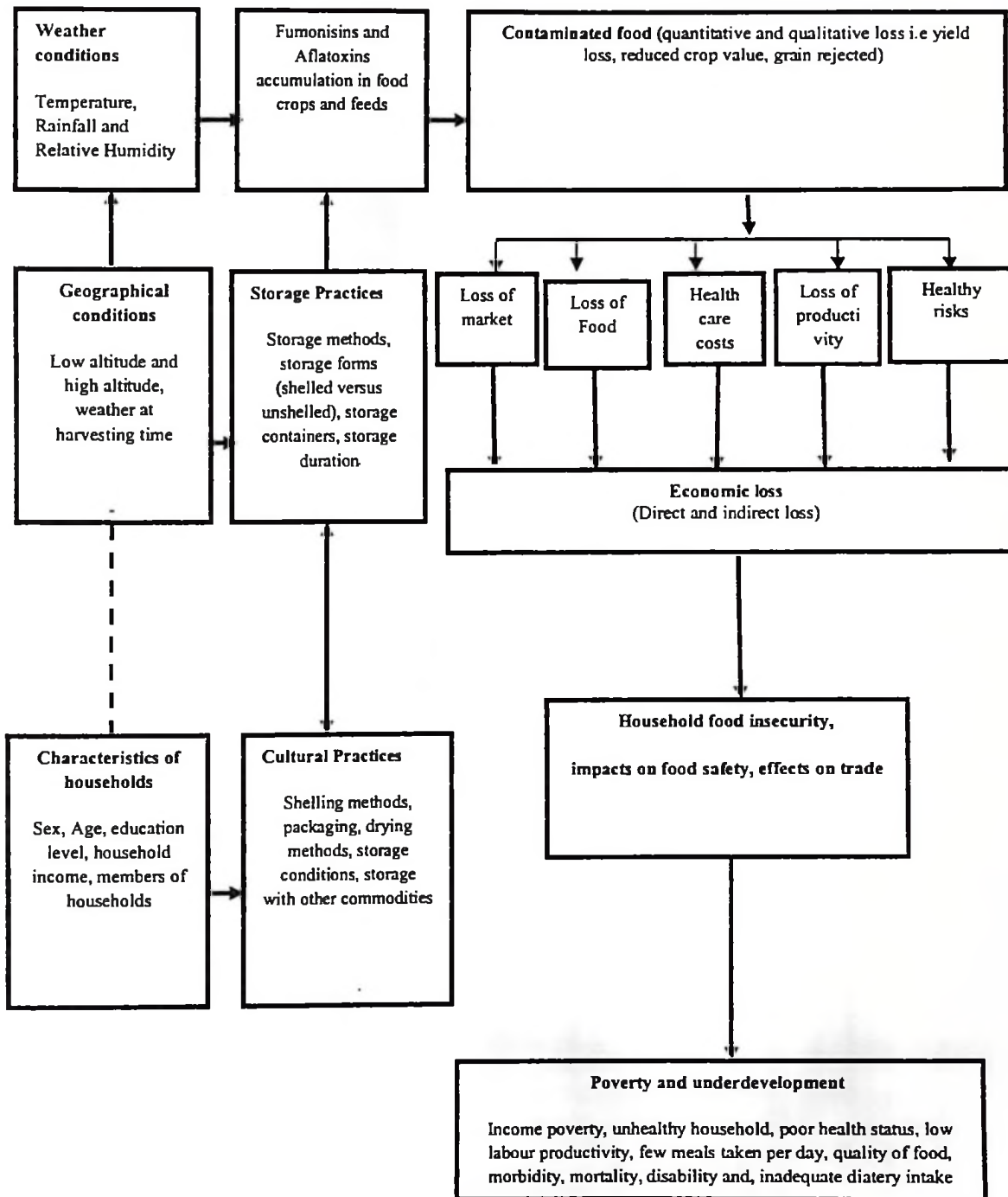


Figure 1: Conceptual framework for factors influencing mycotoxin production and contaminations in household stored maize and groundnuts

1.5 Organization of the Thesis

The thesis is organised into five chapters. Chapter One discusses the background to the research and makes a case for the justification of the study and outlines the objectives of the study. Chapter Two reviews the literature on incidences of mycotoxins in various crops, factors affecting the production of mycotoxin in stored maize and groundnuts and outlines health and economic implications of the mycotoxin on household's food security and safety. The Chapter also reviews the effect of weather on mycotoxin incidences and the effects of moulds. Different approaches used to control mycotoxins are also reviewed. Chapter Three presents the methodology used in the study. This chapter presents the study area and different techniques followed in the study. The study findings are presented and discussed in Chapter Four; in the light of other related works on mycotoxin contamination in stored maize and groundnuts. Chapter Five presents general conclusions and recommendations in the light of the results of the work as a whole. The Chapter also suggests further investigations to be done to enhance knowledge on the mycotoxin contamination.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History of Toxigenic Filamentous Fungi

Filamentous fungi such as *Aspergillus* spp. and *Fusarium* spp. are widespread and found in a number of foods and feeds (Bullerman, 1986). All fungi produce secondary metabolites, but some produce secondary metabolites such as the mycotoxins that are toxic to humans and animals and which cause enormous economic losses to the grain trade and the marketing of foods and feeds annually (Richard, 2007). The most notorious of these toxigenic fungi include *Aspergillus*, *Fusarium*, *Stachybotrys*, *Penicillium* and *Alternaria*. Mycotoxins were recognized as a major problem after the death of thousands of poultry (turkey chicks, over 100 000) and other birds in England in the 1960s (Mohamed, 2011; Kensler *et al.*, 2011). Massive hepatic necrosis, parenchyma cell degeneration, and bile duct proliferation characterized the disease that affects poultry (Mohamed, 2011). The cause of these deaths was eventually traced to the groundnuts (peanut) meal in the feed that contained toxin producing fungus, *Aspergillus flavus*. The mycotoxins that caused the disease was subsequently identified and named aflatoxins (Allcroft and Carnaghan, 1963).

Aflatoxins are known to be hepatotoxic, carcinogenic, and teratogenic. The aflatoxin-producing moulds occur widely in temperate, sub-tropical, and tropical climates throughout the world. The aflatoxins may be produced, both before and after harvest on many foods and feeds especially oilseeds, edible nuts and cereals (Coker, 1997). Although the aflatoxins are predominantly associated with commodities from sub-tropical and tropical origin, their occurrence has also been reported in temperate climates in acid-treated grains (Pettersson *et al.*, 1989). Human fatalities have also occurred from acute

aflatoxin poisoning in India (in 1974), when unseasonal rains and a scarcity of food prompted the consumption of heavily-aflatoxin contaminated maize (Krishnamachari *et al.*, 1975; Lawley 2013). The most widespread of the *Aspergillus* mycotoxins are aflatoxins. At least 6 forms of naturally occurring aflatoxins have been identified: aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁), and aflatoxin G₂ (AFG₂) are found in plant-based food, while aflatoxin M₁ (metabolite of B₁) and aflatoxins M₂ are found in foods of animal origin (Muro-Cacho *et al.*, 2004), aflatoxin B₁ (AFB₁) is the most harmful form due to its direct link to human liver cancer (Leslie *et al.*, 2008; USAID, 2012). The hierarchy of toxicity is in the order of B₁>M₁>G₁>B₂>M₂>G₂ (Frag, 2008). At present, aflatoxin B₁ is considered to be among the strongest natural known carcinogens, and regarded as a quadruple threat, i.e., as a potent toxin, carcinogens, teratogen, and mutagen (Waliyar *et al.*, 2008). World Health Organization (WHO) categorizes aflatoxins as class number 1 carcinogens, as they are highly poisonous, toxic substances (Martinez *et al.*, 1994).

AFM₁ is the hydroxylated metabolite of AFB₁ and is also known to have 2-10% of the carcinogenic potency of the parent compound (Zinedine *et al.*, 2007b; Boudra *et al.*, 2007). The carry over of this carcinogen occur in cows at a transfer ratio (consumed AFB₁ to excreted AFM₁) of 200:1 (Smith and Moss, 1985), which could be as high as 40:0.05 (JECFA, 2001) into human and animal milk that are the main sources of nutrition for infants whose vulnerability due to underdeveloped immune system is obvious. This poses a serious health concern.

The second most commonly found toxigenic fungi in corn are species of *Fusarium*. The first case of moldy-corn poisoning of horses linked to *Fusarium* fungi was reported in the US in the early 1900's and the disorder was identified as equine leukoencephalomalacia (ELEM), a disease of the central nervous system that affects

horses, mules, and donkeys (Prelusky *et al.*, 1994). Outbreaks of the *Fusarium* related disease ELEM have also occurred in South Africa, Brazil and USA (Marasas *et al.*, 1984a). ELEM is an acutely fatal neurological disorder with clinical signs such as ataxia, paresis, hypersensitivity and locomotor derangements. The porcine manifestation is characterised by pulmonary oedema as well as pancreatic and liver damage, with cases confirmed in Brazil and USA (D'Mello *et al.*, 1998). Fumonisin B₁ is classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2002).

Mycotoxins affect a wide range of agricultural products, including cereals, dried fruits, dried cassava, sweet potatoes and banana chips, nuts, coffee beans and oilseeds, which are food security crops and the backbone of most developing countries economies (Reddy *et al.*, 2011). These major crops are highly susceptible to fungal infection which occurs as a result of favourable environmental conditions (such as high humidity and moisture availability) for fungi to grow on the substrates in the field. Other favourable conditions include improper harvesting, high moisture content of the stored food items, storage and processing operations. As defined by Pitt (1996), mycotoxins are fungal metabolites which when ingested, inhaled or absorbed through the skin cause lowered performance of the body, sickness or death to man or animals, including birds. Mycotoxins are considered to be among the most significant food contaminants with negative impact on public health, food safety and security and the national economy of many countries, particularly the developing ones. Huge economic losses are caused by incidences of mycotoxins. European Union regulation on aflatoxins costs Africa huge amount of USD 750 million each year in exports of cereals, fruits and nuts (Otzuki *et al.*, 2001; Diaz Rios and Jaffee, 2008). Grain fungal infection is one of the most serious biotic constraints in the

production of maize and groundnuts. Those moulds and mycotoxins which are currently considered to be of world-wide importance are shown in Table 1.

Table 1: Major species of fungi which produce mycotoxins in food and feed

Fungus	Mycotoxin	Food/feed affected
<i>Fusarium graminearum</i>	HT-2; Type B: DON	Wheat, maize, barley
<i>Fusarium culmorum</i>	Fumonisin	Maize
<i>Fusarium moniliforme</i>	Fumonisin	Maize
<i>Aspergillus flavus</i>	Aflatoxin B ₁ , B ₂	Maize, peanuts
<i>Aspergillus parasiticus</i>	Aflatoxin B ₁ , B ₂ , G ₁ , G ₂ , Cyclopiazonic acid	Maize, peanuts
<i>Aspergillus ochraceus</i>	Ochratoxin A	Cereals, coffee
<i>Aspergillus versicolor</i>	Sterigmatocystin	Fruits, peanuts, local beer
<i>Fusarium crookwellense</i>	Zearalenone	Cereals, rice, local beer, silage
<i>Penicillium verrucosum</i>	Ochratoxin A, Citrinin	Cereals, wheat
<i>Penicillium aurantiogriseum</i>	Penicillic acid, Citreoviridin	Barley
<i>Penicillium citrinum</i>	Cyclopiazonic acid	Nuts, fruits
<i>Penicillium expansum</i>	Patulin	Fruit, vegetables, silage

Source: ECFFC, 2007; EFSA, 2011.

2.2 Types of Aflatoxins and their Physico-chemical Properties

Aflatoxins dissolve in various polar organic solvents including methanol, aqueous acetone and aqueous hexane-acetone-water azeotrope that develop for extraction procedures of natural products and precipitate in petroleum, ether or hexane (Andrallos *et al.*, 1967). Among the 18 identified different types of aflatoxins, the most important are aflatoxin B₁, B₂, G₁ and G₂. Aflatoxins fluoresce under the ultraviolet light. Aflatoxin B₁ and B₂ emit blue fluorescence whereas aflatoxin G₁ and G₂ emit green fluorescence. The quantity and relative proportion of four compounds in culture extracts vary depending on the mould strain, medium composition and cultural conditions. Normally, aflatoxin B₁ is present in the largest amounts whereas B₂ and G₂ are produced in small amounts (Wogan, 1966). Aflatoxins can be produced after ingestion of contaminated food. The hydroxylated aflatoxin derivatives, aflatoxin M₁ and M₂ are detected in the milk of cow that has been fed with toxic meal. The metabolites are toxic as their parent compounds (De Longh *et al.*, 1964).

The molecular formula of aflatoxin B₁ and G₁ are C₁₇H₁₂O₆ and C₁₇H₁₂O₇ respectively. Aflatoxins B₂ and G₂ are the di-hydro derivatives of the parent compounds and molecular formula of these are C₁₇H₁₄O₆ and C₁₇H₁₄O₇, respectively. Some of the physical properties of the compounds are summarized in Table 2. All four aflatoxins have high melting points. The chemical structure of aflatoxin B₁, B₂, G₁ and G₂ were proposed in 1963 (Mohamed, 2011). Structurally, aflatoxin B₁ consists of five rings, those with furofuran moiety (rings B and C), an aromatic six-membered ring (A), a six-membered lactone ring and either a five-membered pentanone or a six-membered lactone ring (D) and either a five-membered pentanone or a six-membered lactone ring (E) as shown in Fig. 2. Physical characteristics of aflatoxins are shown in Table 2. The ultraviolet and infrared absorption spectra are similar in all four compounds. The maximum fluorescence emission of aflatoxin B₁ and B₂ are 425 nm, and for aflatoxin G₁ and G₂ are 450 nm. The emission property can be used for the estimation of aflatoxins concentration by fluoresce technique (Sobolev, 2007).

Several conditions lead to degradation of compounds such as upon standing in methanolic solution. This process is greatly accelerated in the presence of light or heat. The compounds are substantially degraded on chromatograms that are exposed to air and ultraviolet or visible light (Wogan, 1966).

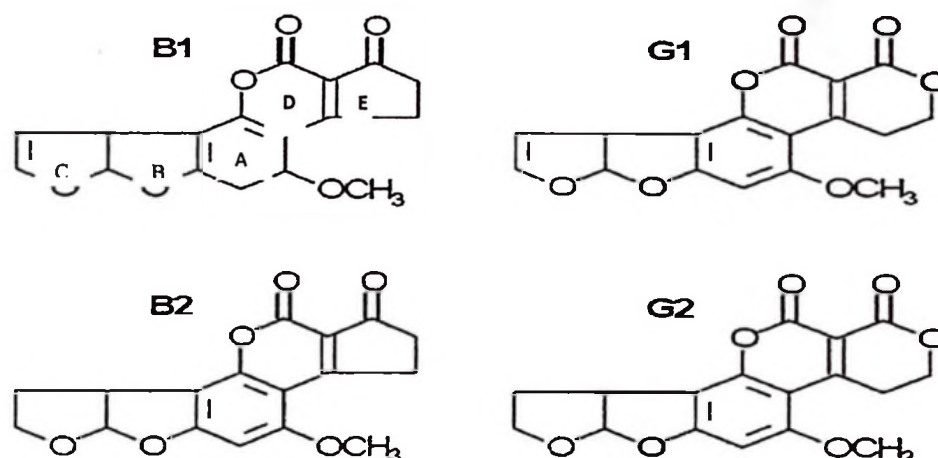


Figure 2: Chemical structures of aflatoxins B₁, B₂, G₁ and G₂

Source: Mohamed, 2011; Fujimoto, 2011

2.2.1 Properties of aflatoxins

Hell (1997) identified four major groups of aflatoxins namely: B₁, B₂, G₁ and G₂ as shown in Table 2. These abbreviations are indicative of the colours they exhibit/or fluoresce under the ultraviolet light (385 nm); thus B is for blue and G is for yellow-green, the M is a hydroxylated metabolic product of B (Bankole and Adebajo, 2003).

Table 2: Physical properties of aflatoxins

Property	Aflatoxin					
	B ₁	B ₂	G ₁	G ₂	M ₁	M ₂
Chemical formula	C ₁₇ H ₁₂ O ₆	C ₁₇ H ₁₄ O ₆	C ₁₇ H ₁₂ O ₇	C ₁₇ H ₁₄ O ₇	C ₁₇ H ₁₂ O ₇	C ₁₇ H ₁₄ O ₇
Molecular weight	312	314	328	330	328	330
Melting point	268-269	286-289	244-246	237-240	299	293
Fluorescence emission	425 nm	425 nm	450 nm	450 nm	425 nm	425 nm
Sorbent	Pentane	m	m	m	Ethy acetate	Methanol
Fluorescence under UV light	Blue	Blue	Green	Green	Blue	Blue

Source: Wogan, 1966

2.2.2 General health hazards of aflatoxins

In general, aflatoxins produce a number of adverse effects in a range of biological systems including plants, animals, and humans (Averkiewa, 2009). Aflatoxin B₁ is the most toxic compound in the series of aflatoxins. It is found to be one of the most potent

carcinogens occurring naturally. Because of frequent contamination of aflatoxin B₁ in agricultural commodities such as peanuts, corn, and animal feed stuffs, aflatoxin problems become a potential hazard to human and animal health (Busby and Wogan, 1979).

2.2.3 Effects of aflatoxins in animals

Aflatoxins have been shown to be lethal to many animals in natural and experimental conditions (Kokić *et al.*, 2009). Animal susceptibility to carcinogenesis by aflatoxins varies with sex, age, species, strain within the species, hormonal and nutritional status of the animal. Consumption of mycotoxin contaminated feed has resulted in a number of disease outbreaks in farm and domestic animals. Outbreaks which are mostly attributable to aflatoxins have been reported in turkeys (Austwick, 1978), pigs (Cook, 1989), horses (Vesonder, 1991), dairy cattle, poultry, rabbits, dogs and camels (Bhat and Nageswara, 1989). Aflatoxins also causes nutrient modifications like vitamin A or D in animals and thus making them unavailable for the normal body physiology and hence leads to nutritional deficiencies (USAID, 2012).

The effects of mycotoxins are usually more obvious in domesticated animals and extrapolations to humans are often based on these observations. These are supported by laboratory-based animal studies. Chronic effects such as (a) decreased productivity, (b) subtle but chronic damage to vital organs and tissues, (c) increased disease incidence because of immune suppression, and (d) interference with reproductive capacity are much more prevalent than acute livestock death (Richard *et al.*, 1983). At least one mycotoxin affects each system in the animal body *via* direct or indirect mechanisms of toxicity. Several important mycotoxins can affect the same system; for example, the immune system; and a given mycotoxin may affect several systems. The true nature of such

intoxications require a more holistic comprehension of the complexities of chemical, biochemical, metabolic, molecular, and environmental bases for mycotoxicoses. Finally, nursing animals may be affected by exposure to AF metabolites secreted in the milk. The guidelines for acceptable aflatoxin levels in feeds are shown in Table 3.

Table 3: Guidelines for acceptable aflatoxin levels in feeds

Animal	Feed	Aflatoxin (ppb)
Finishing beef cattle	Maize and groundnuts product	300
Beef cattle, swine or poultry	Maize and groundnuts products	300
Finishing swine of 100 lb or greater	Maize and groundnuts product	200
Breeding beef cattle, breeding swine or mature poultry	Maize and groundnuts product	100

Source: FDA, 1994.

Note: 1 lb = 453.59265 g or 0.45359 kg

2.2.4 Effects of aflatoxins in humans

Cases in which acute poisoning occurred due to the consumption of foods heavily contaminated with aflatoxins have been reported mainly from Tropical countries such as Nigeria (Aja-Nwachukwu and Emejuaiwe, 2006) and Uganda (Kaaya and Warren, 2005; Kaaya and Kyamuhangire, 2006). In India, an acute aflatoxicosis outbreak occurred and affected man and dogs due to consumption of aflatoxin contaminated maize (Krishnamachari *et al.*, 1975). The data on aflatoxins and human cancer have shown a positive correlation between aflatoxin ingestion and liver cancer in population studies in which aflatoxin intake and the incidence of primary liver cancer were concurrently estimated (Shank *et al.*, 1972). Between 2004 and 2006, nearly 200 Kenyans died after consuming maize contaminated with high levels of aflatoxins. In the 2004 outbreak in Kenya, concentrations of aflatoxins B₁ in maize was found to be as high as 4400 ppb, which is 220 times greater than 20 ppb, limit for food suggested by Kenya authorities (CDC, 2004). Recently, in 2010 over 2 million bags of maize in the Eastern and Central provinces of Kenya were found to be highly contaminated and were not tradeable

(Manyong *et al.*, 2012; Bandyopadhyay and Lopez, 2013). The presence of aflatoxins in human breast milk and cord sera, the teratogenic, carcinogenic and immunotoxic effects of aflatoxins in humans and the possible relationships between aflatoxin exposure and kwashiorkor and Indian childhood cirrhosis are discussed by Raisuddin *et al.* (1993). In 1994, Adhikari *et al.* (1994) investigated the possible effects of consumption of aflatoxin contaminated food on kwashiorkor and reported that the exposure of dietary aflatoxins compounded the effects of kwashiorkor.

The possibility that some cases of Reye's syndrome (encephalopathy with fatty degeneration of the viscera) might be due to aflatoxin ingestion that was reported by Dvorácková *et al.* (1977) in Czechoslovakia, where they detected aflatoxins in the livers of patients with Reye's syndrome. Also aflatoxins cause a pulmonary interstitial fibrosis (Dvorácková and Píchová, 1986). Aflatoxin exposure is also associated with reduced levels of secretory immunoglobulin A (IgA) in children (Turner *et al.*, 2003). Changes in differential subset distributions and functional alterations of specific lymphocyte subsets have been correlated with aflatoxin exposure in Ghanaian adults and indicated that aflatoxins could cause impairment of human cellular immunity that could decrease resistance to infections (Jiang *et al.*, 2005). Aflatoxin B₁ has also been found to be a potent mutagen. Mutagen studies on bacteria suggest that the possible mechanism of initiating mutagenesis is the ability of the aflatoxin to bind the nucleic acids such as DNA.

Aflatoxins are being implicated to cause genetic mutations in people suffering from primary liver cancer particularly in sub-Saharan Africa and Southeast Asia (Bressac *et al.*, 1991). The International Agency for Research on Cancer provides sufficient evidence that AFB₁ and mixtures of AFB₁, AFG₁ and AFM₁ are proven carcinogens, classifying them

as Group 1 carcinogens (IARC, 1993), while AFM₁ and AFB₂ are designated as Group 2B. The deleterious pathway is as follows: AFB₁ is metabolized (by the liver) to AFB₁-8,9-epoxide (AFBO) or to less mutagenic forms which then can either result in (1) cancer, (2) toxicity, or (3) be excreted from the organism (Luch, 2008; Sanchis *et al.*, 2013). The cancer is thus a result of the formation of DNA-adducts by AFBO bonding with genetic material (Sanchis *et al.*, 2013). In general, aflatoxins produce a number of adverse effects in a range of biological systems including plants, animals, and humans (Averkieva, 2009).

2.3 Types of Fumonisin and their Physico-chemical Properties

2.3.1 Properties of fumonisins

Fumonisin is a white hygroscopic powder which dissolve in water to at least 20 g/L, they are soluble in methanol, acetonitrile-water (WHO, 2000). Fumonisins are stable in acetonitrile-water (1:1) at 25⁰C and they are unstable at 25⁰C, forming monomethyl or dimethyl-esters (Visconti *et al.*, 1994). Also they are stable in methanol at -18⁰C (Visconti *et al.*, 1994). They are stable at 78⁰C in buffer solutions at PH between 4.8 and 9.0 (Howard *et al.*, 1998). At least 15 different fumonisins have so far been reported and other minor metabolites have been identified; however most of these have not been shown to occur naturally. They are grouped into four main categories (Torres *et al.*, 2007; Kumar *et al.*, 2008). FA₁, FA₂, FA₃, FAK₁; FB₁, FB₂, FB₃, FB₄; FC₁, FC₂, FC₃, FC₄; FP₁, FP₂ and FP₃. FB₂, FB₃ and FB₄ differ from FB₁ in that they lack hydroxyl groups present in FB₁; FA₁, FA₂ and FA₃ are like FB₁, FB₂ and FB₃, but are *N*-acetylated (Musser and Plattener, 1997). FAK₁ is like FA₁ but is 15-keto functionalized. FCs are like FBs but lack the methyl group adjacent to the amino group. FPs have a 3-hydroxypyridium group instead of the amine group in the FBs (Musser and Plattener, 1997).

In contrast to the other mycotoxins, which are soluble in organic solvents, fumonisins are hydrosoluble, which hinders their study, and it is probable that many other mycotoxins remain undiscovered due to this hydrosolubility characteristic. The fumonisin B₁, the most extensively studied, is a diester of propane 1,2,3- tricarballic acid (TCA) and 2-amino-12,16-dimethyl-3,5,10,14,15- pentahydroxycosane (Bezuidenhout *et al.*, 1988; Thiel *et al.*, 1992). Fumonisin B₁ (FB₁) is usually produced at higher levels than the other three fumonisins (Rheeder *et al.*, 2002). The minimum water activity (a_w) for the growth of *F. moniliforme* is 0.87; the maximum limit is recorded as greater than 0.99. The minimum, optimal and maximum temperatures for the growth of *F. moniliforme* are 22.5-27.5 and 32-37°C, respectively. The physical and chemical properties of FBs are shown in Table 4 and the chemical structure of FB₁, FB₂ and FB₃ are shown in Fig. 3.

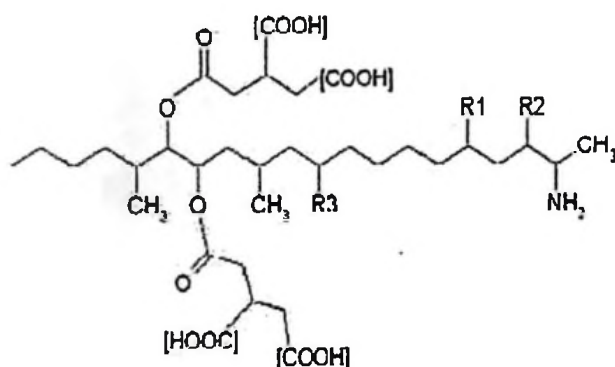
The presence of fumonisins in maize grains has been associated with cases of esophageal cancer in inhabitants of the region of Transkei in southern Africa, in China and in northeastern Italy (Peraica *et al.*, 1999). Fumonisin is also responsible for the leukoencephalomalacia in equine species and rabbits (Marasas *et al.*, 1988); pulmonary edema and hydrothorax in pigs (Harrison *et al.*, 1990); and hepatotoxic, carcinogenic and apoptosis (programmed cell death) effects in the liver of rats (Gelderblom *et al.*, 1988). Fumonisin has been isolated from maize sold in a supermarket in Charleston (South Carolina), the city with the highest incidence of the occurrence of esophageal cancer among Afro-Americans in the USA (Sydenham *et al.*, 1991). Fumonisin is a family of polyketide-derived mycotoxins that are epidemiologically associated with esophageal cancer and neural tube defects in some human populations as well as multiple diseases in livestock animals (Gelderblom *et al.*, 1988; Marasas *et al.*, 2004).



Table 4: Chemical structures and molecular weight of fumonisins

Fumonisin	R ₁	R ₂	Group R ₃	R ₄	R ₅	R ₆	Formula	Molecular weight
FA ₁	TCA	OH	OH	OH	NHCOCH ₃	CH ₃	C ₃₆ H ₆₁ NO ₁₆	763
FA ₂	TCA	H	H	OH	NHCOCH ₃	CH ₃	C ₃₆ H ₆₁ NO ₁₅	747
FA ₃	TCA	OH	OH	H	NHCOCH ₃	CH ₃	C ₃₆ H ₆₁ NO ₁₅	747
FAK ₁	=O	OH	OH	OH	NHCOCH ₃	CH ₃	C ₃₀ H ₅₃ NO ₁₁	603
FB ₁	TCA	OH	OH	OH	NH ₂	CH ₃	C ₃₄ H ₅₉ NO ₁₅	721
FB ₂	TCA	H	H	OH	NH ₂	CH ₃	C ₃₄ H ₅₉ NO ₁₄	705
FB ₃	TCA	OH	OH	OH	NH ₂	CH ₃	C ₃₃ H ₅₇ NO ₁₄	705
FC ₁	TCA	OH	OH	H	NH ₂	H	C ₃₉ H ₆₂ NO ₁₅	707
FP ₁	TCA	OH	OH	OH	3HP	CH ₃	C ₃₉ H ₆₂ NO ₁₆	800
FP ₂	TCA	H	H	OH	3HP	CH ₃	C ₄₀ H ₆₉ NO ₂₀	784
FP ₃	TCA	OH	OH	H	3HP	CH ₃	C ₄₀ H ₆₂ NO ₁₅	784

Source: EFSA, 2005; Musser and Plattner, 1997



Fumonisin B₁: R₁=OH; R₂=OH; R₃=OH

Fumonisin B₂: R₁=H; R₂=OH; R₃=OH

Fumonisin B₃: R₁=OH; R₂=OH; R₃=H

Figure 3: Chemical structures of fumonisin B₁, fumonisin B₂ and fumonisin B₃

Source: Musser and Plattner, 1997; Fujimoto, 2011

2.3.2 General health hazards of fumonisins

Fusarium species are the most encountered toxigenic contaminant in human foods including cereal grains, beans and oil seeds (Chelkowski, 1989a). In maize, *Fusarium* species are both plant pathogens as well as soil saprophytes (Chelkowski, 1989b). There is a growing concern about fumonisins in corn because they are omnipresent in all corn products, including corn-based breakfast cereals, cornmeal, corn flakes, tortillas, tortilla chips, popcorn and sweet corn (Shephard *et al.*, 1996). They have also been found in a survey of tortilla chips and sweet corn from retail outlets in Italy (Doko and Visconti,

1994). Although there are more *Fusarium* mycotoxins found in corn than in any other crop due to the numerous species of *Fusarium* that can attack the plant, *F. moniliforme* is the most common soil-borne pathogen found in corn in all regions of the world. Frequently, *Fusarium* can cause both symptomless infections of corn plants as well as grain infection (Marasas *et al.*, 1984b). It is not unusual to find 100 percent internal kernel infection by *F. verticillioides* (Marasas *et al.*, 1984b). The fungus is transmitted “vertically and horizontally” to the next generation of plants via clonal infection of seeds and plant debris.

In the endophytic phase, the fungus infects the plant vertically, starting from the fungus in the inter-cellular tissues of the seeds spreading throughout the plant during the growing season without showing signs of infection. Thus *F. moniliforme* is a seed transmitted pathogen that does not produce any symptoms of infection (Kedera *et al.*, 1994). Environmental factors that favour kernel infection by *Fusarium* are warm temperatures (20-30°C) and rainfall, especially shortly before silks emerge (Lacey, 1989). Plants remain susceptible to the infection for 7 to 10 days after occurring of the silks. Other environmental factors which increase the likelihood of infection are physical damage to the maturing ear such as from birds or insects, drought stress, warm, dry climate, and the presence of other fungal diseases (Miller, 2001).

2.3.3 Effects of fumonisins in animals

Fumonisins affect animals in different ways by interfering with sphingolipid metabolism (Merrill *et al.*, 1993). They cause leukoencephalomalacia (hole in the head syndrome), a fatal disease in equines (Marasas *et al.*, 1988) and hepatotoxic and carcinogenic effects in rats (Gelderblom *et al.*, 1996). Exposure to fumonisin (FB₁) in maize causes leukoencephalomalacia (LEM) in horses and pulmonary oedema in pigs. Fumonisin B₁ is

also toxic to the central nervous system, liver, pancreas, kidney and lung in a number of animal species.

2.3.4 Effects of fumonisins in humans

The presence of fumonisins in maize has been linked with the occurrence of human oesophageal cancer in the regions of Transkei (South Africa), China and northeast Italy (FAO, 2001). The relationship between exposure to *F. moniliforme*, in home-grown maize, and the incidence of oesophageal cancer has been studied in the Transkei during the ten-year period 1976-1986 (Rheeder *et al.*, 1992). High human esophageal cancer rates associated with fumonisins after the consumption of home-grown contaminated maize have been reported in China (Li *et al.*, 1980), Southern Africa (Marasas, 1996) and Italy (Franceschi *et al.*, 1990). Fumonisins were discovered and characterized in 1988 (Gelderblom *et al.*, 1988; Bezuidenhout *et al.*, 1988) from fusaria. Fumonisin B₁ inhibited the growth and induced morphological features consistent with apoptosis of human cells *in vitro* (Tolleson *et al.*, 1996) hence; fumonisins may cause esophageal epithelial apoptosis. A research conducted by Kimanya *et al.* (2010) in rural areas of Tanzania, showed that the exposure of fumonisin to infants negatively affected growth. Fumonisins (FBs) are also involved in human diseases other than esophageal cancer, but their role is not yet resolved. Fumonsins (FBs) derivatives may also be toxic and exert effects by indirect mechanisms.

2.4 Incidences of Mycotoxins in Various Crops

2.4.1 Incidences of aflatoxins in various foods

Aflatoxins (AFs) are often detected in cereals and their derivatives, groundnuts, and spicies (USAID, 2012). According to an EFSA report (2007) in which 34 326 sample analysed from EU countries were pooled (including export, import, company, and market

control samples), the highest percentage of positive samples occurred in Brazil nuts, pistachios, and spices (LOD) was 0.1–0.2 µg/kg for AFB₁ and 0.2–0.4 µg/kg for total aflatoxins. The susceptibility of groundnuts to infection with aflatoxin producing fungi has been noted elsewhere (Baozhu *et al.*, 2009) and high levels of aflatoxin have been recorded in the nuts (Soler *et al.*, 2010). Similarly aflatoxins have been detected in 19% of the samples of maize and groundnuts products, wheat flour and parboiled rice collected in Khat- mandu (Nepal) and 17% of foods in Indonesia containing groundnuts, a vulnerable ingredient widely used in local cookery (Winarno, 1993). Aflatoxins occurred in 68% of street-vended snacks in Lagos (Nigeria); in particular, corn-based, groundnut-based and wheat- based snacks were found to be contaminated with total aflatoxin concentrations at levels exceeding the maximum limits in foods (15 µg/kg) established by the Codex Alimentarius (Codex Alimentarius, 2008; Ezekial *et al.*, 2012).

Levels of aflatoxin B₁ exceeding the maximum allowable limit set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (5 µg/kg) were found in 70% and 35% of peanut samples collected from Kinshasa (DRC) and Pretoria (South Africa), respectively (Kamika, 2013). Presence of aflatoxins was found also in various street food items in the Cape Coast (Ghana), including meat pie, banku, (a cooked fermented corn dough), beans with gari (roasted fermented cassava flour), dakua (ground, spiced mixture of roasted groundnuts and millet) and fufu (pounded mixture of cooked cassava and plantain) (Annan-Prah *et al.*, 2011). Similarly, *Aspergillus* spp. had 100% occurrence in pre-packaged fruit salad samples comprising pineapple, water melon and pawpaw fruit, from street vendors in Port Harcourt (Nigeria) (Edward *et al.*, 2012).

Aflatoxin contamination in groundnut was reported from Nigeria where the frequency of contamination was over 60% (17/28) with mean value of 105 µg/kg (Opadokun, 1992).

All cottonseed meal samples investigated for aflatoxins in South Africa contained the toxins in which 42 of the 60 samples analyzed exceeded the maximum level for feeds (Reiter *et al.*, 2011). Melon seed another important oilseed in West Africa has been shown to be prone to fungal and aflatoxins contamination at largely unsafe levels (Bankole *et al.*, 2006). Also Opadokun (1992) reported high incidence (73%) of aflatoxins in Nigerian melon seed at mean content of 19 $\mu\text{g}/\text{kg}$. Atehnkeng *et al.* (2008) analyzed 55 samples of maize from 11 districts across three agro-ecological zones of Nigeria for aflatoxins and mean values ranging between 30.9-507 $\mu\text{g}/\text{kg}$ were recovered from the 11 districts which were far beyond all known acceptable levels. Maxwell *et al.* (2000) also, found aflatoxins at alarming concentrations of between 3000-138 000 $\mu\text{g}/\text{kg}$ in Nigeria pre-harvest maize samples. Similarly, stored maize from Uganda contained unsafe aflatoxins level (Kaaya and Warren, 2005; Kaaya and Kyamuhangire, 2006). Very high aflatoxins levels of 46 400 $\mu\text{g}/\text{kg}$ was found in samples from Kenyan local markets (CDC, 2004). Makun *et al.* (2009) also found extremely high AFB₁ contaminations of wheat marketed at Minna, Nigeria at unacceptable levels (range: 40-275 $\mu\text{g}/\text{kg}$) in 27 of the 50 tested samples. In a study by Soleimany *et al.* (2012), 33.3% of analyzed rice from the retail market in Malaysia, had detectable level of aflatoxins ranging from 0.19 to 3.96 ng/kg.

Muhammad *et al.* (2004) found the toxins present in fresh tomatoes marketed in Sokoto, Nigeria. Another vegetable that abhor aflatoxigenic fungi and consequently contain aflatoxins is oyster mushroom (Jonathan and Esho, 2010). Similarly, aflatoxins are most frequently reported in dried figs and raisins worldwide at significant levels of up to 550 and 63 $\mu\text{g}/\text{kg}$ respectively (Fernández-Cruz *et al.*, 2010). Baiyewa *et al.* (2007) showed the presence of the toxin in pawpaw from south western Nigeria.

Cassava and yam are not vulnerable to aflatoxins contamination (Bankole *et al.*, 2006) and even the processed products such as cassava and yam chips and their flour have low contamination rates. Analysis of cassava and yam chips from Benin showed no contamination by aflatoxins (Gnonlonfin *et al.*, 2008) neither was the toxins found in cassava products from Tanzania (Muzanila *et al.*, 2000), Nigeria (Jimoh and Kolapo, 2008) and Ivory Coast (Kastner *et al.*, 2010).

High prevalence (75%) of AFM₁, with all positive samples having levels exceeding the legislated limit of 0.05 µg/L was seen in Libyan cheese (Elgerbit *et al.*, 2004). Amer and Ibrahim (2010) found AFM₁ in 50/150 Egyptian cheese samples at levels between 0.051 to 0.182 µg/L and all the contaminated samples had levels beyond the EU regulated limit. The presence of aflatoxins in eggs from Cameroon (Tchana *et al.*, 2010), at levels of up to 7.68 µg/kg, indicates that poultry animals consume aflatoxins in feeds at alarming concentrations.

Dharmaputra and Putri (1996) reported that the total aflatoxin B₁ content of 39 chicken feed samples collected from 3 markets in Bogor during dry and wet seasons was between 0 to 200.73 ppb. Twenty six out of thirty nine samples (67%) contained aflatoxins more than 30 ppb. A study by Zahari and Tarmudji (1995) on the aflatoxin content of 19 duck feed, 8 mixed duck feed and 8 rice bran samples collected from a duck farm in South Kalimantan reported that in duck feed 100, 100, 52.6 and 15.8% of the samples were contaminated with aflatoxins B₁, B₂, G₁, and G₂, respectively. Their contents were between 4.0 to 160.0 ppb, 4.8 to 60.0 ppb, 0 to 60.0 ppb, and 0 to 8.0 ppb, respectively.

Although fermentation reduces mycotoxins in contaminated food products (Hell and Mutegi, 2011), there is ample evidence to suggest that fermented products in Africa contain significant levels of aflatoxins. Kpodo *et al.* (1996) detected aflatoxins at levels as high as 289 µg/kg in fermented maize dough in Ghana. Detectable (5.2-14.5 µg/kg)

amounts of the toxins were also found in fermented cassava products from Cameroon (Essono *et al.*, 2009). Sorghum based traditional opaque beer from Malawi contained aflatoxins at levels above the Codex permissible limit of 10 µg/kg (Matumba *et al.* 2011). Wagacha and Muthoni (2008) reported incriminating levels of aflatoxins (200 000–400 000 µg/L) in 33% of traditionally brewed beer in South Africa. Levels of up to 50 µg/kg were found in sorghum based local beer from Lesotho (Sibanda *et al.*, 1997).

In other foods, plants and plant products used as medicinal herbs, tea and spices may be commonly contaminated by aflatoxins at significant levels of up to 2230 µg/kg especially in the case of liver curative herbal medicine sold in India (Trucksess and Scott, 2007). According to these authors, contamination of the toxins has been observed in ginger, garlic and capsicum. Zinedine *et al.* (2006) found natural presence of AFB₁ in black pepper, ginger, red paprika and cumin from Morocco at average levels of 0.09, 0.63, 0.288 and 0.03 µg/kg, respectively with the highest level of contamination found in red paprika (9.68 µg/kg). High aflatoxin levels in dried chilli peppers were reported in Thailand with 11% incidence of contamination, and aflatoxin levels reaching a maximum of 966 µg/kg (Shank *et al.*, 1972). In India, an aflatoxin level of 10 to 60 µg/kg was observed in red chilies (Madhyastha, 1985). In another study in Germany, where a total of 185 samples of spices were analysed for mycotoxins, aflatoxins were detected in 4 samples of red chilies, ranging from 8.4 to 24 µg/kg (Woller, 1985). The results of a study on aflatoxin contamination in spices reported up to 120 µg/kg aflatoxin levels in 18 of 125 samples of black pepper, ginger and turmeric collected from drying yards of Kerala, warehouses of Karnataka (India) and some industrial belts of Canada (Seenappa and Kempton, 1980). The samples of common Egyptian foods (17 nuts and seeds, 10 spices, 31 herbs and medicinal plants, 12 dried vegetables and cereal grains) were collected from the markets in Cairo and Giza for aflatoxin analysis. The results indicated the highest

prevalence of aflatoxin B₁ in nuts and seeds (82%), followed by spices (40%), and that the mean concentration of aflatoxin in spices was 25 µg/kg (Selim *et al.*, 1996).

Aflatoxins have also been found recently in other commodities and foods as well. These include wheat (Muthoni *et al.*, 2008; Odoemelam and Osu, 2009; Riba *et al.*, 2010), cassava (Manjula *et al.*, 2009), sorghum (Ghali *et al.*, 2009; Makun *et al.*, 2010; Matumba *et al.*, 2011). Other commodities includes: rice (Makun *et al.*, 2011), local beer (Matumba *et al.*, 2011), powdered milk (Makun *et al.*, 2010), powdered soymilk (Adebayo-Tayo *et al.*, 2009), soybean (Njobeh *et al.*, 2010), maize (Atekhkeng *et al.*, 2008; Akrobortu, 2008). Eggs (Tchana *et al.*, 2010), dried figs (Juan *et al.*, 2008), cowpea (Houssou *et al.*, 2009; Njobeh *et al.*, 2010), Walnuts (Ostadrahimi *et al.*, 2014), Cowpeas (Houssou *et al.*, 2009), Chilli (Paterson, 2007), dried beef (Oyero and Oyefolu, 2010), dried chilli (Hell *et al.*, 2009), smoke-dried fish (Adebayo-Tayo *et al.*, 2008), traditional cured fish (Mugula and Lyimo, 1992) and cowmilk (Kang'ethe and Lang'a, 2009; Tchana *et al.*, 2010; Elzupir *et al.*, 2010; Dutton *et al.*, 2012) Table 5 shows data on aflatoxins incidences in different commodities in some countries from published literature.

Table 5: Incidence of aflatoxin in groundnuts and other commodities reported in the literature

Commodity	Country	Aflatoxin	Incidence	Mean level, \pm SD ($\mu\text{g}/\text{kg}$)	Reference
Maize	Nigeria	AFB ₁	55/55	257.82	Atehnkeng <i>et al.</i> , 2008
	Ghana	AF	30/30	13.596	Akrobortu, 2008
Melon seed	Nigeria	AFB ₁	37/137	14.2	Bankole <i>et al.</i> , 2006
Millet	Nigeria	AFB ₁	12/49	2587.47 \pm 78.23	Makun <i>et al.</i> , 2009
Mouldy sorghum	Nigeria	AFB ₁	93/168	199.51-259.0	Makun <i>et al.</i> , 2009
Powdered milk	Nigeria	AFM ₁	19/100	0.136	Makun <i>et al.</i> , 2009
Powdered soymilk	Nigeria	AFB ₁	30/30	11.53	Adebayo-Tayo <i>et al.</i> , 2009
Raw cowmilk	Egypt	AFM ₁	19/50	0.049 \pm 0.017	Amer and Ibrahim, 2010
Smoke-dried fish	Nigeria	AFB ₁	11/11	3.46	adebayo-Tayo <i>et al.</i> , 2008
Traditional -cured fish	Tanzania	AFB ₁	16%	0-18.5	Mugula and Lyimo, 1992
Sorghum	Tunisia	AFB ₁	58/93	9.9 \pm 11.5	Ghali <i>et al.</i> , 2009
	Malawi	AFB ₁	2/15	2.35 \pm 0.65	Matumba <i>et al.</i> , 2011
Soybean	Cameroon	AFs	2/5	2.1	Njobeh <i>et al.</i> , 2010
Wheat	Kenya	AFB ₁	23/50	1.93	Muthoni <i>et al.</i> , 2008
	Tunisia	AFs	15/51	6.7 \pm 2.4	Ghali <i>et al.</i> , 2008
	Nigeria	AFB ₁		19.0 \pm 1.67	Odoemelam and Osu, 2009
	Algeria	AFB ₁	30/53	> 5	Riba <i>et al.</i> , 2010
Cowpea	Cameroon	AFs	5/15	2.2	Njobeh <i>et al.</i> , 2010
Local beer	Malawi	AFs	5/5	22.3 \pm 4.93	Matumba <i>et al.</i> , 2011
					Kang'ethe and Lang'a, 2009
Animal feeds	Kenya	AFB ₁	703/830	8.9-46.0	
	Sudan	AFs	36/56	130.6	Elzupir <i>et al.</i> , 2009
	South Africa	AFs	17/23	38.9	Mngadi <i>et al.</i> , 2008
Cotton seed meal	South Africa	AFB ₁	42/60	24.9	Reiter <i>et al.</i> , 2011
Pistachio	Morocco	AFB ₁	9/20	158.0 \pm 6.3	Juan <i>et al.</i> , 2008
	Tunisia	AFs	21/40	21.8 \pm 38.0	Ghali <i>et al.</i> , 2009
Poultry feeds	Morocco	AFB ₁	14/21	1.26 \pm 0.65	Zinadine <i>et al.</i> , 2007
Poultry/Livestock feeds	Nigeria	AFB ₁	6/13	15.5	Adebayo and Etta, 2010
Barley	Tunisia	AFB ₁	11/25	18.4 \pm 2.73	Ghali <i>et al.</i> , 2008
Cowmilk	Nigeria	AFM ₁	3/22	\leq 2.04	Atanda <i>et al.</i> , 2007
	Sudan	AFM ₁	42/44	2.07	Elzupir <i>et al.</i> , 2010
	South Africa	AFM ₁	42/42	0.12	Dutten <i>et al.</i> , 2012
	Kenya	AFM ₁	474/613	0.064	Kang'ethe and Lang'a, 2009
Dried Beef	Cameroon	AFM ₁	10/63		Tchana <i>et al.</i> , 2010
	Nigeria	AFB ₁	10/10		Oyero and Oyefolu, 2010
Dried Chilli	West Africa	AFB ₁	1/30	3.2	Hell <i>et al.</i> , 2009
Dried figs	Morocco	AFB ₁	1/20	0.28	Juan <i>et al.</i> , 2008
Dried okra	West Africa	AFB ₁	3/30	5.4	Hell <i>et al.</i> , 2009
Egg	Cameroon	AFs	28/62	0.82 \pm 1.71	Tchana <i>et al.</i> , 2010
Walnut	Iran	AFs		14.4 \pm 8.4	Ostadrhimi <i>et al.</i> , 2014
Chilli	Pakistan	AFs		0.1-96.2	Paterson, 2007
Cowpeas	Benin	AFs		3.52	Houssou <i>et al.</i> , 2009

2.4.2 Fumonisin incidences in various crops

The worldwide occurrence of fumonisins in food has well been documented and reviewed in the literature and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2001. Although fumonisins are found mainly in maize and maize-based products, the sporadic natural occurrence of fumonisins in other food commodities such as sorghum, barley, rice and coffee has been reported (Ghali *et al.*, 2009; Noonim *et al.*, 2010). Fumonisin have also been found recently in other commodities and foods as well. These include wheat (Kushiro *et al.*, 2009; Manova and Mlandenova, 2009), cassava (Manjula *et al.*, 2009), Sorghum (Ayalew *et al.*, 2006), Maize bread (Lino *et al.*, 2007), grapes and grape products such as wine (Logrieco *et al.*, 2009; Mogensen *et al.*, 2010), beer (Romero-González *et al.*, 2009; Aoyama *et al.*, 2010), figs (Moretti *et al.*, 2010), soybeans and millet (Aoyama *et al.*, 2010) and asparagus (Aoyama *et al.*, 2010; Waśkiewicz *et al.*, 2010). Incidence rates of fumonisins in maize samples were also recorded from Italy, Portugal, Zambia and Benin (Doko *et al.*, 1995). These studies were extended to include maize samples from Botswana, Mozambique, South Africa, Malawi, Zimbabwe and Tanzania (Doko *et al.*, 1996). In Honduras, Julian *et al.* (1995) detected FB₁ in all 24 samples maize tested (Table 6). In Argentina, fumonisins were detected during ear development and their occurrence was closely with the natural infection with *Fusarium moniliforme* and *F. proliferatum* (Chulze *et al.*, 1996). Some of the values cited in Table 6 are of toxicological significance since they exceed a tolerable level of 20 ug/kg. Table 6 shows data on fumonisins on maize in some countries from published literature.

Table 6: Incidence of fumonisin in maize and other commodities reported in the literature

Country	Origin	Fumonisin	Analyzed/positive	Mean, µg/kg	SD (µg/kg)	Reference
Brazil	Field	FB ₁	76/76	1.89	1.9	Westhuizen <i>et al.</i> , 2003
China	Households	FB ₁	259/230	1.59	NR	Sun <i>et al.</i> , 2007
Kenya	Maize flour	FB ₁	93/197	0.11-12	NR	Kedera <i>et al.</i> , 1999
Croácia	Field	FB ₁	49/49	0.459	0.311	Domijan <i>et al.</i> , 2005
		FB ₂	49/3	0.109	3.08	
Guatemala	Field, storage, market	FB ₁	1421/572	0.935	NR	Torres <i>et al.</i> , 2007
Italy	Field trials	FB ₁	40/40	15.05	2.08	Cavaliere <i>et al.</i> , 2007
		FB ₂	40/40	6.74	1.02	
Iran	Dried maize	FB ₁	49/48	6.14	3.08	Yazdanpanah <i>et al.</i> , 2006
Spain	From Argentina	FB ₁ + FB ₂	92/92	2.60	1.81	Castells <i>et al.</i> , 2008
Ethiopia	Households	FBs		2117	NR	Ayalew <i>et al.</i> , 2006
South Africa	Market	FBs		142-550	NR	Lino <i>et al.</i> , 2007
West Africa	0 to 6 months of storage	FB ₁ + FB ₂	144	1.90	NR	Fandohan <i>et al.</i> , 2005
Tanzania	Households	FB ₁ + FB ₂	144	954	NR	Kimanya <i>et al.</i> , 2014
Ghana	Household	FB ₁	8/9	0.025-0.165	NR	Doko <i>et al.</i> , 1996
	Field	FB ₁	15/15	0.07-33.1	NR	Kpodo <i>et al.</i> , 2000
Mozambique	Household	FB ₁ + FB ₂ + FB ₃		340-395	NR	Doko <i>et al.</i> , 1996
Malawi	Field	FB ₁	7/8	0.02-0.115		Doko <i>et al.</i> , 1996
China	Harvested Maize	FB ₁ + FB ₂	8	150-4480	NR	Feng <i>et al.</i> , 2011
Argentina	Durum wheat	FB ₁ + FB ₂ + 30		10.5-1245.7	NR	Palacios <i>et al.</i> , 2011
		FB ₃		85-8791	NR	Chulze <i>et al.</i> , 1996
Benin	Cassava flour	FB ₁ + FB ₂ + FB ₃	12	51-836	NR	Ediage <i>et al.</i> , 2011
Honduras	Maize	FB ₁	24/24	68-6555		Julian <i>et al.</i> , 1995
Malaysia	Maize meal	FB ₁ + FB ₂	32	20.5-113.5	NR	Soleinmany <i>et al.</i> , 2012
Morocco	Breakfast cereals, infant food	FB ₁ + FB ₂ + FB ₃	48	6.2-228	NR	Mahnine, <i>et al.</i> , 2012
Spain	Baby food	FB ₁ + FB ₂ + FB ₃	35	10-15	NR	Rubert <i>et al.</i> , 2012
Zambia	Harvested maize	FB ₁ + FB ₂ + FB ₃		20-1710	NR	Doko <i>et al.</i> , 1995

NR= not reported

2.5 Prevalence and Distribution of Aflatoxins Contamination in Tanzania

Aflatoxin contamination of key staple foods; maize and groundnuts is above regulated levels for both total aflatoxins and B₁ in some parts of Tanzania. Focusing on the strain that is most toxic and for which liver cancer impacts are identified, aflatoxin B₁ prevalence data from 2012 indicated contamination above regulated levels (5 ppb) in two zones (Strosnider *et al.*, 2006; Hussaini *et al.*, 2012). In the Eastern zone (Morogoro), 43% of the maize samples were above 5 ppb; and in the Western zone (Shinyanga), 40% of the samples were above 5 ppb, with average contamination of 50 ppb and 28 ppb, respectively. The contamination was much lower in other zones: in the Northern zone (Manyara), 9% of the samples were above 5 ppb; in the Southern Highlands (Iringa, Mbeya, and Rukwa), only 4% were above 5 ppb; and in the Southern zone (Ruvuma), none of the samples were above 5 ppb. Percentages of samples from the Northern, Southern (Mtwara), and Western zones with aflatoxin B₁ contamination above 5 ppb were 20%, 20%, and 8%, respectively, with mean contamination at 20 ppb, 18 ppb, and 20 ppb (Strosnider *et al.*, 2006; Hussaini *et al.*, 2012).

2.6 The impact of Aflatoxins in Tanzania

The importance of cereal grains in human nutrition is widely recognized, as they provide substantial amounts of energy and proteins to millions of people especially in developing countries (FAO, 2010). According to Nuss and Tanumihardjo (2010), cereal provides an estimated 10% and 15% of the world's calories and protein respectively. In Tanzania, maize comprises 41% of the weekly calorie intake of households (Ott *et al.*, 2012). On average Tanzanians eat 521 grams of maize and groundnuts per person per day (Ott *et al.*, 2012). This implies that even at low levels of aflatoxin contamination of key staples, there is measurable health impact because of the high contribution of the staples in the Tanzanian diet. The WHO estimates that in 2010, there were 1209 liver cancer cases in

Tanzania. This implies that at average contamination of 5 ppb in 2010 as many as 45% of annual liver cancer cases in Tanzania could be attributed to aflatoxins. Sensitivity analysis of estimates indicates that, if there were 100% immunization for HBV, the liver cancer cases attributable to aflatoxins contamination could drop by 65% (Ott *et al.*, 2012).

2.7 Magnitude of Mycotoxin Problem in Africa and its Implications

Most of the mycotoxin-poisoning problems occur in the sub-Saharan Africa where maize and groundnuts are a dietary staple (Wu, 2004). About 250 000-hepatocellular carcinoma related deaths occur annually in parts of sub-Saharan Africa due to aflatoxin ingestion alone (Wu, 2004). About 5.2 million cancer deaths occur each year, 55% of which occur in developing countries (GLOBOCAN, 2008). Eighty percent (80%) of cases and deaths of liver cancer occur in these countries particularly in Western and Central Africa while 78% of cervical cancers occur in developing countries (Dow, 1994). Besides the direct health risk and causing premature deaths in Africa, economic losses arising from mycotoxicoses are enormous in the region (Wu, 2004).

One World Bank study estimated that trade losses with the EU alone cost Africa USD 670 million dollars annually in the export of cereals, nuts and foodstuffs (Fapuhunda, 2011). Another study estimated that if all countries were to adopt EU standards on aflatoxins then global trade would decline by USD 3 billion (Dohlman 2008). The Joint FAO/WHO Expert Committee on Food Additives estimated that reducing maximum aflatoxin limits of B₁ from 20 ppb to 10 ppb would reduce cancer by 2 deaths per 1 billion people (Dohlman, 2008). Despite a global effort underway to harmonize mycotoxin and aflatoxin standards there is still plenty of variation in regulatory regimes across countries and continents. Maximum accepted levels of aflatoxins in foods and products for human consumption range from 0.5 ppb in milk to 20 ppb for processed foods (USAID, 2012).

Countries also differ in terms of methods for controlling for aflatoxins. In Nigeria all food and drug imports are sampled by the Port Inspectorate Division under NAFDAC, while export inspection is highly encouraged but voluntary. In Tanzania aflatoxin testing among exports is rarely done and in the United States, aflatoxin testing is not mandatory aside from maize exports (Dohlman, 2008).

2.7.1 Synergies between aflatoxin exposure, Hepatitis B and liver cancer in african countries

A recent study estimated that aflatoxin exposure in Tanzania's population ranged from 0.02 and 50 ng/kg BW/day (Liu and Wu, 2010). In Nigeria, the exposure was 139 to 227 ng/kgBW/day (Liu and Wu, 2010). Excess annual liver cancer incidence estimates for Nigeria were 1.39 to 2.27 cases per 100 000 in hepatitis B virus (HBV)- populations and 41.7 to 68.1 cases per 100 000 in HBV+ populations (Liu and Wu, 2010). Excess annual liver cancer incidence estimates for Tanzania were 0.0002 to 0.50 cases per 100 000 in HBV- populations and 0.06 to 15.0 cases per 100 000 in HBV+ populations (Liu and Wu, 2010). This risk factor is affecting rural populations more strongly (Plymoth *et al.*, 2009).

Hepatitis B Virus (HBV) infection and aflatoxins together lead to a "30-fold higher liver cancer risk as compared to HBV- negative persons" (Groopman *et al.*, 2008; Wu *et al.*, 2011). Also of concern is the effect of aflatoxins on people living with HIV/AIDS (PLWHA). Approximately 1.2 million adults in Tanzania and 3 million adults in Nigeria live with the virus. HIV infection may reduce the body's ability to protect itself from aflatoxins and the common HBV/HIV co-infection may increase biological effects (Wu *et al.*, 2011). There is also concern that aflatoxins may increase risk of developing tuberculosis (TB) in people living with HIV/AIDS (USAID, 2012). Preliminary evidence has suggested an interaction between chronic aflatoxins exposure and immune

suppression and consequently susceptibility to infectious diseases such as malaria and HIV/AIDS (Jiang *et al.*, 2008; Keena *et al.*, 2011). Aflatoxin contamination has been associated with stunting in children, immune suppression, micronutrient deficiencies, and higher prevalence of cancers in sub-Saharan Africa, East Asia, and China (Moturi, 2008; Hell *et al.*, 2010; Smith *et al.*, 2012).

2.7.2 Aflatoxin exposure in pregnant women and infants

Infants exposed to aflatoxin-contaminated foods may be more susceptible to stunting, and malnutrition. Yet exposure to aflatoxins can also incur in utero as evidenced by studies that measured blood albumin–AFB₁ (biomarkers) in pregnant African women (Turner *et al.*, 2007). A study from Ghana suggested that there may be a correlation between aflatoxins and anemia in pregnant women, which is associated with increased risk of maternal mortality and low birth weight in pregnant women (Shuaib *et al.*, 2010). Turner *et al.* (2007) also found that the presence of AFB₁ in the cord blood of newborns was correlated with jaundice, low birth weight and effects on the immune system.

Studies have also found aflatoxins in the form of AFM₁ in the breastmilk of women from several African countries (Zarba *et al.*, 1992). This can have toxic and carcinogenic properties, but also must be communicated and weighed carefully since exclusive breastfeeding for the first 6 months is still considered an essential nutrition action, and is a key for child survival, particularly in poor resource settings where hygiene and sanitation are inadequate (WHO, 2004).

A study in West Africa found that “weaned children had approximately twofold higher mean AF-alb adduct [aflatoxin biomarkers] than those receiving a mixture of breast milk and solid foods and children who were underweight and stunted had 30 to 40% higher

mean AF-alb levels than the remainder of the children” (Gong, *et al.*, 2003). The burden of stunting in Nigeria from consuming maize and groundnuts contaminated with aflatoxins was 0–18.5% of all stunted children under age five in 2010 (Khlungwiset, 2011). Though the WHO recommends that infants up to 6 months of age be exclusively breastfed, premature introduction to complementary foods is common in Tanzania and Nigeria. More than half of Tanzanian infants are introduced to complementary foods prior to the recommended 6 months of age (Kimanya, 2008). Kimanya cites studies on Tanzania stating “in most parts of the country, maize forms the main part of cereals used in complementary foods.” Children are given maize-based porridge or other cereals with water (Mamiro *et al.*, 2005).

2.8 Promising Technologies and Practices for Aflatoxin Mitigation in Tanzania

Many of the technologies for aflatoxin mitigation have been well documented in the recently released Synthesis of the Research on Aflatoxin in Health, Agriculture and Trade (USAID, 2012) and it also draws heavily from the analysis of Liu and Wu (2010) and Khlungwiset (2011) the potential feasibility of these solutions in Tanzania.

2.8.1 Pre-harvest solutions

Many of the pre-harvest solutions currently available to Tanzania are based on Good Agricultural Practices (GAP), which typically include use of insect resistant crops, good tillage and weeding practices, appropriate use of fertilizers, irrigation, and crop rotation (Kimanya, 2008). In addition to GAP, practices such as treating soil with lime and farmyard manure have proven successful at reducing aflatoxin contamination levels (Waliyar *et al.*, 2008). Lime application, use of farm yard manure and cereal crop residues as soil amendments have shown to be effective in reducing *A. flavus* contamination as well as aflatoxin levels by 50-90%, as described by Waliyar *et al.*

(2008). Calcium, which is part of lime, thickens the cell wall and accelerates pod filling, while manure facilitates growth of micro-organisms that suppress soil infections. Manure is known to improve soil water holding storage and can supply a wide range of plant nutrients (Achieng *et al.*, 2010).

2.8.2 Bio-controls

Biocontrols are used in place of traditional chemical pesticides, are environmentally safe and derived from natural means and may include beneficial insects, plant extracts, or the introduction of other natural organisms. Use of bio-controls is promising with reductions of aflatoxin B₁ by as much as 83% (USAID, 2012). Plants sprayed with the atoxigenic strain were 97% free of the aflatoxins at follow-up, and inoculation of soil (which may be more feasible for smaller farmers) has also proven to be a highly effective method to prevent aflatoxins pre-harvest (USAID, 2012). International Institute for Tropical Agriculture (IITA) is currently exploring options for possible bio-control development that could be adopted by Mali, Ghana and Tanzania (USAID, 2012). IITA and its partners seek to develop a scalable, natural scientific solution for aflatoxin contamination in the form of a biological control that naturally is both adapted to and native to local environments. In Nigeria, the inoculation of maize with atoxigenic isolates of *A. flavus* reduced aflatoxin levels in soils from 70.1% to 99.9% (Atehnkeng *et al.*, 2008).

2.8.3 Post-harvest stage

During the post-harvest stage, thorough drying, prompt storage and transport using clean, dry containers are the basic elements of aflatoxin prevention and control. Timely harvest is also critical for aflatoxin prevention (Kimanya, 2008). One study found that aflatoxins in maize increased 4-7 fold after a 3-4 week delay in harvest after maturity (Hell, 2008). IITA has made recommendations for aflatoxin mitigation in maize for subsistence

farmers, who often lack the resources or access to drying and storage equipment. It based its recommendations on surveys with subsistence farmers throughout in Nigeria and other parts of Sub-Saharan Africa. The key elements of these recommendations include sorting, cleaning, drying, packaging, adherence to hygiene and sanitary conditions in storage and transport, as well as through raising farmers' awareness about these practices (Hell *et al.*, 2008). Different drying methods were also analyzed by Hell *et al.* (2008) which suggested that for maize, drying on a platform may be the most effective way to reduce aflatoxins.

2.8.3.1 Storage

Clean, dry, insect and rodent free storage conditions are critical to prevent aflatoxin growth (Hell *et al.*, 2000; USAID, 2012). Making storage options inexpensive and accessible is of paramount importance for consistent, long term utilization. Turner *et al.* (2007) also investigated low-technology post-harvest handling options for groundnut in an aflatoxin susceptible zone in Guinea. The package of interventions investigated included: hand sorting, storage in jute bags, education on improved sun drying, wooden pallets for drying, locally-made natural fiber mats and insecticides. The estimated cost of this intervention package was USD 50 (including USD 10 for the wooden pallet); a sizeable but potentially manageable cost where the GNP per capita is USD 1100. Five months after harvest, this combination of storage methods led to a 50% reduction of aflatoxin biomarkers among the households in the intervention group as compared to the control group (Turner *et al.*, 2007).

2.8.3.2 Processing stage

Even in resource-constrained communities, there are several processing methods that can reduce aflatoxins in maize. The most promising processes reviewed included cleaning the cereal/groundnut by sorting, washing the food before processing and dehulling grain

mechanically. Cleaning and dehulling were also noted to be safer as these methods are unlikely to produce other toxins that would be harmful to human health (Fandohan *et al.*, 2008). Roasting also had some promising reductions in aflatoxins, up to an 85% reduction in aflatoxin B₁ in one quoted study (Bankole *et al.*, 2005). While cooking reduced aflatoxins, it was noted that generally temperatures that are not achieved during home cooking (195 °C for aflatoxins reduction) would be needed to sufficiently affect aflatoxin levels (Fandohan *et al.*, 2008).

2.8.3.3 Industrial processing

Industrial detoxification processes include using inorganic salts and organic acids, and ammoniation which can eliminate the aflatoxin producing fungus with ammonia vapor as well as natural acids, salts and plant extracts. Ammoniation (using either ammonium hydroxide or gaseous ammonia) appears to have the most practical application for the decontamination of agricultural commodities (Norred *et al.*, 1991). In animal feed, an anticaking/binding agent like hydrated sodium calcium aluminosilicate may reduce AFM₁ residues in milk, depending on the initial concentration of AFB₁ in the feed (USAID, 2012).

2.9 The Risks of Aflatoxins Exposure in Tanzania

2.9.1 Risk characterization for agriculture and food security

By definition, risk characterization is the qualitative and/or quantitative evaluation of the nature of the adverse effects associated with mycotoxins, which may be present in food. Much of Sub-saharan Africa is at risk of unsafe levels of aflatoxin exposure that can negatively affect human health, food security and economic trade (William *et al.*, 2004). In Tanzania, the perceived impact of aflatoxin contamination on agriculture and food security has so far been negligible because aflatoxin contamination often does not cause

visible damage to crops. The market does not differentiate between aflatoxin-free and aflatoxin-contaminated food; therefore, farmers do not incur any costs for mitigating aflatoxins (Leslie *et al.*, 2008). This in turn results in increased risk that aflatoxin-contaminated grains leave the farmers' fields and enter the food and feed supply (Kimanya *et al.*, 2009).

The production and consumption of traditional/local beers is a widespread practice in most of the Africa countries including Tanzania (Haggblade and Holzapfel, 1989). Maize, sorghum and finger millet are the most common ingredients for the brewing process. These commodities are prone to pre- and post-harvest toxigenic fungal colonization and mycotoxin contamination. The risk of mycotoxin contamination increases during the malting step which involves raising moisture content of the grains and the humidity of the environment (Schwarz *et al.*, 1995). The risk is much greater in an African setting since malting is done under typical home environments where fungal colonization is not controlled (Novellie and De Schaepdrijver, 1986).

Farmers are not aware of aflatoxins, nor of measures to control aflatoxins in the field, which begin with good agricultural practices (GAP). There is no set agenda for agricultural extension services to include aflatoxins, mycotoxins, food safety, or GAP in their messaging, nor is there a strategy or guidelines for crop-specific GAP (Kimanya, M. E. personal communication, 2012). Although the use of quality seed is a fundamental means to mitigate aflatoxins, the country and economic assessment found that nationally, only 18% of agricultural households use improved seeds for maize, and 3% use improved seeds for groundnuts. Since healthy plants can better resist disease, the use of irrigation, fertilizers, and crop protection chemicals also matters in aflatoxin control. Yet only 2% of

the area cultivated under maize is irrigated, and the area irrigated for groundnuts is negligible.

In Africa, crops are cultivated under rainfed condition, with low levels of fertilizer and practically no pesticide application. These management practices promote *A. flavus* infection in fertility stressed plant. Any action taken to interrupt the probability of silk and kernel infection will reduce aflatoxins contamination (Dierner *et al.*, 1987). In Tanzania, use of fertilizer is at 17% for maize and 1% for groundnuts (Minot, 2009). Use of pesticides is at 11% for maize and 3% for groundnuts (Msuya, M. personal communication, 2014). Drying of maize, groundnuts, and other crops is typically done on the ground, although there is some evidence of use of brick and mud structures that are above the ground. Storage units are often self-made, and commodities are stored without means of monitoring the temperature and humidity of such local storage units (Udoh *et al.*, 2000).

2.9.2 Risk characterization for domestic commerce and international trade

Aflatoxin-contaminated grain can enter the domestic markets and the informal international markets (e.g., Kenya and Zambia for maize) because of lack of awareness and difficulty faced in enforcement of existing standards. Tanzania Food and Drugs Authority (TFDA) enforces commodity standards but only for packaged foods and foods bound for the formal export market; thus, the vast majority of foods consumed by the Tanzania population are not regulated for aflatoxin. In Tanzania, aflatoxins testing among export is rarely done (Dohlman, 2008). Country assessment field research in Kongwa, Njombe, and Bukombe found no evidence of testing for aflatoxins in the domestic maize and groundnut markets in Tanzania. There is low awareness about aflatoxins and their

health impact among most farmers, traders, and market sellers (Kimanya, M. E. personal communication, 2012).

In the animal feed sector, there is aflatoxin control even though there are no regulations on aflatoxins in this sector. However, since there is no mandate for withdrawal and destruction of contaminated commodities, grain deliveries rejected by large commercial operations are likely to be sold by a trader to smaller feed manufacturers that do not test for aflatoxins. Maize chaff generated by large millers is used by the feed industry as raw material and is not regulated or tested for aflatoxins, which raises concern for aflatoxin contamination in animal products, particularly milk and possibly eggs (Stene *et al.*, 2011).

2.9.3 Risk characterization for human health

Consumers' level of aflatoxin knowledge is still very low in Tanzania, while the contribution of maize to calorie intake is very high. Thus, maize consumption can be less than 10g/person/day in various European countries, but can rise to an average of 400-500g/day in rural Africa (Shephard *et al.*, 2002), with a 90 percentile value of over 700g/person/day (Shephard *et al.*, 2007). Cereals is eaten an average of more than 5 days per week (Smith and Subandoro, 2007). In Africa, cereals contribute 46% of the total energy intake; however, this figure could be as high as 78% in some African countries (FAOSTAT, 2010). Although groundnuts do not account for a large share of calorie intake, they are widely promoted as ingredients for weaning foods (Kimanya *et al.*, 2010). This implies that even small levels of aflatoxin contamination in maize and groundnuts could present a high risk of aflatoxin exposure, particularly in mainland Tanzania. Maize and groundnuts together account for 44 % of the calorie intake (41% for maize and 3% for groundnuts) and maize is the major crop grown (FAO, 2012).

2.10 Factors Responsible for Mycotoxins Production in Stored Maize and Groundnuts

In terms of storage conditions, grains with moisture content above 9% and stored at moderate temperatures (28⁰C to 30⁰C) increase the risk of infection by fungi which are mycotoxin producers (Haheshwar *et al.*, 2000). Grain damage by insects, rodents, birds, drought stress and mechanical injury which predispose the crop to colonization by the fungus can lead to mycotoxin occurrence in groundnuts and maize (Williams *et al.*, 2004). However, the likelihood of acute aflatoxicosis, as seen in Kenya in 2004 (CDC, 2004) in humans include limited food availability, optimal environmental conditions for fungal development in crops and commodities, coupled with inadequate regulatory systems for their detection. Socio-economic factors that influence crop management practices such as labour shortages, poor access to mechanization, and existence of credit mechanisms, power relations and social structure of livelihood pattern influence, all have some implications on mycotoxins contamination.

In the field conditions, several additional factors may influence the production of mycotoxins. These may include agricultural practices like tillage and crop rotation (Lipps and Deep, 1991), lodging (Langseth and Stabbetorp, 1996) fungicide used (Moss and Frank, 1985), plant variety (Kiecana *et al.*, 2002) and geographical differences (Langseth *et al.*, 1996). Also, organic cultivation practices may pose a risk of increased mycotoxin production (Edward, 2003).

2.11 The Effect of Weather on Mycotoxin Incidence

The environmental factors affecting mycotoxin production are of physical, chemical, or biological origin (Van Osenbruggen and Petterson, 2002). However, these factors rarely have an impact in an independent manner (Abramson *et al.*, 2002), thus their interactions

are usually more important than what would be expected from simple summations of the actions on their own (Moss, 1991).

The timing of the humidity determines the risk for mycotoxin contamination. Rainfall at the time of anthesis sensitizes crops to *Fusarium*-infection and subsequent mycotoxin contamination (Langseth and Stabbetorp, 1996). The concentrations of a trichothecene mycotoxin and deoxynivalenol (DON) in Norwegian oats were affected by the annual weather. The highest amount detected was after warm and dry springs with heavy rainfalls at the time of the anthesis. Cold summers with moderate rainfalls, instead lead to low concentration levels of DON (Langseth *et al.*, 1999). In Finland, cold and rainy summers have led to the presence of lower levels of DON than those occurring in warmer and partly dryer growing seasons (Yli -Mattila *et al.*, 2004). Weather conditions are also favourable for the occurrence of *Fusarium* species, where the frequency of identification of these fungi is as high as 93.5% (Lugauska *et al.*, 2004).

2.12 Effects of Moulds

Fungal invasion and mycotoxins contamination of agricultural products lead to losses in terms of quantity, market value, quality of food and feed production due to changes in colour, texture and taste (Mutegi *et al.*, 2009) and reduction of seed germination, energy and nutritional value changes in terms of loss of carbohydrates, proteins, amino acids and vitamins and increases in fatty acids may also occur (Ominski *et al.*, 1994). Moulds are able to grow on all kinds of food: cereals, meat, milk, fruit, vegetables, nuts, fats and products of these. The mould growth may result in several kinds of food spoilage: off-flavours, toxins, discolouration, rotting and formation of pathogenic or allergenic propagules (Tipples, 1995). Moulds rank second only to insects as a cause of damage in stored grains (CAST, 1989). They cause detrimental changes in grain appearance, quality,

and dry matter (Ng *et al.*, 1998), and they reduce the energy content and ethanol yield for corn (Hardy *et al.*, 2006). Moulds are destructive agents causing losses of agricultural commodities in many zones of the world, ranking alongside insects and weeds for the reduction of crop loss or yield. They can occur on the in-field grown crops as well as on the harvested commodities, leading to a damage ranging from rancidity, odour, flavour changes, loss of nutrients, and a destruction of germ layer. This can result into a reduction of the quality of grains as well as gross spoilage and possible mycotoxin production (Marín *et al.*, 1998).

Direct crop losses in the field are caused by plant pathogens through a reduction of crop yields (Sauer, 1988). Some plant pathogens may produce mycotoxins either as incidental products or as a chemical associated with the infection process as is the case with some species of *Fusarium*. Plant pathogens may also render the crop have a lower grade by causing blemishes, blights or other quality problems (Mills, 1989). Some spoilage fungi can also produce mycotoxins, for example *Penicillium*, although many penicillia associated with grains are pathogenic. *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* are amongst the most common mycotoxin-producing fungal species associated with growth in and damage of food crops in the field, and in the store, if poor storage conditions prevail after harvest, or previously dried commodities become rewetted.

2.12.1 Adverse quality changes

Growth of spoilage moulds on the surface of seeds often results into a dull rather than a bright appearance as in normal seeds (Sforza *et al.*, 2006). Dull appearance is sometimes considered a degrading indicator. The presence of spoilage fungi on seeds is also often associated with musty odours, which are a degrading factor (CGC, 2002). Other important effects of spoilage fungi on seeds include reduction in germinability and discolouration of

whole seeds or portions of them, including the germ (Chuck-Hernández *et al.*, 2012). Infection of maize grain by storage fungus results in discoloration, dry matter loss, chemical and nutritional changes and overall reduction of maize grain quality (Chuck-Hernández *et al.*, 2012).

Under the right moisture conditions, spoilage fungi invade the germs with no visible signs of molding, weaken the seeds, and eventually cause seed death. Some strains of the spoilage molds *Aspergillus restrictus*, *A. candidus* and *A. flavus* can cause severe damage and kill the germs quickly. As fungal invasion of the germs of seeds continues, the tissues of the germ become brown and then black (Christensen and Sauer, 1982). Discolouration caused by fungi results into lowering of the grade of the product (CGC, 2002).

Generally, quality is assessed and the products graded on the basis of appearance, shape, size, and the like, but smell and flavour are sometimes included. Foreign matter content and contaminants are factors for loss of quality (Khlangwiset *et al.*, 2011). Foreign matter may be in the form of insect fragments, grass, rodent hairs, and excrete; weed seeds, parts of plants, earth, stones, glass, and the like. Contaminants that cannot be readily removed include soluble excretions of pests, oils, pesticides, pathogenic organisms spread by vectors, and toxins arising from fungal infections. Chemical changes may also be important, for example, in oilseeds. Infestation in groundnuts may cause an increase in the free fatty acid level leading to rancidity in the oil and in the maize meal as well.

2.12.2 Nutritional loss

This is the combination of both the quantitative and qualitative losses. Quantitative loss is a physical loss of a substance and is shown by a reduction in weight or volume. It is the form of loss that can most readily be measured and valued. Nutritional loss and loss of

seeds are both aspects of quality losses. A considerable proportion of the grains rejected by the farmer are often discarded because of mould; and the presence of infected grain causes a drop in quality grading. Shah *et al.* (2002) explained that changing temperature and relative humidity not only promotes moulds growth, but also causes considerable nutrient losses of grain (Fandohan *et al.*, 2003). Weight loss during storage (which is not due to a loss of moisture) is a measure of food loss but the latter may be proportionately larger owing to selective feeding by the pests. Rodents and moth larvae may preferentially attack the germ of the grain thus removing a large percentage of the protein and vitamin content, whereas weevils feeding mainly on the endosperm will reduce the carbohydrate content (Ominski *et al.*, 1994). Many pests may eat the bran of cereals reducing vitamins such as thiamin. Other storage factors such as moisture and fungal infection also lead to changes in vitamin content (Anyanwu, 2004). Moulds are considered the principal reason for the destruction of fats in grain during storage, which occurs more rapidly than the destruction of other nutrients, such as carbohydrates and proteins. Estimated losses in metabolizable energy in maize due to mould growth range from 5% to 25 % depending on the mould species involved (Bartov *et al.*, 1982; Tindall, 1983). Although mould growth reduces all the amino acids in the grain, cysteine, and lysine appear to be more severely affected than the others (Kao and Robmson, 1972).

2.12.3 Monetary loss

Weight loss is an economic loss is any downgrading of produce due to poor quality. Any control measure that has to be employed to render or keep the commodity saleable can be counted as an economic loss and is perhaps the most easily accountable loss. Losses in packaging and the costs of repacking due to rodent and handling damage, repairs and stoppages in machinery, as well as damage to the fabric of the store. These are all economic losses that can be the result of infestation (Bandyopadhyay *et al.*, 2007;

Manjula *et al.*, 2009). The presence of moulds and mycotoxins leads to price discounts or rejections of shipments by buyers. The annual loss due to mycotoxins from US exports of Bt maize is estimated at about USD 23 million (Wu, 2006). Over USD 100 billion of exported commodities all over the world are susceptible to mycotoxin contamination (Cardwell *et al.*, 2001). It is estimated that, annual economic losses in Asia and Africa due to mould are in excess of USD 130 millions (Chandrasheker *et al.*, 2000). Globally, about USD 1.2 billion in commerce is lost annually due to aflatoxins contamination, with African economies losing USD 450 million each year (Atehnkeng *et al.*, 2008; Bandyopadhyay and Lopez, 2013).

2.12.4 Heat damage

Spoilage fungi including *Aspergillus* species such as *A. candidus* (white or cream) and *A. flavus* (yellow green) can, through their respiration, raise the temperature of stored products up to 55°C (Mulinge and Apinis, 1969). Development of these molds frequently occurs in the pockets of increased moisture within bulks. The pockets result into moisture migration, high-moisture weed seeds, plant debris, heavy rains, or melted snow (Mills, 1989). The chemical process involved in heat generation is predominantly aerobic oxidation of carbohydrates such as starch. The viable grain kernels, insects, molds, mites and other organisms in the stored grain are living organisms and they respire; during the respiration process, oxygen is consumed and carbon dioxide, water and heat are produced (Bern *et al.*, 2013). The carbon dioxide, moisture, and heat produced through respiration of the grain causes an increase in temperature and dry matter loss of the stored grain (Lee, 1999). Heating occurs when this energy is released faster than it can escape from the cereal substrate (Fleurat-Lessard, 2002; Magan *et al.*, 2004). The elevated temperatures result into internal browning or blackening of seeds, reduced seed quality, and lower or no germination (Mills, 1989). The effects of heat damage become progressively worse if

the initial mold heating is succeeded by chemical heating (Freeman, 1980). The presence of heated brown or black seeds and/or a burnt odour in a sample of grain lowers the grade. Seed lots with elevated levels of heated seeds cause problems for the processor, as oil from heat-damaged oilseeds requires extra decolourizing procedures during processing and this leads to extra costs.

Heat-damaged externally blackened kernels are classified as either bin-burnt or fire-burnt, depending on the severity of the heating. Fire-burnt beans are often shiny black on the outside, with large internal cavities, whereas bin-burnt beans, although often black on the outside, are brown to dark brown in the cross sections, with no large, internal cavities. Furthermore, the fire-burnt beans are often fused together (Christensen and Kaufmann, 1977). Ultrastructure and mineral distribution in sound and heat-damaged canola/rapeseed have been studied by Mills and Chong (1977). Heat-damage may also result from improper artificial drying. Seeds damaged by excessive heat in drying have reduced viability, are darker, and may have blistered pericarps, or seed coats. If heat-damage is extreme the seeds may explode or partially pop (Freeman, 1980).

2.12.5 Effects to residential surroundings

Moulds release tiny spores to reproduce, just as some plants produce seeds. Mould spores can be found in both indoor and outdoor air, and settled on indoor and outdoor surfaces. When mould spores enter a damp environment, they can begin growing and digesting whatever they land upon in order to survive (Agency, 2011). Reports of a black, sooty substance located in close proximity to distilled spirit warehouses have been reported worldwide. One such report in Bonnybridge, Scotland, showed residential homes located near whiskey warehouses were severely blackened, along with blackening of local vegetation to the extent that it has died off (BBC News, 2009). For years residents in

Dumbarton, Scotland, have battled a fungal substance that they called “whisky black” (Kemp, 2011). The residents claim the mold is linked to the warehouses near their neighborhood, where the black fungus clings to brick, wood, and metal. Similar blackened buildings can be found in places like West Lothian, UK (Mcwhirter, 2011) and Ontario, Canada (Smith, 2011).

2.12.6 Toxins

Under suitable conditions of moisture and temperature, spoilage moulds produce poisonous substances, called mycotoxins, on stored grains and processed feeds (Mueller *et al.*, 2013). When mycotoxin-contaminated grains are eaten by susceptible animals, disease conditions called mycotoxicoses can result. The effects of mycotoxins on animals vary depending on the species and age of the animal, and the type and the amount of toxins present in the feed (Hussein and Brasel, 2001). Disease effects include lack of weight gain (Smith *et al.*, 1992), formation of tumors, and loss in productivity (Khlanguiset *et al.*, 2011). Others include fetal abnormalities, and sudden death.

In the USA, aflatoxins, produced by *Aspergillus flavus* sometimes occurs in poultry feeds (Hamilton, 1985). Aflatoxins have also been reported in grain dust, posing health problems for workers handling aflatoxin contaminated corn in Georgia, the USA (Burg *et al.*, 1982). Long time ago, aflatoxin was shown to occur in fragments of fungal mycelium and other mycotoxins in the fungal spores in grain dust. An overview of the worldwide risks from mycotoxins is expressed by Mannon and Johnson (1985). It is possible that other toxic substances including carcinogens are produced when grain and grain products become heated and/or burnt. The production of such toxic substances and their effects on animals when heat-damaged products are incorporated into animal feeds require further investigation.

2.12.7 Allergens

Spoilage fungi present in and on stored grains cause allergic health problems in both humans and animals (WHO, 2009; Shih-Wen, 2013). In some people an allergic reaction to fungal spores may take the form of asthma, or occasionally a condition known as hypersensitivity pneumonitis or extrinsic allergic alveolitis. At least 70 allergies have been well characterized from spores, vegetative parts and small particles from fungi (Kurup *et al.*, 2000). Two types of fungus-related health problems have been described in humans: bronchial asthma and farmer's lung. Such health problems are caused by allergic reactions in the respiratory tract stimulated by allergens primarily from fungal spores (Wardlaw and Gedes, 1992). In 1968, over 70% of the grain in the province of Saskatchewan in Western Canada was harvested or initially stored in a tough or damp condition because of unusual harvest conditions. Subsequently, 20 out of 3200 farmers and elevator managers who had worked with the damp, heated, or spoiled grain developed acute farmer's lung syndrome (Dennis, 1973). For a review on the nature of grain dust, work exposure to the dust, and related health disorders were explained by Manfreda and Warren (1984). Mould exposure may also be related to non-specific chest problems (Kim *et al.*, 2013).

2.13 Economic Impact of Mycotoxins

Fungi can damage the product in a number of ways: they can produce chemicals called enzymes which may stop seeds from germinating, they decrease the quality of the products for food, through discolouration or change in taste (bad flavour or smell), and they decrease the nutritive value and some fungi produce substances which are poisonous to people and animals (mycotoxins). The costs to farmers include reduced income from outright food or feed losses and lower selling prices for contaminated commodities (Oliveira *et al.*, 2014). Both quantity and quality of meat, milk, and egg production

decreases. According to statistics up to 25% of the world's crops are mycotoxin-contaminated and over 4.5-5.0 billion people around the world especially in less developed nations are at the risk of chronic exposure to mycotoxins (Bhat, 2008; FAO, 2010; Tiffany, 2013).

For instance, the estimated losses in wheat and barley attributed to the *Fusarium* mycotoxins in the United State alone are about 2 900 million USD a year, while the financial losses caused by mycotoxins due to decreases in the productivity of farm animals are however difficult to assess (Windels, 2000; CAST, 2003). According to Cardwell *et al.* (2001), the estimated losses due to *Fusarium* toxins deoxynivalenol (DON) and Zearalenone (ZEA) to Ontario pork producers alone was put USD at 9 000 000 as a result of reproductive problems arising from zearalence, while that due to DON (Vomitoxin) was put to USD 12 000 000 as a result of reduced growth rate in growing and finishing hogs because the animals eat less feed when contaminated with DON. Contamination of grains by aflatoxins alone inflicts annual losses of more than USD 750 million in Africa and is a major economic and health problem for the continent (Goyal *et al.*, 2003). Mycotoxins have attracted worldwide attention due to the significant losses associated with their impact on human and animal health and consequent national economic implications (Makun *et al.*, 2009).

In certain African countries, approximately 60% of the crops are lost due to fungal spoilage and mycotoxin contamination, thereby contributing to food insecurity Cardwell *et al.* (2004). The economic losses affect the entire chain of food and feed production by the reduction of marketable grains, discounts for contaminated grains, increased cost of drying, decreased weight gain in animals (ill-thrift), fertility problems and increased costs of animal health (Jemmali,1987). In the USA, it is estimated that the annual direct

economic burden from aflatoxins, fumonisins and trichothecenes amounts to USD 1.5 billion to USD 2.5 billion (Steyn, 2008).

Economic losses occur because of several reasons such as (i) yield loss due to diseases induced by toxigenic fungi (Mutegi *et al.*, 2009) (ii) reduced crop value resulting from mycotoxin contamination (iii) losses in animal productivity from mycotoxin-related health problems and (iv) human health costs (Bhat and Miller, 2010). Additional costs associated with mycotoxins include the cost of management at all levels— prevention, sampling, mitigation, litigation, and research costs. These economic impacts are felt all along the food and feed supply chain: crop producers, animal producers, grain handlers and distributors, processors, consumers and society as a whole (due to health care impacts and productivity losses). The estimates of the costs of control of mycotoxins in food products in the United States vary; one report estimated the costs to be at the tune of USD 0.5 to USD 1.5 billion/year and another estimated the costs to be at the tune of USD 5 billion/year for the USA and Canada (Steyn, 2008). Neither of these estimates included human health impacts or crop yield losses. Aflatoxins in maize in the USA have been estimated to cause losses of up to USD 225 million/year, excluding mitigation costs (USD 20-30 million/year just for testing). Aflatoxins in the USAs' peanuts were estimated to cause over USD 25.8 million losses per year during 1993 to 1996, with most of the costs (nearly USD 23 million) being shouldered by the shelling segment of the industry (Miller, 1995). Grower losses were estimated at about USD 2.6 million per year (Munkvold, 2003).

In developing countries, few estimates are available, but based on the elevated levels of aflatoxins regularly found in the developing world; it is likely that losses consistently exceed those occurring in the United States. As an example, losses due to aflatoxins in

three Asian countries (Indonesia, Philippines, and Thailand) were estimated at USD 900 million annually (Lubulwa and Davis, 1994). It is observed that in Indonesia, the Philippines, and Thailand, 5% of the maize and groundnuts produced were discarded because of aflatoxin contamination (Lubulwa and Davis, 1994). The annual cost of contamination due to aflatoxin and other molds in these countries in terms of product spoilage, human health effects, and losses in the poultry and pork sectors were calculated to be 477 million Australian dollars about a decade ago (Vasanth *et al.*, 2003). On the other hand, a study done in Germany on 'quantification of the economic impact of EU aflatoxins standards on developing and transition countries' shows 1% tightening of the standards would reduce trade flows from these countries by 1.07% (Khachatryan *et al.*, 2005).

Exports of agricultural products particularly groundnuts from developing countries have dropped considerably in recent years resulting in major economic losses to producing countries (Otzuki *et al.*, 2001; Bhat and Vashanti, 2003). Mycotoxin losses to livestock and poultry producers from mycotoxin-contaminated feeds include death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency. Other adverse economic effects of mycotoxins include lower yields for food and fibre crops.

Reduced crop value is a significant component of the losses caused by mycotoxins. This affects crops entered into local trade as well as crops intended for export. In aflatoxin-outbreak years in the USA, many producers are turned away by grain elevators and other buyers because their crops exceed the 20 ppb limit. They are forced to either accept a lower price on the local feed market or dispose of their crop. Internationally, a standard limit of 4 ppb (adopting the European Union limit) for

aflatoxins in peanuts would be estimated to cost about USD 450 million annually in lost exports (William *et al.*, 2004). The impact of export losses is worsened by the situation in which developing countries, whose populations are most at risk for aflatoxin exposure, may be forced to export their highest quality maize and retain the poorer grain for domestic use (Wu, 2004). Of the reported USD 900 million impacts of aflatoxins in Southeast Asia, USD 500 million of the costs were related to human health effects (Schmale and Munkvold, 2003). On a global scale, human health is the most significant impact of mycotoxins, with significant losses in monetary terms (through health care costs and productivity loss) and in human lives lost.

2.14 Pre-harvest Strategies for Mycotoxin Management

The pre-harvest strategy for mycotoxin management includes weeding, tillage practices, fertilizer application, weed control, and irrigation systems (Kimanya *et al.*, 2008a). Crop rotation (Devreese *et al.*, 2013) and management of crop residues are also important in controlling *A. flavus* and *F. moniliforme* infection in the field. Crop rotation is important and is focused on breaking the chain of infectious material, for example by wheat-legume rotations. Including maize in the rotation should be avoided, as maize is very susceptible to *Fusarium* infestations. Furthermore, plant breeding is also encouraged in managing mycotoxins in the field. Crop management practices such as weeding reduce water usage and assist in reducing moisture stress and may therefore contribute to reduced mycotoxin contamination of the grain (Moreno and Kang, 1999). Irrigation is also valuable to prevent fungi infestation by reducing plant stress. All plants in the field need an adequate water supply; however, excess irrigation during flowering (anthesis) makes conditions favorable for *Fusarium* infection (Codex Alimentarius, 2002).

In Africa, crops are cultivated under rain fed conditions, with low levels of fertilizer and little or no pesticide application. These conditions promote mycotoxin infection of fertility stressed plants; and any action taken to reduce the probability of silk and kernel infection would reduce mycotoxin contamination (Setamou *et al.*, 1998).

The selection of cultivars of cereal crops resistant to *Fusarium* and *Aspergillus* pathogens is currently viewed as a viable and sustainable option for reducing the contamination of grain (D'Mello and MacDonald, 1997). Interestingly, it has been observed that the resistance of some cultivars of agriculturally important crops to fungal infection has been correlated with their content of phenolic compounds before or after infection (El Modafar *et al.*, 2000). As mentioned earlier, when properly used to control fungal diseases of cereal plants, fungicides minimize the possibility of mycotoxin production (D'Mello and MacDonald, 1997; Placinta *et al.*, 1999). A worrying trend of resistance to fungicides by *Fusarium* pathogens has now been observed which may in time drastically reduce their overall impact (Placinta *et al.*, 1999).

Insecticides, which indirectly reduce mycotoxin production by reducing insect damage, may also directly inhibit fungal growth and mycotoxin production. An example is Naled which has been found to inhibit growth and aflatoxin production by *A. parasiticus* (Draughton and Ayres, 1981). Payne *et al.* (1986) demonstrated in an extensive four year study that the reduction of moisture stress was associated with lower levels of aflatoxin contamination. Despite the important link between moisture stress (drought) and higher mycotoxin levels and the fact that droughts occur commonly, breeding for drought resistance has received little or no interest (Moreno and Kang, 1999). Drought damaged plants are shown to be more susceptible to infection, so crop planting should be timed to avoid high temperatures and drought (Kabak *et al.*, 2006).

Other management practices such as mixed cropping with vegetables have been found to reduce aflatoxin contamination of maize, whereas intercropping with cassava, groundnuts or cowpeas and ear damage on the field were found to increase aflatoxin contamination (Hell *et al.*, 2003). Munkvold and Desjardins (1997) observed that, genetic engineering of plants to produce antifungal proteins or to detoxify mycotoxins *in planta* was a feasible approach to minimizing the risk they pose. For example, transgenic corn has mostly been genetically manipulated to include the gene from *Bacillus thuringiensis* (Bt) which responsible for the production of the protein Bt cry1Ab, known to be toxic to insects. This corn is now widely known as Bt corn and has been demonstrated to have higher yields and lower levels of insect damage and infection by *Fusarium* compared to non-transgenic corn (Gatch and Munkvold, 2002). In addition, it has been observed that total fumonisins were reduced in some cases by as much as 30 to 50 times in Bt maize hybrids compared to the levels in non-Bt hybrids (Hammond *et al.*, 2004). Bt maize hybrids provide effective control of larvae of crambid moths such as *C. partellus* and provide partial to very good control of the noctuids *S. calamistis* and *B. fusca* (Tende *et al.*, 2010). The use of genetically modified maize is still marginal in most parts of the world due to the uncertainty about the long term safety of consuming such products. In addition, the use of Bt maize is limited in developing countries where poor communal/rural farmers tend to produce seeds from the previous harvest. Moreover, the emergence of insect resistance is now threatening the continued use of Bt maize (Linacre and Thompson, 2004).

Another benefit of Bt maize is reduced occurrence of ear moulds. Because insect damage provides a site for infection by moulds, Bt-protected maize can have lower levels of toxins produced by moulds (i.e., mycotoxins), especially fumonisin and deoxynivalenol (Dowd, 2000; Munkvold *et al.*, 1999). Since then, various Bt maize hybrids have been developed that contain transgenes of either the Cry1 or Cry2 family and has always been

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considered an important pest of maize. Reduced pest status was reported throughout South Africa since the release of Bt maize in 1998 (Gouse *et al.*, 2005). Bt maize has revolutionized pest control in a number of countries, but there still are questions about its use and impact.

2.15 Post-harvest Strategies for Mycotoxin Management

Unlike the pre-harvest period, most of the conditions during the post-harvest period can actually be controlled. Good agricultural practices (GAP) that have been found to limit mycotoxin production from the point of harvesting and during storage include rapid drying to moisture contents of about 13-14% (Devreese *et al.*, 2013; Hussein and Brasel, 2001); storage in dry cool rooms with adequate aeration to avoid 'hotspots' from building up and also making it capable of preventing the entry of pests such as rodents and insects (Jayas and White, 2003), application of effective pesticides and fungicides (D'Mello and MacDonald, 1997; Placinta *et al.*, 1999). During the post-harvest stage, thorough drying, prompt storage and transport using clean, dry containers are the basic elements of aflatoxin prevention and control. Before storage, visibly damaged or infected grains should be removed (Jard *et al.*, 2011). Timely harvest is also critical for aflatoxin prevention. One study found that aflatoxins in maize increased 4 to 7 fold after a 3 to 4 week delay in harvest after maturity (Hell *et al.*, 2008). Prolonged harvesting, and long drying periods on the field should be avoided as they have been associated with higher aflatoxin levels in corn in Benin (Hell *et al.*, 2003).

2.16 Control Measures of Mycotoxins

A broad category of preventive measures have been attempted. They include; physical decontamination, biological decontamination, chemical decontamination plant breeding, good agronomic practices, and detoxification.

2.16.1 Physical decontamination

Physical methods used for removal or elimination of mycotoxins from contaminated commodities include; density segregation and floatation, cleaning and washing, sieving, dehulling, hand picking and electronic sorting, irradiation, milling; thermal degradation, solvent extraction, and adsorption (Ma *et al.*, 2002). The segregation of commodities into various particles, sizes, and subsequent removal of the fractions that contain higher toxin concentration reduces the level of mycotoxin in the entire commodities (Desjardins *et al.*, 2000).

Mould damaged and mycotoxin contaminated kernels exhibit different physical properties with respect to undamaged, therefore, they may be separated by density segregation in certain liquid or fractionation by specific gravity separator (Desjardins *et al.*, 2000). Corn screenings or broken corn kernels usually contain fumonisin level about 10 folds higher than is the case with corn. Therefore, the separation of the screenings based on size, has been suggested as a candidate method for decontamination (Gbodi *et al.*, 2001; Desjardins *et al.*, 2000).

Irradiation (γ -irradiation, X-rays, ultraviolet light, and visible light) has been used for inactivation or destruction of some mycotoxins. Gamma irradiation reduced T-2 toxin, zearalenone and deoxynivalenol levels of wheat, corn, and soyabeans by 16%, 25%, and 33% respectively and DON and fumonisin in corn by 13% and 20% respectively (Fanelli *et al.*, 2003). Detoxification of 70% to 90% of trichothecenes was observed in contaminated corn in Austria by applying ultrasonication without altering its original taste and appearance (Glaston *et al.*, 2000).

Addition of nutritionally inert sorbent (hydrated sodium calcium aluminosilicates, zeolite activated carbon, bentonite, clays and special polymers) in the diet reduces the absorption of mycotoxins from the gastro intestinal tract, thereby avoiding the toxic effects of livestock and their carryovers into animal products (Siame *et al.*, 1998). The efficiency of the absorption depends on the chemical structure of both the adsorbent and the mycotoxin.

2.16.2 Biological detoxification

Biological detoxification involves the enzymatic degradation or transformation of toxins leading to less toxic products. Biological detoxification can be regarded as any microbial based (whole cell or enzyme) system which results in the biotransformation or degradation of mycotoxins giving rise to metabolites that are either non-toxic or less toxic than the parent molecule (Avantaggiato, 2012). *Flavobacterium aurantiacum* has been observed to metabolize aflatoxin B₁ to water soluble products and CO₂ in various foods (Line *et al.*, 1994). A black yeast fungus *Exophiala spinifera* was able to grow on fumonisin B₁ and a sole carbon source (Blackwell *et al.*, 1999). It hydrolyzed fumonisin B₁ (HFB₁) yielding free tricarballylic acid and aminopentol (AP1) and the hydrolysis was followed by oxidative deamination of the resulting aminopentol (Duvick *et al.*, 1998). Fumonisin esterase and deaminase enzymes were isolated from the *Exophiala spinifera* and expressed in transgenic corn plants showing a complete metabolization of fumonisin B₁ with the release of carbon dioxide (Duvick *et al.*, 1998; Choudhary and Kumar, 2010). While yeast expressing mycotoxin-degrading enzymes may offer a natural way of providing these activities, transgenic plants are being proposed as an economic approach to reduce fumonisin contamination of maize (Diaz and Smith, 2005).

Enzymatic reactions also offer a specific, often irreversible, efficient and environmentally friendly way of detoxification that leaves neither toxic residues nor undesired by-products (Kolossova and Stroka, 2011). These mycotoxin degrading enzymes are primarily produced by microorganisms. Epoxidases are enzymes which are able to detoxify trichothecenes by transforming their epoxy group into diene groups (Schatzmayr *et al.*, 2006). For example, DON can be detoxified to its de-epoxy form, DOM-1. Takahashi-Ando *et al.* (2002) reported that ZON is converted into a less estrogenic product by the cleavage of the lactone structure. The responsible enzyme is a lactonohydrolase, originating from the fungus *Clonostachys rosea* IFO 7063. Recently, two genes of *Sphingopyxis*. MTA 144 responsible for the detoxification of FB₁ have been identified, and recombinant enzymes have been produced (Hein *et al.*, 2010). The degradation of FB₁ consists of two consecutive pathways. FB₁ is first metabolized to HFB₁ by a carboxylesterase, followed by an aminotransferase, which deaminates HFB₁, leading to an even less toxic compound (Hein *et al.*, 2010).

2.16.3 Chemical decontamination

The wide variety of chemical decontamination processes include radiation, oxidation, reduction, ammonization, alkalization, acidification and deamination (Kabak *et al.*, 2006). Chemical substances used for decontamination of mycotoxins are acids, alkali, oxidizing reagents, reducing agents, and chlorinating agents (Colovic *et al.*, 2013). These substances can cause mycotoxin content to be reduced by up to 99% (Colovic *et al.*, 2013). Although they are very effective, chemical treatments are not widely used due to practical problems: they are expensive and time consuming; they can change palatability and nutritive value of material; they decrease material quality, and can induce the formation of toxic by-products (Avantaggiato, 2012).

Moist ozone and dry ozone were able to reduce deoxynivalenol concentration in contaminated maize by 90% and 70% respectively (Rosa, 2003). Ozonation is reported to be used to destroy AFB₁ in food. Apart from the efficacy of AFB₁ degradation, ozone can kill pests in food and also has the potential advantages for inactivation of microbes including bacteria, fungi, and viruses (Tiwari *et al.*, 2010). In addition, ozone has a high penetration capacity and can be quickly decomposed to oxygen without producing any toxic residuals, making it have numerous potential applications in the food industry. Ammoniation treatment combined with heat and pressure was able to reduce fumonisin level by 79% in maize contaminated with 86 mg/kg of fumonisin B₁ (Park *et al.*, 2004). Deoxynivalenol level was reduced by 9% and 85% in corn when exposed to 100% ammonia for 1 hour and 18 hours respectively (Rosa, 2003). Ammonia hydroxide (3%) was able to reduce zearalenone by 64% in naturally contaminated corn (33.5 mg/kg) after 16 hours of exposure (Charmley and Prelusky, 1994; Hope *et al.*, 2003).

The use of ammonia to detoxify grains has been studied by a number of workers and is considered to be the most acceptable and efficient method in an industrial scale (Moss, 1998). Norred *et al.* (1991) have shown that ammoniation at atmospheric pressure and ambient temperature only slightly reduced the fumonisin levels by hydrolysis to aminopentol. However, when ammoniation is done under high pressure and ambient temperature larger than 50 degree celcius, it reduces fumonisin and aflatoxins content by 79% (Park *et al.*, 1996) and 93% (Martinez *et al.*, 1994), respectively. Weng *et al.* (1994) determined that the degradation of aflatoxins by ammoniation was irreversible, helping to ease concern that the degradation products could be converted back to active carcinogens in the stomach. The major limitations of ammoniation are kernel discolouration and a strong ammonia odour, which need to be resolved to boost the chances of industrial success of this form of detoxification (Lillehoj and Wall, 1987).

Nixtamalization is a traditional method practiced in Mexico to detoxifying aflatoxins in corn by boiling and soaking in calcium oxide (lime) to produce tortilla flour (Maciorowski *et al.*, 2007). Nixtamalization, the treatment of maize with lime (calcium hydroxide) and heat to produce masa/tortilla flour, has been shown to reduce fumonisin B₁ levels by hydrolysis to hydrolysed fumonisin B₁ (Sydenham *et al.*, 1995). The Maillard reaction (non-enzymatic browning) between fructose and the amino group of fumonisin B₁ has been shown to result into a significant decrease in the level of detectable fumonisin B₁ (Lu *et al.*, 1997). The reaction results into the removal of the primary amide from the fumonisin, giving rise to a product that has been determined to be non-toxic and non-cancer initiating elements in rats. Park *et al.* (1996) have also reported the reduction of fumonisin concentrations by up to 100% in contaminated corn with treatment of a combination of hydrogen peroxide (H₂O₂) and sodium bicarbonate (NaHCO₃).

The reaction products were found to have a much lower toxicity than the untreated maize. In view of the plethora of proposed chemical detoxification techniques, Munkvold and Desjardins (1997) correctly stated that before any of these methods can be used industrially a great deal of work needs to be done to ensure that the products retain their functionality and that their sensorial qualities are not severely compromised. In addition to this, the consequences of the application of these methods on the nutritional and safety aspects of the products need to be determined.

2.16.4 Plant breeding

Cultivating a variety of crops that are resistant to infestation by certain mycotoxin-producing moulds will minimize the problem of mycotoxin contamination (Ma *et al.*, 2002). For instance, the problem of ergot contamination of cereals and millet has been

successfully minimized by cultivating a variety of rye, wheat, pearl millet that are resistant to the disease (Torres *et al.*, 2001; Simpson *et al.*, 2002). However, there has been little success in providing resistant varieties of maize and groundnuts to minimize aflatoxins.

2.16.5 Agronomic and good agricultural practices

Agronomic practices such as avoiding water stress, minimizing insect infestation and reducing inoculum potentials help to minimize mold contamination and mycotoxin production (Langseth *et al.*, 1996). According to Lipps and Deep (1991), good agricultural practices at both pre-harvest and post-harvest such as appropriate drying techniques, maintaining proper storage facilities and avoiding exposure of grains or oilseeds to moisture during transport and marketing are all control measures to help minimize mycotoxin contamination through minimizing mould growth. Sequestering contaminated, moldy, shriveled or insect infested seeds from sound seeds have usefully minimized aflatoxin contamination in groundnuts (Krysinska-Traczyk *et al.*, 2001).

2.16.6 The detoxification of mycotoxins

Mycotoxin detoxification refers only to postharvest treatments designed to remove, destroy (decontaminate) and ultimately reduce the toxic effects of mycotoxins (detoxify). The potential ability of a plant to detoxify mycotoxins *in situ* is treated as a prevention strategy. The mycotoxin content of grains can further be reduced during processing. Automatic colour sorting, often in combination with manual sorting, is widely used to segregate kernels of abnormal appearance (which are considered more likely to contain aflatoxin) during the processing of edible grade groundnuts (Whitaker *et al.*, 1999). A further segregation process involves the removal of aflatoxin from animal feeds after ingestion. Here, mycotoxin binding agents-hydrated sodium calcium aluminosilicate,

zeolite, bentonite, kaolin, spent canola oil bleaching clays which are included in the diet formulation, are reported to be able to remove aflatoxin, by adsorption from the gut (Huwig *et al.*, 2001; EFSA, 2009).

Clay products, including bentonites, zeolites and Hydrated sodium calcium aluminosilicate (HSCAS), are the most common feed additives effective in binding aflatoxins *in vitro* as well as *in vivo* (Kabak *et al.*, 2006). Because of their fairly nonpolar properties, they lack the ability of adsorbing *Fusarium* mycotoxins, such as fumonisins, zearalenone (ZON) and trichothecenes, as well as ochratoxin A (OTA) (Kabak *et al.*, 2006; Phillips *et al.*, 2008). HSCAS has a lamellar interlayer structure in which the planar aflatoxin B₁ (AFB₁) can be bound. The interaction is based on the negative charge of the clay with the partly positive charged dicarbonyls of AFB₁ (Phillips *et al.*, 2008). Although the mentioned clays have proven to be effective in preventing aflatoxicosis in various animal species, several disadvantages should be considered. They do not exert any binding potential towards other mycotoxins, they can adsorb vitamins and minerals, and the risk of natural clays to be contaminated with dioxins should also be considered (Huwig *et al.*, 2001).

Ammonia, both as an anhydrous vapour and as an aqueous solution, is the detoxification reagent which has attracted the widest interest and which has been exploited commercially by the feed industry for the destruction of aflatoxin (Park *et al.*, 1988). Commercial ammonia detoxification (ammoniation) facilities exist in the USA, Senegal, France and the UK, primarily for the treatment of groundnut cake and meal. Commercial ammoniation involves the treatment of the feed with ammonia at elevated temperatures and pressures over a period of approximately 30 minutes.

2.16.7 Biological control of mycotoxins

The biological control of aflatoxin using the competitive exclusion approach has been demonstrated under field conditions in cotton (strain AF36) and maize (*A. flavus* K49) in the United States (Cotty, 2006; Abbas *et al.*, 2011). Also, in Nigeria using four non aflatoxigenic *A. flavus* strains formulated into a biocontrol product named Aflasafe™ (Atehnkeng *et al.*, 2008). Specifically in peanuts, a non-toxigenic *A. flavus* strain NRRL 21882 has been successfully commercialized as Afla-Guard™ brand biological control agent (Horn and Dörner, 2009). The first commercial use of Afla-Guard® in USA, resulted in an aflatoxin reduction averaging 85% in farmers' stock peanuts and as high as 98% in shelled stock (Dörner *et al.*, 2009). The same strategy was applied to prevent the aflatoxins contamination in peanuts from Australia and Argentina (Pitt and Hocking, 2009; Chulze, 2013). The natural, non-toxic, biocontrol product "aflasafe" uses indigenous strains (morphotypes) of *A. flavus* that do not produce aflatoxins (called atoxigenic strains). Atoxigenic strains can be directed at reducing aflatoxins contamination in several crops throughout an area simultaneously (Bandyopadhyay *et al.*, 2007). Manipulation of the composition of fungal communities (i.e replacing high aflatoxins-producers with their cousins that do not produce aflatoxins) so that high aflatoxins-producers are less common, as viable approach for reducing aflatoxins contamination throughout all crops grown in a target area (Cotty, 1989). These are applied to 'push out' their toxic cousins from the field in a process called 'competitive exclusion'. Competitive exclusion works by applying selected native atoxigenic strains to out compete and exclude aflatoxins-producers during colonization of grains, thereby reducing levels of aflatoxins contamination (Cotty and Antilla, 2003).

When appropriately applied prior to plant flowering, these native atoxigenic strains completely exclude aflatoxin producers. Aflasafe™ consists of a mixture of four native

atoxicogenic strains specifically targeted for a particular country or agroecosystem. Multistrain product such as Aflasafe™ may be superior to single- strain products because they display both immediate and long-term efficacy in diverse environments (Probst *et al.*, 2011). Field trials of the biocontrol method (aflasafe) on Nigerian stations in Zaria, Ikenne, Mokwa and Ibadan resulted in a 50 to 90% reduction in aflatoxin contamination in maize (Viray and Hollingworth, 2009). Biological control of mycotoxins using Aflasafe has proved to be a practical and effective method of reducing aflatoxin in the field (Cotty and Antilla, 2003).

Product development is currently also underway in Ghana, Mozambique, Tanzania, and Zambia. Use of bio-controls is promising with reductions of B₁ aflatoxins by as much as 83% (USAID, 2012). Plants sprayed with the atoxicogenic strain were 97% free of the aflatoxins at follow-up, and inoculation of soil (which may be more feasible for smaller farmers) has also proven to be a highly effective method to prevent aflatoxins pre-harvest (USAID, 2012).

Biocontrol technologies, in conjunction with other aflatoxin management tools, can profitably link farmers to markets, improve health of people and animals, and increase food safety. Widespread biocontrol adoption cannot occur, however, without first creating a flexible and enabling system for biopesticide regulation in tandem with other policy and institutional support. Licensing and stewardship of biocontrol products must receive attention to ensure that the quality and affordability of the products are not compromised.

2.16.8 Biological control of mycotoxins using lactic acid bacteria

Several methods are available for degrading toxins from contaminated food, for example, using alkaline ammonia treatment to remove mycotoxins from food. However, these

methods are harsh to food as they involve use of chemicals which are potentially harmful to health or may impair/reduce the nutritional value of food. Cooking food does not remove mycotoxins either, as most of them are heat-stable. Detoxification of mycotoxins in food through LAB fermentation has been demonstrated over the years (Chelule *et al.*, 2010; Dalié *et al.*, 2010). Using LAB fermentation for detoxification is more advantageous in that it is a milder method which preserves the nutritive value and flavor of decontaminated food (Bata and Lásztity, 1999). In addition to this, LAB fermentation irreversibly degrades mycotoxins without leaving any toxic residues. The detoxifying effect is believed to be through toxin binding effect (Hernandez-Mendoza *et al.*, 2009; Nikbakht-Nasrabadi *et al.*, 2013). Other authors allude to a possibility of an enzymatic interaction, although this was not thoroughly investigated (Zinedine *et al.*, 2005). As in the case of mycotoxin detoxification, LAB fermentation has also been successfully used to detoxify cassava toxins (cyanogens) following fermentation of cassava food products (Caplice and Fitzgerald, 1999). In addition to cyanogen detoxification, cassava fermentation contributes to the preservation and improvement flavour and aroma of cassava ferment (Holzapfel, 2002). Although cooking has been used as a method of cyanogens detoxification, it has a number of problems as it leaves residual cyanogens in processed cassava, which exist as glucoside, cyanohydrin or free cyanide, which are equally toxic as their parent compounds in uncooked food (Ravi and Padmaja, 1997).

Lactic acid bacteria (LAB) is a group of organic mycotoxin binders, which have recently become of interest (Gerbardo *et al.*, 2012). Lactic acid bacteria (LAB) are gram-positive, catalase-negative, non-sporulating, usually non-motile rods and cocci that utilize carbohydrates fermentatively and form lactic acid as major end product (Gerbardo *et al.*, 2012). These bacteria are mainly divided into four genera: *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Pediococcus*. They have been used in the food processing industry for

decades because of their fermentative and food preserving abilities (Mugula *et al.*, 2001). They have also displayed mycotoxin binding abilities (Dalié *et al.*, 2010).

Several authors have concluded that the strength of the mycotoxin-LAB interaction is influenced by the peptidoglycan structure and, more precisely, by its amino acid composition (Dalié *et al.*, 2010). The most extensively investigated mycotoxin binding LAB are strains of *Lactobacillus rhamnosus*. *L. rhamnosi* strains have demonstrated in vitro binding capability of DON, T-2, ZON, FB₁, AFB₁ and OTA (Niderkorn *et al.*, 2006; Piotrowska and Zakowska, 2005). There is recent evidence that some lactic acid bacteria have the ability to bind aflatoxin B₁ (Hernandez-Mendoza *et al.*, 2009). Hence, inclusion of culturally appropriate fermented foods in the diet may be a feasible method of partially reducing aflatoxin risk. Other methods of food processing, such as extrusion processing at temperatures greater than 150 °C can moderately reduce aflatoxins and other mycotoxins (Bullerman and Bianchini, 2007).

2.16.9 Dietary and food processing interventions

Reliance on a single crop especially cereals such as maize by natives in Africa as well as insufficient foods in both quantity and quality have been advanced as some of the factors which impair food security in the continent (Kana *et al.*, 2012) and predispose the natives to attacks from mycotoxins (Bryden, 2007). A variety of dietary interventions can reduce aflatoxin-related health risks. One simple dietary intervention, where feasible, is to consume less maize and groundnuts in favour of other food crops that have significantly lower aflatoxin contamination, such as rice, sorghum, and pearl millet (Bandyopadhyay *et al.*, 2007). Where it is not easy to make such a dietary shift, however (such as where maize and groundnuts have traditionally been staples), other dietary interventions may prove helpful.

One class of dietary interventions involves adsorption of aflatoxins. Adsorbent compounds, such as NovaSil clay (NS), can prevent aflatoxicosis in many animal species when included in their diet. They do so by binding aflatoxins with high affinity and high capacity in the gastrointestinal (GI) tract (Phillips *et al.*, 2008). Green tea polyphenols (GTPs) have been shown to inhibit chemically-induced cancers in animal and epidemiological studies (Fujiki *et al.*, 2002). Chlorophyllin sequesters aflatoxins during the digestive process and hence impedes its absorption (Egner *et al.*, 2001). A variety of substances have the potential to reduce aflatoxin-induced liver cancer by inducing phase 2 enzymes that convert aflatoxins' carcinogenic metabolite into a less harmful form that can be excreted (Kensler *et al.*, 1999).

2.16.10 Surveillance and awareness creation

This could be a long-term intervention strategy as has been advocated by WHO (2006) and James (2007). It is imperative for African countries to strengthen nationwide surveillance, increase food and feed inspections to ensure food safety, and local education and assistance to ensure that food grains and animal feeds are harvested correctly, dried completely, and stored properly. Awareness of what mycotoxins are and the dangers that they pose to human and animal health could be done through government bodies, private organizations, non-governmental organizations, national media networks such as radios and television programs as well as features in newspapers and magazines. Seminars and workshops could be used as avenues and bridges of information exchange and dissemination between researchers and the population respectively (WHO, 2006).

Control measures also include education of the population on the dangers of mycotoxin contaminated diet, early harvesting, rapid drying, sorting, sanitation use of improved storage structure, insect control, use of botanicals and synthetic chemicals, biological

control, and use of resistant varieties (Munkvold *et al.*, 1999; Jennings *et al.*, 2000). Monitoring of human population groups for diseases attributable to mycotoxins have to be carried out throughout the world to ensure a supply of safe food which is free from naturally occurring contaminants (Missmer *et al.*, 2006; Bhat and Miller, 2010).

2.17 The Effect of Spoilage on Food Security

Food spoilage may be defined as any change that renders food unfit or unsafe for human consumption. Microbiological spoilage is a significant problem with respect to the shelf life of raw materials and processed foods and is a key contributor to food waste. Future food security will necessitate that less food is wasted. More than 25% of the food that is bought is wasted because of delays in the food chain, poor storage, and human behaviour (FAO, 2010). Contamination and spoilage may occur at any stage along the food chain from harvest to retail, where bacteria, yeasts and moulds cause microbial spoilage. The availability of safe food is a prerequisite for the well being of people and the development of national economies. The low quality and safety of foods in Africa have a significant impact on human and animal health, and are a major constraint to export trade (Manjula *et al.*, 2009).

Similarly, food loss or waste is generally defined as edible material intended for human consumption, arising at any point in the food supply chain that is instead discarded, lost, degraded or consumed by pests between harvest and at the point of reaching the consumer (FAO, 1981). According to Bloom (2010), food waste occurs when an edible item goes unconsumed as a result of human action or inaction and is often the result of a decision made farm-to-fork by businesses, governments, and individual consumers. Food is lost or wasted at all stages of the food chain, from production on the farm or pond, to the food being served on a plate, and the causes of the losses are varied. For example, pests and

diseases can considerably decrease crop production with current losses estimated to be more than USD 150 billion worldwide (Oerk, 2006) contributing to global food insecurity.

There are several reasons why food loss in general and food waste in particular are important. The first reason is that the world population is growing and we will need more food to feed people. The United Nations predicts that the world population will reach 9.3 billion by 2050 (UN, 2011) and this growth will require at least a 70% increase in food production. The second reason is that, food waste represents significant amounts of money and other resources invested throughout food's entire lifecycle to produce, store, transport, and otherwise handle something that does not ultimately meet its intended purpose of feeding people (Buzby *et al.*, 2011). By lifecycle, it means all the way from the initial production of food through the disposal of any uneaten food. These resources include arable land, labor, energy, fresh water, agricultural chemicals (e.g., fertilizer, pesticides) and other inputs. Thirdly, is that, there are negative externalities that arise throughout the entire lifecycle of food (including food waste) and adversely impact society and the environment. In general, food that is produced, regardless of whether it is consumed or wasted, has contributed to pressure on the availability of fresh water and other natural resources (Lundqvist *et al.*, 2008), including land needed for urbanization, forests, and protected areas some of which is necessary for biodiversity and wildlife. In short, food production can result in the co-production of negative externalities. At the beginning of food's life cycle, negative externalities begin to arise when food is produced and these externalities are produced unnecessarily when food is wasted. A few examples of these externalities include: (1) greenhouse gas emissions from cattle production (Lundqvist *et al.*, 2008), (2) air pollution caused by farm machinery and trucks that transport food, (3) water pollution and damage to marine and freshwater fisheries from

agricultural chemical run-off during crop production, and (4) soil erosion, salinization, and nutrient depletion that arise from unsustainable production and irrigation practices (Nellemann *et al.*, 2009).

Disposing of uneaten food at the end of food's life cycle also poses negative externalities. For example, incinerating food waste creates emissions that can negatively impact human health and the environment. Landfilling food waste also negatively impacts the environment through the methane gas generated when food waste decomposes anaerobically. Methane has 25 times the global warming potential of CO₂ (over a 100-year time horizon) (IPCC, 2007).

2.18 Routes for Yield Loss of Maize

Current global production levels could far exceed demand if the loss of yields which occur during the pre-harvest period, during harvesting and in store could be minimized. The total yield loss in an agricultural season has been reported to be as high as 30% in the tropical humid countries and about 10-15% in cooler temperate areas (FAO, 1992). Poor post-harvest practices tend to exacerbate the situation resulting into losses in excess of 50% often being reported in many parts of the world (Sode *et al.*, 1995). A good example is the storage losses of up to 58% that have been reported in Nigeria as a result of insect and mould attack (Okereke and Nwosu, 1987), whereas physical and mechanical damage (that occur mostly during harvesting) contribute to yield loss, pests in the form of insects and rodents (Udoh *et al.*, 2000) together with moulds (Ominsk *et al.*, 1994) share the greatest responsibility for yield losses. Their intertwined roles are discussed below.

2.18.1 Role of insects in the yield loss of maize

Insects and other pests such as rodents are considered to be the principal causes of grain losses (Udoh *et al.*, 2000; Abebe *et al.*, 2009). They are able to cause severe damage at any stage in the pre-harvest or post-harvest periods. In addition, the damage from insect feeding provides preferential sites for penetration by fungi, with some insects also acting as vectors of fungi (Munkvold *et al.*, 1997; Sobek and Munkvold, 1999). A prominent example being the European corn borer (ECB) (*Ostrinia nubilalis* Hb), a major pest of corn in central Europe which causes severe physical damage and yield losses as a result of tunneling into the stalks and ears, in addition to promoting the infection of corn with *Fusarium* spp and *A. flavus* (Magg *et al.*, 2002). Moths of corn earworm (*Helicoverpa zea*) and the sap beetle (*Sitophilus zeamais*) have also been determined to be vectors of *A. flavus* (Rodriguez-del-Bosque *et al.*, 1998). Cereal grains are the most important staple crops in Africa. One of the key constraints to improving food and nutritional security in Africa is to reduce losses of cereals due to pests and weather factors, which are estimated at USD 4 billion annually (FAO, 2010).

2.18.2 Role of fungi in the yield loss of maize

Post-harvest losses to storage insect pests such as the maize weevil, *Sitophilus zeamais* Motschulsky, have been recognized as an increasingly important problem in Africa (Boxall, 2002). Affordable and effective control methods for reducing *S. zeamais* damage are needed in these countries. Infestation by this weevil commences in the field (Abebe *et al.*, 2009; Demissie *et al.*, 2008), but most damage is done during storage. Damaged grains have reduced nutritional values, low percent germination and reduced weight and market values (Giga *et al.*, 1991). The contamination of cereal grains by fungi is often an additive process, which begins in the field and potentially increases during harvest, drying and storage (CAST, 2003). Fungi are generally ranked as the second most important

cause of grain yield loss (Ominski *et al.*, 1994). Maize in particular is regarded as the most vulnerable to degradation by fungi (Munkvold *et al.*, 1997). In addition to grain yield losses, the fungal infection of maize has been determined to decrease the processing and nutritional quality of the grain (Bottalico *et al.*, 1989). The extent of reduction in grain quality is logically related to the degree of fungal development (Vieira, 2003). The losses incurred as a result of fungal growth are not only of economic importance but are also of significant public and animal health concern due to the possible production of mycotoxins by these fungi (de Campos *et al.*, 1980).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Descriptions of the Study Area

3.1.1 Location and size

Kilosa District is one of six districts that comprise Morogoro Region. It is located in Eastern Central Tanzania, 300 km west of Dar es Salaam, and between latitudes 5°55" and 7°53" South of the Equator and longitudes 36°30" and 37°30" East of the Greenwich.

3.1.2 Climate and topography

The climate of Kilosa District, as described in detail by Kimaro (1989), is characterized by a dry tropical climate of the semi-arid type. The mean annual temperature of the District is 25°C. Annual rainfall ranges from 800 mm in low-lying areas (500 m.a.s.l) to about 1300 mm in high altitude areas (1500 m.a.s.l). The district experiences an average of 8 months of rainfall (October to May) with the highest levels between February and March. The rainfall distribution is bimodal, with short rains (October to January), followed by long rains (Mid-February to May). The mean annual rainfall ranges between 1000 and 1400 mm in Southern flood plains, while the North (currently Gairo District) has a mean annual rainfall of 800 to 1100 mm. The district is divided into three physiogeographic units, which also constitute different agro-ecological zones (Gilliard-Byers, 1994). A map of the study area is presented in Fig. 4.

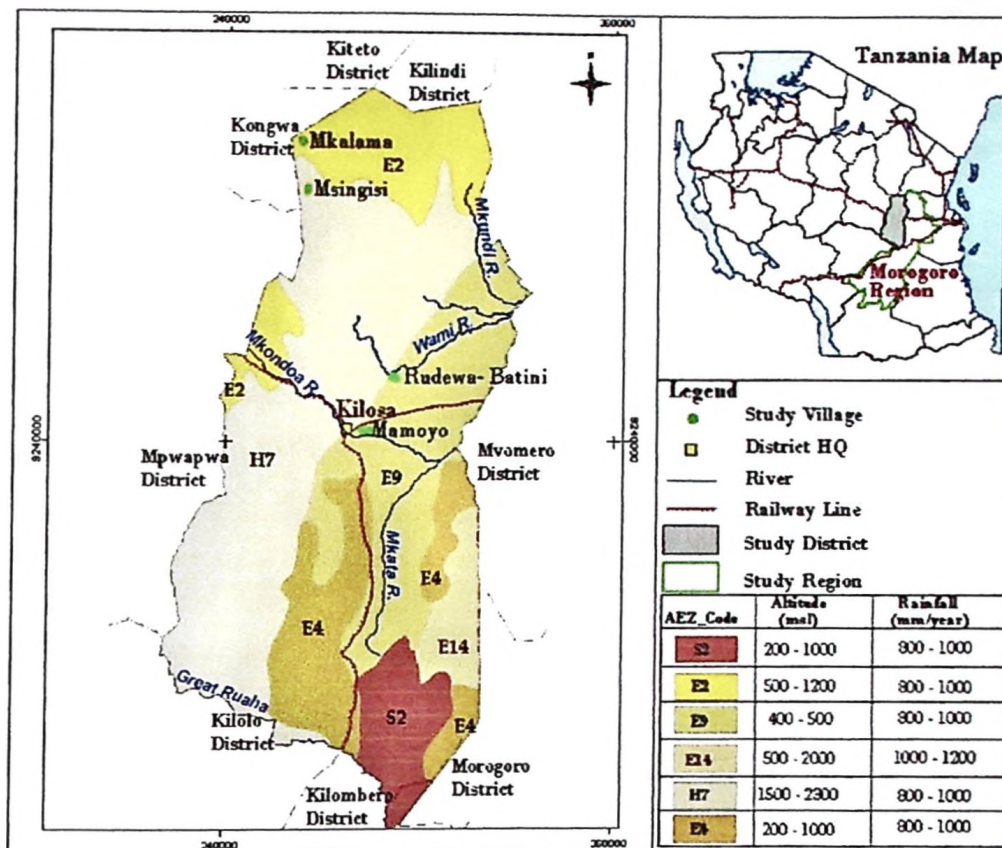


Figure 4: Kilosa District map showing the study areas

3.1.3 Soils and vegetation

The soil ranges from dark-reddish-brown to red sand loam in most parts and sand clay in the valleys. The vegetation is complex and dominated by the miombo woodlands and savannah grasses.

3.1.4 Socio-economic characteristics

In terms of socio-economic activities, Kilosa District is one of the districts in Tanzania with great potential of economic development and prosperity. The district has a very good climate and the land is favourable for agriculture and other economic investments. Kilosa District has relatively higher income compared to other districts in the country (PHDR, 2005). The contribution to the Gross Domestic Product of Tanzania by the year 2007, Kilosa ranked 6 with a contribution of 5.4% of the total.

The main occupation of the residents in the district is crop farming, which is predominant in the rural areas. The main crops grown include cereals such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and paddy (*Oryza sativa* L.); roots and tubers such as cassava (*Manihot esculentus* L.) and sweet potatoes (*Ipomea batatas* L.); oil seeds crops such as groundnut (*Arachis hypogaea* L.), and coconut (*Cocos nucifera* L.); and pulse crops such as cowpea (*Vigna unguiculata* L.), pigeon pea (*Cajanus cajan* L.), greengram (*Vigna radiata* L.) beans (*Phaseolus vulgaris* L.), and okra (*Abelmoschus esculentus* L.). Other crops include fruits such as orange (*Citrus sinensis* L.), banana (*Musa* spp.) and mangoe (*Mangifera indica* L.); and lastly vegetable crops such as onion (*Allium cepa* L.), tomatoe (*Lycopersicon esculentum* L.), and amaranthus (*Amaranthus* spp.) to mention a few.

3.1.5 Area, topography and agro-climatic zone

Kilosa District covers a total area of 14 567.9 square kilometers, of which 536 590 ha is suitable for agriculture, 483 390 ha is under natural pasture, 323 000 ha is reserved for Mikumi National Park, 80 150 ha is under forest cover and 14 420 ha is water and swamps (KDC, 2010). The district topography ranges from central and southern flood plains of the Wami, Mkata and Ruaha rivers which stand at 400 m.a.s.l to the cultivation steppe in the north around Gairo (currently, Gairo District) reaches 1500 m.a.s.l. The district is divided into three physio-geographic units, which also constitute different agro-ecological zones. The highest parts of the district found in the Ukaguru (2200 m.a.s.l), Rubeho (2200 m.s.s.l), and Vidunda (1100 m.a.s.l) mountains, which get annual rainfall of between 800 mm and 1100 mm; and the area is characterized by moderately fertile well drained soil, comprising of sandy (clay) loam soil. The lowest parts of the district are found in the central and southern plains, which experience an

average rainfall of 1000 mm and 1400 mm with poorly drained black clay and loamy soils which are suitable for maize, paddy, sisal, sugarcane and onion cultivation (KDC, 2010).

The mean annual temperature in Kilosa is between 25°C and 30°C. Table 7 shows the agro-ecological zones of Kilosa District, Tanzania. The highlands are characterized by cool climate and short rainy (rainfall is not bimodal) seasons with rainfall deficiencies for crop production. The lowlands experience high annual precipitation and warm climatic conditions during and towards the end of the rainy season.

Table 7: Agro-ecological Zones of Kilosa District

Zone	Characteristics Altitude (m.a.s.l)	Average rainfall (mm) per annum
Mountain/Highlands	>2200	300-1100
Plains/Lowlands	300-600	1000-1400
Medium Highland	<2200	900-1400

Source: URT, 2002; KDC, 2010 with modifications on altitude (m.a.s.l) and average rainfall (mm)

3.1.6 Demographic patterns

3.1.6.1 Population

As per 2012 Population and Housing Census, the district had 438 175 people out of whom 218 378 were males and 219 797 were females, with an average of 4.6 people per family and the growth rate of 2.5% (NBS, 2013). The district's population density was 34 persons per square km.

3.1.6.2 Site selection and justification

The survey was conducted in four villages of Kilosa District between 2010 and 2011 representing lowlands (moist) and highlands (dry) agro-ecological zones. Four villages in the study areas were selected: Mamoyo, Rudewa-Batini, Msingisi and Mkalama. Two villages per agro-ecological zone were chosen for the study since maize and groundnuts are well raised/grown in these areas.

The villages for the study were selected based on the following reasons: (i) The villages grow maize and groundnuts as the main source of income; and these were the crops, which they were used in the study of mycotoxins contamination, (ii) The assessment of the incidence of mycotoxins in stored maize and groundnuts and its implications on household food security has not been done in these villages despite the fact that, maize and groundnuts are the basic staple foods of people in Kilosa, (iii) The study area of Mamoyo, Rudewa-Batini, Msingisi and Mkalama are in two different agro-ecological zones, with respect to altitude and thus can provide a basis for comparison in the level of mycotoxin contaminations in Kilosa District. In Mamoyo and Rudewa-Batini, maize and groundnuts are mainly grown in the low altitude zone. In contrast, in Msingisi and Mkalama, maize and groundnuts are mainly grown in the higher altitude areas. These reasons were considered to capture the diversity in maize and groundnuts production systems in Kilosa and generate background information to build on for further studies.

3.2 Assessment of Incidence and Level of Mycotoxins in Stored Maize and Groundnuts

The sampling criteria for the selection of farmers who participated in the study followed the method described by Kaaya *et al.* (2006) with some modifications with question wording to suit the purpose of the study. In each village, 18 maize farmers and 18 groundnuts farmers were sampled once. Maize which was stored as cobs was shelled into grains and, the grains mixed to obtain a homogeneous (aggregate) sample. The coordinates of each site was recorded by using geographical position system (GPS) shown in Appendix 1. The samples were collected from different areas of a container and then mixed to produce a composite sample. The samples were taken with a grain sampling spear, at three levels: top, middle, and bottom. The samples were collected after 3 months of storage. The samples of approximately 1.0 kg of a well-mixed composite sample were packaged in a well-sealed container, and transported to the Tanzania Food

and Drugs Authority (TFDA) laboratory in Dar es Salaam for analysis. Data collected include weight of collected samples. Similarly, a 1.0 kg groundnuts sample was obtained from each interviewed household for aflatoxin testing. The sample was drawn from different parts of the farmer's storage container and thoroughly mixed.

3.3 Aflatoxins and Fumonisin Analysis

3.3.1 Aflatoxins analysis

From each farmer a representative sample of groundnuts (1.0 kg) was collected for aflatoxins analysis. Groundnuts stored as pods were shelled and samples were mixed thoroughly to obtain a homogeneous sample. A 250 g sub-sample was drawn from each 1.0 kg sample and ground in the laboratory using a dry mill kitchen grinder. The flour/groundnut paste was triturated in a blender in 70% methanol (70 ml absolute methanol in 30 ml distilled water, v/v) containing 0.5% potassium chloride (w/v) until thoroughly mixed. The extract was then filtered through Whatman No.1 filter paper and diluted in phosphate buffered saline (PBS) containing 500 µl Tween-20 (PBS-Tween). Analysis was performed using an HPLC instrument consisting of two chromatographic pumps, sampling system, and fluorescence detector (HPLC-FLD). The purified extract was then transferred to another tube and evaporated to dryness in a 60 °C water bath under vacuum. HPLC reverse-phase analysis was performed to maximize the fluorescence of aflatoxins in the aqueous mobile phase. The mobile phase was water and methanol (25:10, v/v). The flow rate was 1 ml min⁻¹. Fluorescence excitation and emission wavelengths were set at 365 nm and 440 nm. Detection and quantification limits (LOD and LOQ) were 0.2 and 1 µg/kg. The limits of detection (LOD) is defined as the lowest concentration that the analytical process can reliably differentiate from background levels. Only AFB₁ was considered for data analysis, being the most toxic and almost always dominant aflatoxin (Giorni *et al.*, 2007).

3.3.2 Fumonisin analysis

Dry-shelled maize samples were mixed and then 250 g was ground using a Romer Mill and homogenised and stored at -20°C until analysis. The process of extracting fumonisins was performed according to the method of Shephard *et al.* (1996). A portion of 25g of the ground maize sample was extracted with 40 ml of methanol: water (3:1, v/v) in 100 ml glass bottle fitted on a horizontal laboratory shaker. The extract/slurry was filtered through Whatman filter paper No.1 and the bottle rinsed with 10 ml of the mix of methanol and water (3:1, v/v). The pH of the filtrate was measured and values were within the pH range of 5.8-6.5. The mixture was centrifuged for 10 min at 2000 rpm.

A 10 ml aliquot of the filtered extract was applied to a strong anion exchange (SAX) cartridge fitted to a solid phase extraction manifold. Before applying the extract, the SAX cartridge was conditioned with 5 ml methanol, followed by 5 ml of a methanol-water mix (3:1, v/v). After application of the extract, the SAX cartridge was then washed with 8 ml of the methanol-water mix (3:1, v/v), followed by 3 ml of methanol. The fumonisins were eluted with an acetic acid-methanol solution (5:95, v/v) and the eluates were collected and evaporated to dryness with gentle stream of N_2 in 60°C water bath under vacuum.

For quantification and determination, samples for fumonisins analysis were cleaned up by Multisep® 211 column and also detected by the high- performance liquid chromatograph with fluorescence detection (HPLC-FLD). Fluorescence excitation and emission wave lengths were set at 335 nm and 465 nm. An aliquot was derivatized with a solution of 40 mg ortho-phthaldehyde (OPA) in a mixture of 1 ml of methanol, 5 ml of 0.1 M disodium tetraborate solution (3.8g $\text{Na}_2\text{B}_4\text{O}_7 \times 10 \text{H}_2\text{O}$ dissolved in 100 ml water) and 50 mL of β -mercaptoethanol. 100 ml of the mixture was injected within 4 min. into the HPLC for analysis using a reversed-phase HPLC fluorescence detection system. Fumonisin are

polar molecules, soluble in water and in polar solvents and thus ideally suited for determination by reversed-phase HPLC. Methanol-0.1M sodium dihydrogen phosphate (75:25, v/v) mixture adjusted to pH 3.35 with orthophosphoric acid was used as mobile phase. The flow rate of the mobile phase was 1 ml/min at a 65 °C reaction temperature. Fluorescence of the fumonisin OPA derivatives was detected at wavelengths of 335 nm (excitation) and 465 nm (emission) using a fluorescence detector. The LOQ were in the range of 0.02 to 0.5 µg/kg. Only FB₁ were considered for data analysis, being the most toxic and always dominant.

3.4 The Influence of Storage Practices on Mycotoxin Contaminations

3.4.1 Effect of grain protectants on mycotoxin contaminations

To study the effect of grain protectants on mycotoxin contaminations, two independent experiments were conducted for maize and groundnuts. Both studies were conducted in a randomized completely block design (RCBD) with four treatments replicated four times (for estimating experimental error) in a split-plot arrangement. The main factor was storage duration (3, 6 and 9 months). The sub-factor was grain protectant. Grain protectants were 2.0 g of actellic super dust, 30 g of wood ash, 80 g of dried neem leaves per bag and untreated control. Fresh neem leaves of same size and colour (indicative of same age) were collected from the neem tree in the surrounding area of the study villages. The leaves were dried outdoors in the shade and were then crushed and finally used. Maize variety TMV₁ was chosen since it is a commonly grown variety among smallholder farmers in the study area. Before the experiment, the harvested maize was checked for the existence of fungal spoilage and whether it was free from insect damage and placed in a polythene bag (2.0 kg of maize each). Grain sampling was carried out regularly every 12 weeks. The sampling intervals were 3 months, 6 months, and 9 months after storage. The bags were randomly placed in the store as shown in Appendix 2 and the

layout of the experiment is shown in Appendix 3. At each sampling, 0.5 kg of grain was collected from each polythene bag using a grain sampling spear for laboratory analysis.

For groundnuts, treatments were sorting, use of wood ash and untreated control. The approaches used in experiment explained above for the case of maize were also used in this experiment. At each sampling, 0.5 kg of groundnuts was collected from each polythene bag for laboratory analysis. The layout of the experiment was similar with that in experiment 1. The linear model for split-plot with the main plots arranged as an RCBD used in the experiment is shown in Equation 1.

$$Y_{ij} = \mu + D_i + \beta_j + \varepsilon_a + \beta D_{ij} + E_b \dots \dots \dots (1)$$

where:

- Y_{ij} = An observation in the i^{th} storage duration at the j^{th} treatment
- μ = The general effect
- D_i = The i^{th} storage duration effect
- β_j = The j^{th} treatment effect
- ε_a = Main plot error
- $\beta D_{(ij)}$ = The interaction between the i^{th} storage duration and j^{th} treatment
- E_b = Experimental error

3.4.2 Effect of storage methods on mycotoxin contaminations

A split-plot arrangement with 4 replications was used in this study. Studies were conducted in a randomized completely block design (RCBD) with three treatments replicated four times in a split-plot arrangement. The main factor was storage duration (3, 6 and 9 months). The sub-factor was maize storage method: storing maize on the floor, storing maize grains in polythene bags, and storing maize grains in cribs. Once every 3 months of storage, 1.0 kg of maize and groundnuts were collected from the respective

storage containers for laboratory analysis and the weight from each storage container was measured.

3.4.3 Effect of grain storage form on mycotoxin contaminations

The effect of grain storage form (shelled vs. unshelled) of maize and groundnuts under different storage times (3 versus 6; 6 versus 9 and 3 versus 9 months) was studied by determining the weight of maize samples after every 3 months. The t-test was used to compare the effect of storage form.

3.5 Assessment of the Effect of Weather on Mycotoxin Incidences in Maize and Groundnuts

Weather data were recorded for two phases for two years (2010 and 2011) where; phase 1 started from July to September (as dry season) and phase 2 started from October to December (as a wet season). Temperature and relative humidity were recorded daily from the first day of storage until the final day of storage using data loggers (Monarch Instruments RHTemp Track-It B Logger) recording at 1 hr intervals. Also, rainfall data were collected from nearby meteorological stations. The range of temperatures and relative humidity were recorded for two years' cycles. The data collected include: maximum and minimum air temperature ($^{\circ}\text{C}$), relative humidity (%), rainfall (mm) and geographical positioning system (GPS) coordinates (altitude, longitude and latitude). The weather variables were averaged across villages.

To study the effect of season on mycotoxin contaminations, storage months (July, August, September, October, November and December) were considered in the experiment, replicated in four villages. The covariates were weather variables, and the response was

the level of toxin. Log-linear model with Poisson distribution was used to determine the influence of weather on mycotoxin contaminations in stored maize and groundnuts.

3.6 The Effects of Mycotoxin Contamination and Loss due to Food Spoilage on Household Food Security

3.6.1 Design of experiment

The sample of approximately 10 kg of maize was stored in a small polythene bag for 9 months. The losses were estimated by the count and weighing method. Replicates in this case were the number of observations on monthly basis. The data collected were rotten and discoloured maize grains which were further counted as spoiled grains expressed in percentages. The damaged kernel characteristics which were determined during this study include: mouldy kernel, and off colour kernels.

3.6.2 Count and weigh method

This method was used to separate each sample, separates into undamaged and damaged portions, counts and weighs each, and calculates the percentage weight loss. It was assumed that the undamaged portion was totally undamaged. The method was used for unshelled and mold-damaged grains; and it provided a useful means of estimating loss at moderate infestation levels with a minimum of apparatus including spring balance with a range of 0.5 g and a tally counter. To control the initial moisture content (mc), freshly harvested maize was dried until below 14 moisture content as a range commonly recommended for safe storage.

3.6.3 Procedure used in estimating % weight loss of grains

The grains were separated into undamaged and damaged categories, the latter being separated according to cause. The grains in each category were counted and weighed

using Gwinner *et al.* (1996) method. The resultant data were substituted in the formula shown in Equation 2.

$$\% \text{ weight loss} = \frac{(W_u * N_d) - (W_d * N_u)}{W_u * (N_d + N_u)} * 100 \dots \dots \dots (2)$$

where:

- W_u = weight of undamaged grains
- N_u = number of undamaged grains
- W_d = weight of damaged grains
- N_d = number of damaged grains

The estimate annual revenue loss in USD for groundnuts was calculated using the mathematical formula shown in Equation 3.

$$ARL = AVR * AVC * \left(\frac{USD}{1500}\right) * 1000 \dots \dots \dots (3)$$

where:

- ARL = Annual revenue loss (in USD)
- AVR = Average annual production (in Tons)
- AVC = Average contamination (%)

3.6.4 Empirical models in estimating spoilage loss

In arriving at a final loss figure, the value of damaged grain in any alternative or secondary use was considered. If the grain intended for human consumption was damaged and, therefore used to feed cattle, the loss suffered by the farmer would be given using the following mathematical expression shown in Equation 4.

$$L_n = L_f - L_s \dots \dots \dots (4)$$

where:

- L_n = Net loss;

L_f = Value as food, and

L_c = Value as feeding stuff

The export loss of maize/groundnuts (Wu, 2004) is given by the following mathematical expression shown in Equation 5.

$$Export\ Loss_{ijk} = P_i \times W_{i,j} \times r_{ijk} \dots\dots\dots(5)$$

Where; $r_{ijk} = 1 - \int PDF_{ijk} dk$

- i = Crop (maize or groundnuts),
- j = Nation,
- k = International mycotoxin standard (fumonisin, aflatoxin),
- P_i = World price for food crop i per unit volume,
- $W_{i,j}$ = Total export weight (in metric tons) of crop i from nation j ,
- $r_{i,j,k}$ = Fraction of export volume of crop i from nation j ,
rejected at international mycotoxin standard k and
- $\int PDF_{ijk} dk$ = Probability density function

3.7 Farmers Practices that Contribute to Contaminations of Maize and Groundnuts by Mycotoxins

3.7.1 Sampling procedures

Four villages were selected for the study in which 18 farmers were selected from each village for the interview making a total of seventy-two (72) respondents. A structured questionnaire was administered to the farmers (Appendix 4). The basic questionnaire was adapted from a similar study by Kaaya *et al.* (2006) in Uganda. The farmers were asked, among other things, questions on the type of storage problems they experienced, when they noticed these problems, the type of seeds, and the storage systems they used. The study also took some personal observation to get salient information that would help in identifying problems faced by the farmers. In addition, the samples of maize and

groundnuts were collected from 18 prominent groundnuts and maize growing households in each village. The samples were mixed and stored in a labeled brown paper envelop and sent to the laboratory for fumonisins and aflatoxins laboratory analysis.

3.7.2 Measuring levels of awareness of mouldy infection in food crops among farmers

The questionnaire was self-administered and consisted of sociodemographic and socio-economic backgrounds as basic information of respondents (age, gender, marital status and household incomes). In regard to the knowledge and awareness of mouldy infection in food crops, a number of statements was created and they were measured using an index-scale (2=know, 1=not sure and 0= don't know). All statements were created with the reference to mycotoxins occurrence in Tanzania based on the literature review. Principal component analysis (PCA) was performed on three constructs index-scales to check factor dimensionality. Awareness and knowledge were extracted from a factor analysis conducted on each set of items defining each one in particular. A SCREE test with varimax rotation was used to obtain more interpretable factors (Hatcher, 1994). Items with factor loading of 0.40 or greater were retained for further analysis. To test the reliability of the questionnaire, a pre-test was conducted using the Crobach's alpha techniques.

3.8 Statistical Data Analysis

Objective 1: The data were organized using the Microsoft Excel 97 software program. Statistical analyses were performed using the SAS 9.1 software programme (SAS Institute, Cary, NC, USA). The distribution (%) of fumonisins and aflatoxins and levels ($\mu\text{g}/\text{kg}$) in maize and groundnuts kernels were calculated. Descriptive statistics such as percentages and frequencies were used to summarize the data. Tests of

significance were carried out using analysis of variance (ANOVA) at 5% probability level. The Chi-square test was performed to establish the relationship between variables (aflatoxins and fumonisins concentrations) and agroecological zones. To test if the resulting frequency distributions were similar for the four villages, the data were subjected to Kolmogorov-Smirnov (K-S) test as well as for equality of variances (Sprent and Smeeton, 2001).

Objective 2: The data on fumonisin and aflatoxin contamination levels due to different storage treatments were subjected to analysis of variance (ANOVA) using the PROC ANOVA procedure of Genstat Statistical Programme Version 13 (VSN International, UK) and the differences among the treatment means were compared using Fisher's Protected Least Significant Difference (LSD) test at 5% probability level in case the F-statistic was significant. The t-test was used to test the significance of relationships across variables. Levene's Test of Homogeneity of variance was carried out to determine whether the variance in mean level of fumonisins in maize and aflatoxins in groundnuts were the same for each of the two forms (shelled versus unshelled).

Objective 3: The data revealed in the study were subjected to statistical analysis using log-linear analysis with Poisson distribution.

Objective 4: The damaged kernels were determined and calculated as percentages using the Microsoft Excel 97 Software program.

Objective 5: Socioeconomic data were analyzed using the Statistical Package for Social Sciences (SPSS Version 14). All categorical data were coded as discrete binomial dummy variables for subsequent analysis. Frequencies of responses were calculated and

correlation analysis was carried out on the survey variables. T-test, ANOVA and Chi-square tests were used to test the significance of relationships across variables. The linear multiple regression model shown in Equation 6 was used in the analysis of factors influencing mycotoxins production in the study area. Linear multiple regression model check was conducted to:

- i. examine collinearity diagnostics for multicollinearity using tolerance and variance inflation factor (VIF) . More checking was done when the tolerance was less than 0.20 or VIF was greater than 5 (Cohen *et al.*, 2003).
- ii. examine residual plots for error variance assumptions (i.e., normality and homogeneity of variance)
- iii. examine influence diagnostics (residuals, dfbetas) for outliers. If the results showed no standardized Dfbeta values of < -2 or > 2 , it was concluded that the dataset does not include outliers or influential cases.
- iv. examine significance of coefficient estimates to trim (i.e., removing insignificant predictors) the model and revising the model and rerun the analyses based on the results of steps i-iv and finally, the final regression equation was interpreted using the coefficient estimates.

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \beta_5x_5 + \beta_6x_6 + \beta_7x_7 + \beta_8x_8 + \beta_9x_9 + \varepsilon_i \dots(6)$$

Where:

- Y = is a continuous variable denoting mycotoxin contaminations in $\mu\text{g}/\text{kg}$
- β_0 = is the intercept (the value of the response variable when the explanatory variable is 0)
- β_1 - β_9 = are independent variable coefficients showing the marginal effects of the unit change in the independent variables on dependent variable
- X_1 - X_9 = are independent variables
- ε_i = is an error term which represents unobservable factors assumed to be independently distributed over the survey period.

The explanatory variables used in the analysis are;

$SORT(X_1)$ = Sorting before storage

$LAT(X_2)$ = Leaving to dry after three weeks

$SUM(X_3)$ = Shelling using machinery

$IND(X_4)$ = Insect damage

$SSR(X_5)$ = Storing produce in the same room

$HOF(X_6)$ = Heaping produce on the floor

$SPS(X_7)$ = Storing the produce in shelled form(groundnuts)

$FAI(X_8)$ = Farmers awareness of insect in storage room

$UTP(X_9)$ = Use of traditional and botanical grain protectants (wood ash and dried neem leaves)

3.9 Statistical Data Analysis for Awareness of Mouldy Infections

Data was analyzed statistically using the software package for social sciences (SPSS); Version 14.0. The differences in mean total score of knowledge and awareness between the socio-demographic and socio-economic factors were determined by t-test (independent t-test). Measurements of association were carried by Chi-square test (χ^2) for categorical variables. The Chi-square test was used to see if significant relationships exist between the total knowledge and awareness score and the variables. The right response for each question received a score of 1, with 0 for the wrong response. Ordinal logistic regression analysis was used to examine the correlations between gender, education level, marital status and income level compared with knowledge scores of respondents. Independent variables were coded either 1 or 0. Age: less than 35 years = 0, 35 years and above = 1; Gender: Male = 0, Female = 1; Education status: less than high school = 0, high school and above = 1; Marital status: Single, divorced or widow = 0, Married = 1; Household annual income: Below USD 66.7 (100 000 Tanzanian Shillings) = 0, Above

USD 66.7 (above 100 000 Tanzanian Shillings) = 1. The dependent variable (knowledge) scores were coded either 1 or 0 (below the average score = 0, above the average score =1). Statistical significance was set at 0.05. The p-value of less than 0.05 was considered significant.

3.10 Hypotheses Testing

The hypothesis tested by this study was to determine whether storage practices (storage treatments, storage methods and storage forms) have no impact on mycotoxin contamination in stored maize and groundnuts. Two-way Analysis of variance (ANOVA) and independent t-test were used to measure the level of significance. A 95% confidence interval ($p < 0.05$) was used to determine significance, and conclusions were inferred based on all significant findings. When F-test was significant and less than 5%, the null hypothesis was rejected and the alternative hypothesis was accepted at 5% significance level. In addition, multiple linear regression analysis examined the linear relationships between factors contributing to mycotoxins productions in stored maize and groundnuts. For the effect of weather on mycotoxin productions, a Z -test was used to test the null hypothesis. The Z-statistic was obtained by dividing the estimate of the parameter for the asymptotic standard error (i.e. square root of the variance of the parameter estimate). Values of Z-statistics greater than $|1.96|$ were used to reject the null hypothesis at a significant level of 0.05 and therefore the alternative hypothesis was accepted at 5% significance level.

3.11 Limitations of the Study

The study only analysed samples of maize and groundnuts collected from farmers' stores. This limitation was based on the fact that only maize and groundnuts which were grown by farmers themselves were considered to be a good indicator for the incidence of

mycotoxins in a study area rather than taking into considerations of samples from the market which might come from different geographical areas of the country and therefore it would have been difficult to interpret such results. As a result, the study did not include maize and groundnuts samples from the market sellers and other retailers.

Also, the study did not actually measure the moisture content of maize and groundnuts but used the simple method of detecting moisture in grains such as salt and bottle technique which, according to literature, indicates the moisture content of the grain. Only four out of 164 villages were included in the study. This was because of limited time and funds available to include more villages. Eighteen households from each village were also included in the study. This is due to the homogeneity which exists among the various villages in terms of crop productions, food storage patterns and cultural values. Only households producing maize and groundnuts growers were included in the study.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Incidences of Mycotoxin in Household Stored Maize and Groundnuts

4.1.1 Incidences of mycotoxin contamination in stored maize and groundnuts

The incidence of fumonisins B₁ (FB₁) in stored maize samples (n = 72) is presented in Table 8. The results show that 100% of the examined samples were contaminated with FB₁. Out of the 72 samples collected, 4 (5.6%), 16 (22.2%) and 52 (72.2%) were contaminated with FB₁ as shown in Table 8.

Table 8: Maize samples containing fumonisin B₁ (µg/kg)

Concentration (µg/kg)	No. of samples	Percent
21-99	4	5.6
100-150	16	22.2
>150	52	72.2
Total	72	100.0

The contamination levels of maize with fumonisin for the four villages were as follows: maize samples from Mamoyo Village were found contaminated with mean fumonisins of 165.75 ± 7.42 µg/kg whereas the level of contamination in samples collected from Rudewa-Batini, Msingisi and Mkalama villages were 162.30 ± 6.63 µg/kg, 152.18 ± 7.12 µg/kg and 151.28 ± 7.28 µg/kg respectively (Table 9). The greatest FB₁ was detected in samples collected from Mamoyo ranging from 70.95-213.15 µg/kg followed by samples from Rudewa-Batini ranging from 75.46-188.93 µg/kg. Samples from Msingisi and Mkalama were contaminated with FB₁ ranging from 70.83-195.55 and 63.26-207.97 µg/kg respectively (Table 9).

Table 9: Descriptives statistics showing average fumonisin B₁ (µg/kg) in stored maize for the four villages surveyed in Kilosa District

Village	No. of samples	Mean	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Mamoyo	18	165.75	7.42	150.09	181.4096	70.95	213.15
Rudewa-Batini	18	162.30	6.63	148.31	176.2869	75.46	188.93
Msingisi	18	152.18	7.12	137.36	167.3920	70.83	195.55
Mkalama	18	151.28	7.28	135.92	166.6349	63.26	207.97
Total	72	157.93	3.56	150.83	165.0255	63.26	213.15

Levels of fumonisin contamination determined by this study compares very well with the levels reported in other studies on home stored maize in rural areas of Africa. For example, Doko *et al.* (1996) reported fumonisin contamination of 165 µg/kg and 225 µg/kg for samples of maize from Tanzania. The same authors reported fumonisin levels of 370 µg/kg in maize collected from Botswana, and 135 µg/kg for maize from Malawi. Other studies as reported by Shephard *et al.* (1996) analysed maize from market outlets, which contained this level of contamination as compared to home grown maize. High levels of fumonisins (up to 20 µg/kg) have also been found in maize based food (maize, maize flour and polenta) in Italy (Visconti *et al.*, 1998). Elsewhere, even higher levels of fumonisin contamination have been reported. For example, the results of a survey carried out in China indicated that fifty six per cent of samples (134/240) were found to contain fumonisin B₁ at levels ranging from 50 to 34868 µg/kg (Ueno *et al.*, 1997). Germany reported detection of fumonisins in 27% of samples (86/317) and the levels ranged from 67 to 132 µg/kg (Meister *et al.*, 1996). Continuous monitoring of these mycotoxins is required in order to assure that maize products containing high levels of fumonisins are not consumed by animals and humans.

The data on aflatoxins contamination in groundnuts show that out of 72 samples collected, 5 (6.9%), 45 (62.5%) and 22 (30.6%) were contaminated with aflatoxins B₁ (AFB₁) (Table 10).

Table 10: Groundnuts samples containing aflatoxin B₁

Concentration (µg/kg)	No. of samples	Percent
21-99	5	6.9
100-150	45	62.5
>150	22	30.6
Total	72	100.0

The contamination levels of groundnuts with aflatoxin for the four villages were as follows: groundnuts samples from Mamoyo village were found contaminated with mean aflatoxins of 148.84 ± 6.03 µg/kg whereas the level of contamination in samples collected from Rudewa-Batini, Msingisi and Mkalama villages were 146.06 ± 6.11 µg/kg, 133.89 ± 6.89 µg/kg and 132.46 ± 4.98 µg/kg respectively. The greatest aflatoxin B₁ was detected in samples collected from Mamoyo ranging from 83.20-195.05 µg/kg followed by samples from Rudewa-Batini ranging from 74.40-184.55 µg/kg. Samples from Msingisi and Mkalama were contaminated with aflatoxin B₁ ranging from 72.97-195.17 µg/kg and 81.29-175.49 µg/kg respectively (Table 11).

Table 11: Descriptives statistics showing average aflatoxin B₁ (µg/kg) level in stored groundnuts for the four villages surveyed in Kilosa District

Village	No. of samples	Mean	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Mamoyo	18	148.84	6.03	136.13	161.55	83.20	195.05
Rudewa-Batini	18	146.06	6.11	133.18	158.95	74.40	184.55
Msingisi	18	133.89	6.89	119.35	148.42	72.97	195.17
Mkalama	18	132.46	4.98	121.95	142.97	81.29	175.49
Total	72	140.31	3.08	134.18	146.45	72.97	195.17

Thus, 100% of the groundnuts samples had aflatoxins above the permissible level of 20 µg/kg destined for human consumption (FDA, 2001). Such high toxin levels (above 20 µg/kg) in groundnuts were reported in earlier studies (Adebanjo *et al.*, 1994). A recent study found that 90% of groundnut cake samples from five states in Nigeria had aflatoxins levels that exceeded 20 ppb (Ezekiel *et al.*, 2012). Yameogo and Kassamba (1999) reported that seeds of groundnut from Burkina-Faso inoculated with *A. flavus*

excreted all the four major aflatoxins (B₁, B₂, G₁ and G₂) which peaked at 170 µg/kg after six days. Another study carried out by Haryadi and Setiastuty (1994) on aflatoxin contamination in 30 samples of raw groundnuts collected from various traders reported that aflatoxin B₁ was detected in 8 out of 15 samples collected. In Botswana, Mphande *et al.* (2004) reported the presence of aflatoxin and cyclopiazonic acid in meals with half of those tested containing total aflatoxins at concentrations above 20 ppb set by the WHO. Aflatoxin levels exceeding 20 ppb must be considered unsatisfactory, while those exceeding 100 ppb must be considered totally unsatisfactory, capable of inducing acute toxic effects in both human and animals.

The results of the present study indicate that the frequency and level of aflatoxin contamination in stored maize and groundnuts studied are quite high in Kilosa District; these commodities are used as food and are also used in various food preparations almost daily, and are consumed in significant amounts. Under these circumstances, the consumption of contaminated maize/groundnuts leads to high levels of aflatoxins/fumonisin intake and the effects of toxicity is high in malnourished hosts. Hence, it is essential to take steps to prevent mycotoxin contamination in maize, groundnuts and other commodities. Tables 12 and 13 shows One-way ANOVA for the concentration of fumonisin and aflatoxins in maize and groundnuts samples analysed.

Table 12: One-way ANOVA Table for the level of fumonisin B₁ (µg/kg) in maize samples analysed

Source	df	SS	MSS	F-value	Pr>F
Model	20	58291.36448	2914.56822	22.77	<0.0001
Error	51	6528.41032	128.00805		
Corrected Total	71	64819.77479			
	R-Square	CV	Root MSE	Average FB ₁	Mean
	0.89928	7.164188	11.31406	157.9253	<0.0001
Source	df	Type III SS	MS	F-Value	Pr>F
Replication	17	55 495.71059	3264.45356	25.5	<0.0001
Block	3	2795.65388	931.88463	7.28	0.0004

Both replications and blocks are significantly different at 5% level of significance

Table 13: One-way ANOVA Table for the level of aflatoxin B₁ (µg/kg) in groundnuts samples analysed

Source	df	SS	MSS	F-value	Pr>F
Model	20	38029.10279	1901.4551	9.36	<0.0001
Error	51	48391.2104	203.1786		
Corrected Total	71	64819.77479			
	R-Square	CV	Root MSE	Average AFB ₁	
	0.7859	10.1588	14.2541	140.3121	
Source	df	Type III SS	MS	F-Value	Pr>F
Replication	17	34270.6770	2015.9222	9.92	<0.0001
Block	3	3758.4258	1252.8086	6.17	0.0012

Both replications and blocks are significantly different at 5% level of significance

4.1.2 Levels of FB₁ and AFB₁ compared to regional and international accepted standards

Figures 5 and 6 show the relationship between fumonisin and aflatoxin concentration and different international standards. Maize and groundnuts samples analyzed were grouped into four categories: samples with 0-4 ppb, samples with 4-20 ppb, samples with 21-100 ppb, and samples with > 100 ppb. The upper limit of aflatoxin and fumonisin content for groundnut and maize destined for EU market is 4 ppb while 20 ppb is the maximum permissible level set by United States FDA and WHO. After 3, 6 and 9 months of storage, the proportion of samples with FB₁ of 21-100 ppb were 5.6% and the samples with >100 ppb were 94.4%. The proportion of samples with 21-100 ppb and >100 ppb after 6 months of storage were 8.33% and 91.67% respectively, while after 9 months of storage the incidences were 5.6% and 94.4% respectively. Similarly after 3, 6, and 9 months of storage; the proportion of samples with AFB₁ of 21-100 ppb were 8.3% and those with >100 ppb were 91.7%. The proportion of samples with 21-100 ppb and >100 ppb after 6 months of storage, were 8.3% and 91.7% respectively, while after 9 months of storage the incidence were 6.9% and 93.1% respectively.

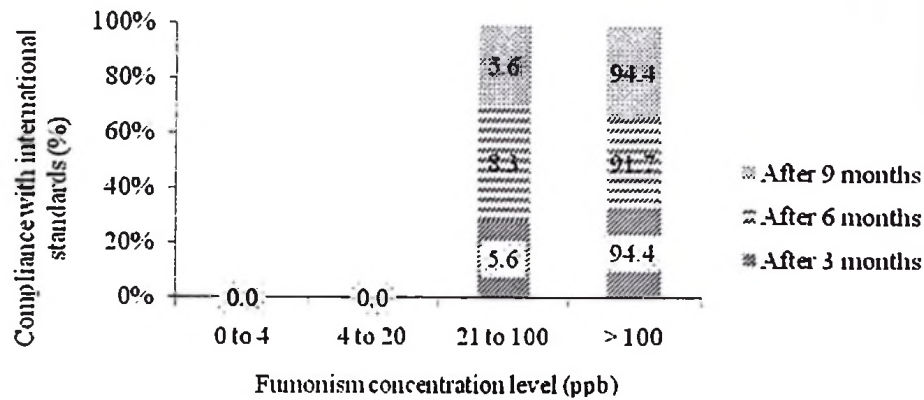


Figure 5: Percentage of samples analyzed in relation to different international standards

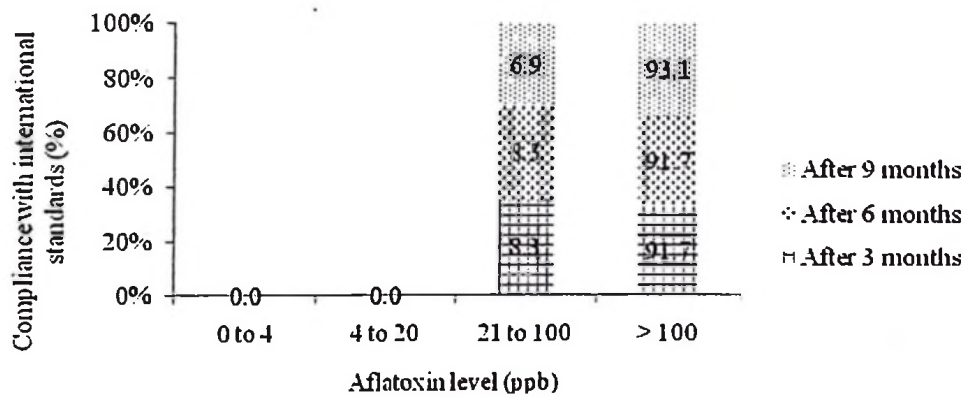


Figure 6: Percentage of samples analyzed in relation to different international standards collected from four villages, Kilosa District

The results from Fig. 5 and 6 show that a large number of the samples tested (94.4%) had fumonisin and aflatoxin concentration levels of more than 100 ppb and only 5.6% were within 21-99 ppb. About 94.2% of the samples had a level greater than the tolerance limit (20 ppb or $\mu\text{g}/\text{kg}$) set by the United States Food and Drug Administration (FDA). The levels of fumonisins in some samples collected far exceeded the maximum levels of 20 $\mu\text{g}/\text{kg}$ set by the US Food and Drug Administration for food intended for direct human consumption. This was possibly as a result of poor agricultural practices such as lack of fungal disease control mechanism, planting dates, harvesting dates, storage, crop residue disposal, land tillage methods, crop rotation, seed sources that might have promoted the growth of *Fusarium* spp. that produce fumonisins.

Timing of harvest can have consequences for the final level of mycotoxin contamination. Generally, earlier harvest results in lower concentrations of mycotoxins (Jones *et al.*, 1981). The risk of fumonisin contamination may begin very early during ear development and production increases throughout the development and physiological maturation of the infested maize ear. Obst *et al.* (1997) noted that minimum tillage instead of ploughing resulted in a 10-fold increase in DON content in wheat crop. In the same way, Steinkellner and Langer (2004) noted that the deeper the tillage the lower was the concentration of *Fusarium* spp. in the soil. Schmidt and Nitzsche (2004) showed that cultivation technique affected mycotoxin contents of maize. Maize is the plant most susceptible to contamination by *Fusarium* spp. Spores released from mature sporangia of *Fusarium* spp. are spread far by wind, and then settle on the soil (Jouany, 2007). They can stay in soil for a long period or grow on dead plant residues such as straw or stubbles, which increase the soil contamination level. Then conidiospores and ascospores can infect ears and leaves of the next crop after being spread by wind or rain-splash droplets. Ascospores found in the asci of fungi are important for infection of maize ears by *F. graminearum*. They can develop in fruiting bodies called perithecia that form on infected organic matter lying on the soil surface and are then released outside when the perithecia ripen. As ascospores are usually dispersed over a short distance, infections in a particular field are generally initiated by spores within that field (Jouany, 2007). This explains why repeated monocultures of maize in the same field will enrich the soil with fungal spores, thus increasing the risk of contamination. During the growing season, infected plant tissues can serve as sources of secondary conidial inoculum, which colonize new non-infected plant tissues (Abbas *et al.*, 2009).

Soil serves as a reservoir for primary inoculum of *A. flavus* and *A. parasiticus*, and groundnuts pods are in direct contact with soil population of aflatoxigenic fungi

(Razzaghi-Abuyaney *et al.*, 2010). A recent study documented that the distribution of aflatoxin contamination in different agro-ecological zones of Senegal established that the soil is a reservoir for field infections in much the same way as poor storage practices (Tiffany, 2013). The disease cycle and epidemiology of *A. flavus* were recently reviewed by Amaike and Keller (2011). *Aspergillus flavus* lives in soil as conidia or sclerotia and in plant tissues as mycelia. Sclerotia survive in the soil under severe environmental conditions and produce conidia and possibly ascospores (Horn *et al.*, 2009), leading to a population increase under hot and drought weather conditions (Payne, 1998). Sclerotia germinate as mycelia, which then form conidiophores and conidia.

Rotating between crops that are susceptible to the same mycotoxigenic fungus should be avoided in order to reduce both plant disease and increased mycotoxin formation due to increased inoculum. For instance, the continued cultivation of peanuts on the same land may contribute to a built-up of high *A. flavus* population in the soil, with the consequent increase of infection and aflatoxin contamination (Ortiz *et al.*, 2011). When maize was intercropped with cowpeas the likelihood of aflatoxins contamination increased (Hell, 1997).

Plant residues act as a reservoir for mycotoxigenic fungi. Crop residues are clearly the most important source of inoculum for *F. graminearum*, which causes Gibberella ear rot and DON contamination of maize. So, crop rotation and tillage are recommended to control plant contamination with *Fusarium* spp. (Payne, 1999) but these agricultural practices are not always recognized as efficient. Proper residue management is critical for reducing the risk of mycotoxin contamination.

Post-harvest aflatoxins contamination is most attributable to improper storage of the pods and seed. It is well established that mold invasion is facilitated because of increased moisture levels of stored commodities (Abramson, 1998). Inappropriate kernel moisture during storage can proceed from leaky roofs, condensation because of improper ventilation in the warehouse, high-moisture foreign material associated with stored peanuts, and high-moisture peanuts initially going into storage (Davidson *et al.*, 1982). The minimum moisture content for *A. flavus* growth on groundnut is 8–10% at around 82% relative humidity, and aflatoxin production is generally correlated with kernel moisture contents of 10% or higher (Diener and Davis, 1970). It is well known also that stock piling of peanuts can cause heat built-up and moisture accumulation, resulting in mold growth and aflatoxin contamination. Other studies reported that the maximum moisture content for storage of groundnuts (unshelled) is 9% while that for shelled peanut is 7%. At these moisture contents, if the environment relative humidity is maintained at 70% and temperature at 25–27 °C, safe storage of nuts is guaranteed for approximately one year (Odogola, 1994; Waliyar *et al.*, 2008).

Based on WHO standards, the majority of the samples in the study villages (100%) were not safe for consumption because they exceeded 20 ppb. In addition, most of the samples (98.3%) contained higher than the permissible levels of mycotoxin in animal feed (100 ppb), while only 1.7% of the samples had less in mycotoxin levels. It is interesting to mention that about 100% of the samples analyzed were not within the Tanzanian maximum tolerable levels. Tanzania regulates maximum limits of AFB₁ and total aflatoxins at 5 ppb and 10 ppb (TBS, 2004). The consumption of AFB₁ in this commodity on daily basis may be dangerous to animals and also to humans who consume these animal products since AFB₁ has been found in animal tissues and may be metabolized into aflatoxin M₁ which is excreted in animal milk (Viljoen, 2003).

4.1.3 Relationship between fumonisins and aflatoxins concentration with altitude

Tables 14 and 15 present the Chi-square tests showing the relationships between altitude and fumonisins and aflatoxins concentrations. A positive and significant correlation ($r=0.89$, $p<0.01$) was observed between agroecological zones. There was a significant ($\chi^2=4.431$, $df =1$, $p = 0.035$,) association ($\Phi =0.248$) between altitude and level of fumonisin contaminations. The high levels of fumonisins ($\mu\text{g}/\text{kg}$) from the low altitude zone could be attributed to the prevailing environmental conditions during production and storage periods. The low altitude zone has a sub-tropical climate characterised by relatively high rainfall (900 mm), high humidity (88%), and relatively high temperatures (25.5°C). In contrast, the high altitude zones have a tropical climate and the maize production season is characterised by relatively low monthly rainfall (675 mm), low humidity (67%), and low temperatures (18°C). The effect of relative humidity on fumonisin production has been reported by a number of researchers (Kimanya *et al.*, 2009).

Similarly, a significant correlation ($r=0.91$, $p<0.01$) was observed between aflatoxin levels and agroecological zone. There was a significant association ($\chi^2 = 3.869$, $df=1$, $p=0.049$) between altitude and the level of aflatoxins contaminations (Table 15) Aflatoxins level was significantly higher in low altitude than in high altitude areas ($p<0.05$). As explained for the case of high incidence of fumonisin contamination, the warm and humid conditions prevailing in Mamoyo and Rudewa-Batini villages could be attributed to these observations. This finding is in agreement with the findings by Rheeder *et al.* (1992) who reported the variations between one location and the other as being a result of variation in climatic and environmental conditions. Significant correlations were found to exist between agroecological zones and mycotoxin levels, whereby a wet and humid climate tends to aggravate aflatoxin and fumonisin levels. In Uganda, for example,

aflatoxin levels in maize samples were higher in more humid areas than it was in drier areas (Kaaya *et al.*, 2006). Similar results are in a survey of groundnuts samples from Nigeria by Atehnkeng *et al.* (2008) who found significantly higher odds of groundnuts from the low altitude being contaminated as compared to those from the high altitude, and partly attributing this to the distribution of agroecological zones within the districts; the wetter and dry zones.

It is difficult to associate the specific causes of higher levels of aflatoxin and fumonisin in the low altitude areas in Kilosa District; however, it is probably that high moisture contents of grain during storage does not allow for sufficient drying of groundnuts and maize. Maize and groundnuts which are in most cases not sufficiently dried in Mamoyo and Mkalama villages have been associated with increasing levels of mould and fumonisin contamination. This is feasible due to frequent rainfall during the groundnuts and maize harvesting months of February and March in these villages unlike in the case of other harvesting periods of May and June in the high altitude area where rainfall is minimal at that period. Possibly because of these reasons, the percentage of AFB₁ and FB₁ positive samples in the low altitude areas was higher than that in the highlands. On the other hand, fumonisins and aflatoxins develop rapidly in high air humidity and moderate temperature environments (Munkvold and Desjardins, 1997).

The harvesting of maize and groundnuts in Mamoyo and Rudewa-Batini is normally done in February-May, a period which coincides with the rain period. Under rainfall conditions, mature maize experiences prolonged period of high water content that may favour mould growth and mycotoxin formation during storage (Kimanya *et al.*, 2008a). Harvesting of maize during wet days in these areas in the low altitude increases the grain moisture content and susceptibility of grain to infection by fungi and subsequent

mycotoxin contamination. Moreover, Mamoyo and Rudewa-Batini are generally warm and humid villages and experience high rainfall of 1000-1600 mm/year unlike the high altitude villages of Msingisi and Mkalama which receive 800-1000 mm/year. It has also been reported that an increased aflatoxins formation was registered by heavy rains during storage, delayed storage and high moisture contents (Kumar *et al.*, 2008). Furthermore, the storage conditions and their effects on aflatoxins production were studied by Saleemullah *et al.* (2006).

The current study indicates that stored maize and groundnuts were more prone to fumonisins and aflatoxin contaminations because of poor agricultural as well as storage practices. Normally, maize is harvested during January through March every year in Kilosa District. The harvested maize is spread on the drying floor for natural drying under the bright sunshine. After drying and grading of maize and before packing into gunny bags, farmers do not check for the moisture content required for maize. Due to lack of knowledge, then the maize with moisture is packed in the bag and taken to the storage before it is used. At the storage, the maize with this moisture is prone to *Fusarium moniliforme* infection and fumonisin contamination. To avoid fumonisins contamination during storage, there is a need to develop suitable packaging and storage practices for maize in Kilosa District. This measure needs to be adopted with strict surveillance and quality control of stored maize and groundnuts.

It is also important to emphasize that, prevalence and contamination levels of mycotoxins vary greatly according to several factors, i.e. geographical location, harvest year and commodity (Scudamore and Patel, 2009). The variations in contamination levels are dependent on factors such as weather differences and extent of processing (Miller, 1995). Differences in geographical and environmental conditions might be responsible for

differences in fungal distributions and concentration observed among different locations (Roigé *et al.*, 2009).

Table 14: Chi-square tests showing the relationships between altitude and fumonisins contaminations in stored maize

Measures of association/relationships	Value	df	Asymp.(2-sided)	Exact Sig.(2-sided)
Pearson Chi-Square	4.431	1	0.035	
Continuity Correction	3.392	1	0.066	
Likelihood Ratio	4.527	1	0.033	
Fisher's Exact Test				0.064
Linear-by-Linear Association	4.369	1	0.037	

Pearson Chi-Square = 4.431, $p = 0.035$; Likelihood Ratio = 4.527, $p = 0.033$

Table 15: Chi-Square tests showing the relationships between altitude and aflatoxins contaminations in stored groundnuts

Measures of association/relationships	Value	df	Asymp.Sig. (2 - sided)	Exact Sig. (2-sided)
Pearson Chi-Square	3.869	1	0.049	
Continuity Correction	2.916	1	0.088	
Likelihood Ratio	3.920	1	0.048	
Fisher's Exact Test				0.070
Linear-by-Linear Association	3.815	1	0.051	

Pearson Chi-Square = 3.869, $p = 0.049$; Likelihood Ratio = 3.920, $p = 0.048$

Table 16 and 17 shows the descriptive statistics (i.e the percentage counts) of the relationship between altitude and fumonisins contaminations in stored maize and groundnuts. Studies conducted in Benin, Brazil, Zimbabwe and Tanzania have all reported high levels of fumonisins in maize grown in the most humid areas (Fandohan *et al.*, 2005; Kimanya *et al.*, 2008b). However, it is important to note that other environmental factors, such as dry weather at or just before pollination and physiological stress in the final stages of maize development, may also promote fumonisins production before the maize is harvested. Furthermore, the maize production period in the low altitude zone is long, extending from October to March. Such a long production period, in addition to the prevailing environmental conditions probably exposes maize to *Fusarium* infection and subsequent fumonisins contamination. Udoh (1995) in a two-year survey of stored maize of 25 farmers, each in five agroecological zones observed that in the mid-

altitude zone the sample was contaminated with aflatoxins and in the Northern Guinea Savanna three samples showed low aflatoxin contamination.

Table 16: Descriptive statistics analyses showing the relationships between altitude and fumonisins contaminations in stored maize

Agroecological zone		Fumonisin level ($\mu\text{g}/\text{kg}$)		Total
		>100	<100	
Low altitude	Count	30	6	36
	Expected count	26.0	10.0	36.0
	% within altitude	83.3	16.7	100.0
	% within fumonisin level	57.7	30.0	50.0
	% Total	41.7	8.3	50.0
High altitude	Count	22	14	36
	Expected count	26.0	10.0	36.0
	% within altitude	61.1	38.9	100.0
	% within fumonisin level	42.3	70.0	50.0
	% Total	30.6	19.4	50.0
Total	Count	52	20	72
	Expected count	52.0	20.2	72.0
	% within altitude	72.2	27.8	100.0
	% within fumonisin level	100.0	100.0	100.0
	% Total	72.2	27.8	100.0

Table 17: Descriptive statistics analyses showing the relationships between altitude and aflatoxins contaminations in stored groundnuts

Agroecological zone		Aflatoxin level ($\mu\text{g}/\text{kg}$)		Total
		>100	<100	
Low altitude	Count	26	10	36
	Expected count	20.5	15.5	36.0
	% within altitude	72.2	27.8	100.0
	% within fumonisin level	63.4	32.3	50.0
	% Total	36.1	13.9	50.0
High altitude	Count	15	21	36
	Expected count	20.5	15.5	36.0
	% within altitude	41.7	58.3	100.0
	% within fumonisin level	36.6	67.7	50.0
	% Total	20.8	29.2	50.0
Total	Count	41	31	72
	Expected count	41.0	31.0	72.0
	% within altitude	56.9	43.1	100.0
	% within fumonisin level	100.0	100.0	100.0
	% Total	56.9	43.1	100.0

4.2 Storage Practices and their Influence on Mycotoxin Contaminations in Stored Maize and Groundnuts

4.2.1 Effect of grain protectants on mycotoxin contaminations

The differences between different methods of treating grains were noted and were highly significant ($p=0.001$, $LSD=4.996$, $df=3$, $n=4$) for all grain protectants (Table 18). After 3, 6, and 9 months of storage, a significant difference existed for the fumonisin content measured in the different grain protectants ($p=0.001$, $LSD=4.996$, $df=3$, $n=4$). No significant difference existed for fumonisin and aflatoxin content measured in the different storage times ($p>0.05$) (Table 18). The results of the ANOVA table (Table 18) shows that storage duration ($p=0.391$, $LSD=4.996$, $df=3$, $n=4$) had no significant effect on fumonisins contamination in stored maize. Grain storage protectants differed significantly ($p=0.001$, $LSD=4.996$, $df=3$, $n=4$) on fumonisins contaminations. Similarly, there was no significant effect of interaction between storage duration and storage treatments ($p=0.860$, $LSD=4.996$, $df=6$, $n=4$) on fumonisin contamination.

Table 18: Two-way ANOVA of effects of grain protectants on fumonisin contaminations using Split-Plot procedure

SV	df	SS	MS	Vr	Fpr.
Block	3	264.89	88.30	0.61	
Storage duration (A)	2	318.59	159.30	1.10	0.391
Error (A)	6	867.11	144.52	4.06	
Grain protectants (B)	3	102 398.92	34132. 97	959.59	<0.001
Storage duration x Grain protectants (AxB)	6	89. 22	14.87	0.42	0.860
Error (B)	27	960.40	35.57		
Total	47	1 04 899.14			

Similarly, the results of ANOVA Table 19 shows that treatments in groundnuts differed significantly ($p=0.001$, $LSD=3.665$, $df=2$, $n=3$) on aflatoxin contaminations. Table 19 shows that storage duration ($p=0.105$, $LSD=3.665$, $df=2$, $n=3$) had no significant effect on aflatoxins contamination in stored groundnuts. Also, interaction of storage duration and storage treatments ($p=0.829$, $LSD=3.665$, $df=2$, $n=3$) had no significant effect. The hypothesis tested by this study was to determine whether storage methods have no significant impact on mycotoxin contamination in stored groundnuts.

Table 19: Two-way ANOVA of effects of groundnuts protectants on aflatoxin contamination using Split-Plot procedure

SV	df	SS	MSS	Vr	Fpr.
Block	3	144.08	48.03	1.42	
Storage duration (A)	2	227.08	113.54	3.35	<0.105
Error (A)	6	203.07	33.85	1.85	
Protectants (B)	2	4 861.96	2 430. 98	133.14	<0.001
Storage duration x Protectants (AxB)	4	26. 77	6.69	0.37	0.829
Error (B)	18	328.65	18.26		
Total	35	5 791.62			

The use of dried neem leaf as a means of grain protectant showed low level in fumonisins contamination (75.62 µg/kg) in grains unlike in other treatments. The other three methods were the use of wood ash (157.18 µg/kg), Actellic super dust (173.88 µg/kg) and storing maize without any treatments (198.86 µg/kg) which showed a high level of fumonisins contamination in maize as shown in Table 20. Table 21 shows the levels of fumonisins B₁ and aflatoxins B₁ (µg/kg) in maize and groundnuts under different storage times. The results of the ANOVA shown in Tables 18 and 19 indicate that since F-test is significant at less than 5%, the null hypothesis is rejected and, therefore, the effects of the storage practices are statistically different at the 5% level.

The use of dried neem leaf in maize seems to be good storage protectants as compared to other treatments. It is reported that, *Aspergillus* fungi would not grow on local medicinal plants, and could not lead to aflatoxins on them (Reddy *et al.*, 2009). Recently, Anjorin *et al.* (2008) reported the effect of neem extract on control of *F. verticillioides* in maize. More recently Mondali *et al.* (2009) studied the efficacy of different extracts of neem leaf on seed borne fungi, *A. flavus*. In this study the growth of fungi was inhibited significantly and controlled. Thus, the mixing of plant substances with stored maize grain may actually reduce the risk of mycotoxin development and controlling it. Plants may reduce relative humidity inside the grain store through their biomasses, and consequently reduce fungal growth. The use of ash seemed to be a good second storage protectant in

maize. Ash is used as both inert filler and for its other negative effects on insects. As inert filler, ash works by filling up the space around the seeds and impeding the movement of insects as well as in sealed containers, reducing the volume of air available to the insects for respiration. Ash has been reported to damage the cuticle of insects causing them to dehydrate and also has detrimental effect on egg development (Almekinders, 1999).

Actellic super dust is also known to break down quite rapidly after several months, leading to a spike of losses in long-term storage with only single dosages (Biliwa and Richter, 1990). The study revealed that; the presence of locally available traditional storage protectants could have important economic implications for use in the district. According to the literature, the neem, *A. indica*, has been known as a bio-pesticide against various insects (Boeke *et al.*, 2004) and most of its parts contain azadirachtin (active ingredient), which has most insecticidal activity among other limonoids, or tetranortriterpenoids present in neem trees (Morgan, 2004). Its properties such as toxicity, repellence, feeding deterrence, and insect growth regulator activity contribute mainly toward insecticidal activity (Schmutterer, 1990). The concentration of azadirachtin content in neem leaves has been reported as 0.0244% (w/w), i.e. about 250 g of azadirachtin per gram of neem leaves (Radhakrishnan *et al.*, 2007). The differences between different types of treating groundnuts were noted and were highly significant for all storage treatments ($p < 0.001$, $LSD = 3.665$, $df = 2$, $n = 3$). Sorting practices as a means of treatment before storage showed low level in aflatoxins contamination (125.21 $\mu\text{g}/\text{kg}$) in groundnuts as compared to other treatments. The other two methods were the use of kitchen ash (142.22 $\mu\text{g}/\text{kg}$) and storing groundnuts without any treatments (153.49 $\mu\text{g}/\text{kg}$) which showed a high level of aflatoxins contamination in groundnuts as shown in Table 20.

Table 20: Levels of fumonisins B₁ and aflatoxins B₁ (µg/kg) in maize and groundnuts under different grain protectants

Treatment	Fumonisin level (µg/kg)	Treatment	Aflatoxin level (µg/kg)
1. Neem leaf	75.62a	1.Sorting	125.21a
2. Wood ash	157.18b	2.Ashing	142.22b
3. Actellic Super Dust	173.88c	3.Control	153.49c
4. Control	198.86d		
Grand mean	151.40		140.31
SE±	5.964	SE±	4.273
LSD	4.996	LSD	3.665
CV (%)	3.9	CV (%)	3.0

Means within the same column with different letter differ significantly at $p \leq 0.05$.

Table 21: Levels of fumonisins B₁ and aflatoxins B₁ (µg/kg) in maize and groundnuts under different storage times

Storage time	Fumonisin level (µg/kg)	Aflatoxin level (µg/kg)
1. After 3 months	148.18ab	138.50ab
2. After 6 months	151.49ab	138.56ab
3. After 9 months	154.49ab	143.86ab
Grand mean	151.39	140.31
SE±	6.011	3.359
LSD	10.40	5.812
CV (%)	4.0	2.4

Means within the same column with same letter do not differ significantly at $p \leq 0.05$.

The reduction of aflatoxin levels resulting from sorting has been observed in other studies (Rheeder *et al.*, 1992; Desjardins *et al.*, 1998). As sorting has been done manually by hand, its success in reducing aflatoxins levels would be subjective. Afolabi *et al.* (2006) proposed the visible sorting of maize grain as a technique to reduce fumonisin levels by subsistence farmers. Fandohan *et al.* (2005) noted that hand sorting of visibly mouldy grains with the aim of reducing mycotoxin levels is likely to depend on the ability of the people responsible for this activity.

4.2.2 Effect of storage methods on mycotoxin contaminations

The differences between different methods of storing grains were noted and were highly significant ($p=0.001$, $LSD=4.375$, $df=2$, $n=3$) for all storage methods (Table 22). The results of the ANOVA Table 22 shows that storage duration of maize ($p=0.001$, $LSD=4.375$, $df=2$, $n=3$) had a significant effect on fumonisins contamination in stored maize. Also, storage duration and storage methods ($p=0.080$, $LSD=4.375$, $df=4$, $n=3$) had

no significant interaction. The hypothesis tested by this study was to determine whether storage types have no significant impact on mycotoxin contamination in stored maize. The results of the ANOVA Table shown in Table 22 indicate that since F-test is significant and less than 5%, the null hypothesis is rejected and therefore, the effects of the storage methods are statistically different at the 5% level.

Table 22: Two-way ANOVA of the influence of storage methods on fumonisin contaminations in stored maize

SV	df	SS	MSS	Vr	Fpr.
Block	3	84.75	28.25	1.97	
Storage duration (A)	2	2 193.30	1 096.65	76.60	<0.001
Error (A)	6	85.91	14.32	0.55	
Storage methods (B)	2	11 427.13	5 713.56	219.62	<0.001
Storage duration x Storage methods (AxB)	4	258.95	64.74	2.49	0.080
Error (B)	18	468.27	26.02		
Total	35	14 518.31			

Similarly, the results of the ANOVA table (Table 23) shows that storage duration of groundnuts ($p=0.001$, $LSD=3.412$, $df=2$, $n=3$) had a significant effect on aflatoxins contamination in stored groundnuts. Also, storage duration and storage methods $p=0.040$, $LSD=3.412$, $df=4$, $n=3$) had significant interaction. The hypothesis tested by this study was to determine whether storage methods have no significant impact on mycotoxin contamination in stored groundnuts. The results from Table 22 show that the storage duration ($p=0.001$, $df=2$) and the storage methods ($p=0.001$, $df=2$) had a significant effect on fumonisin contaminations. Storage duration and storage methods had no significant interaction ($p=0.08$, $df=4$) on fumonisin contamination.

Similarly, the results from Table 23 show that storage duration ($p=0.011$, $df=2$) and storage methods ($p=0.001$, $df=2$) had significant effect on aflatoxin contaminations. Also, storage duration and storage methods had significant two-way interaction ($p=0.040$, $df=4$) on aflatoxins contamination (Table 23). The hypothesis tested by this study was to determine whether storage practices (methods) have no significant impact on mycotoxin contamination in stored maize.

Table 23: Two-way ANOVA of the influence of storage methods on aflatoxins contaminations in groundnuts

SV	df	SS	MSS	Vr	Fpr.
Block	3	52.27	17.42	1.26	
Storage duration (A)	2	285.94	142.97	10.33	<0.011
Error (A)	6	83.07	13.84	0.88	
Sub-Plot (B)	2	449.34	224.67	14.20	<0.001
Storage duration xStorage methods (AxB)	4	199.51	49.88	3.15	0.040
Error (B)	18	284.79	15.82		
Total	35	1 354.92			

Significant differences were observed between storage methods in groundnuts ($p=0.001$, $LSD=3.412$, $df=2$) for all storage methods. Higher aflatoxin levels were detected when groundnuts were stored in nylon bags ($145.31 \mu\text{g/kg}$) followed by storage in old-plastic tins with aflatoxin level of $138.09 \mu\text{g/kg}$. The storage container by using polythene bags was the least with aflatoxin level of $137.57 \mu\text{g/kg}$ (Table 24). The results of the ANOVA table shown in Table 22 and 23 indicate that since the significant F-value is less than 5%, the null hypothesis is rejected and therefore, the effects of the storage methods in maize and groundnuts are statistically different at 5% level. Table 25 shows the levels of fumonisin B₁ and aflatoxin B₁ ($\mu\text{g/kg}$) in maize and groundnuts under different storage times.

Table 24: Levels of fumonisin B₁ and aflatoxin B₁ ($\mu\text{g/kg}$) in maize and groundnuts under different storage methods

Methods	Fumonisin level ($\mu\text{g/kg}$)	Treatment	Aflatoxin level ($\mu\text{g/kg}$)
1. Crib	135.91a	1. Polythene bags	137.57a
2. Polythene bag	158.43b	2. Old-plastic tins	138.09a
3. Heaping on floor	179.54c	3. Nylon bags	145.31b
Grand mean	157.93		140.32
SE \pm	8.84	SE \pm	8.33
LSD	4.375	LSD	3.412
CV (%)	12.8	CV (%)	14.5

Means within same column with different letter differ significantly at $p \leq 0.05$.

Table 25: Levels of fumonisin B₁ and aflatoxin B₁ ($\mu\text{g/kg}$) in maize and groundnuts under different storage times

Storage time	Fumonisin level ($\mu\text{g/kg}$)	Aflatoxin level ($\mu\text{g/kg}$)
1. After 3 months	144.7a	127.3a
2. After 6 months	157.3ab	140.1ab
3. After 9 months	171.8b	153.6b
Grand mean	157.93	140.33
SE \pm	2.29	5.25
LSD	3.96	9.08
CV (%)	8.7	3.7

Means within same column with different letter differ significantly at $p \leq 0.05$

From Table 24 results, among the three storage methods, the average fumonisins level in maize when the maize was stored as a heap on the floor was the highest (179.5 µg/kg) and was significantly different from when the maize was stored in a polythene bag and in a traditional crib ($p < 0.05$). The average fumonisins level of the crib was the lowest (135.9 µg/kg). It is documented that certain storage methods are more effective than others in preventing high grain moisture levels, which may lead to mould and fungi contamination, including aflatoxin proliferation (Hell *et al.*, 2000). For example, clay structures may be overly damp and dark for optimal drying, while improved cribs allow for open air flow and have been shown to reduce moisture contents from 20% to 14% over three months and are associated with decreased aflatoxin contamination in Nigeria and Benin (Hell *et al.*, 2000). Field fungi concentrations such as *Fusarium* generally decrease with duration of storage period as moisture content declines (Fandohan *et al.*, 2005). Storage on the floor and in non-ventilated facilities, however, is not recommended due to ineffective drying and high residual levels of *Fusarium* and contamination of fumonisins (up to 40.3% of kernels infected) (Fandohan *et al.*, 2005). It has to be pointed out that some of these traditional methods protect the product reasonably well and need at most slight improvements. On the other hand, it is possible that some traditional methods are unsatisfactory and lead to high losses.

In East and Southern Africa, farmers store crops in small bags with cow dung ash, in wood and wire cribs, pits, metal bins, wooden open-air or roofed cribs, and in raised platforms and roofed iron drums enclosed with mud (Wambugu *et al.*, 2009; Kankolongo *et al.*, 2009). In tropical and subtropical countries, a large proportion of the grain (such as maize) is harvested and stored under hot and humid conditions, and most farmers lack proper knowledge, equipment and methods of drying grains (Weinberg *et al.*, 2008). Subsequently, the maize is stored while still relatively moist and warm; both warmth and

high moisture contents can result in rapid deterioration of the grains and promote the growth of microorganisms (e.g. fungi and bacteria) and insects in the grains (Ekechukwua and Norton, 1999). Farmers in Africa use traditional granaries to store their grains which are not effective against storage pests. The lack of suitable grain storage structures, storage management technologies force maize growers to sell their produce immediately after harvest. Consequently, farmers receive low market prices for any surplus grain they may produce (Kimenju *et al.*, 2009).

4.2.3 Effect of grain storage form on mycotoxin contaminations

The statistical paired comparisons of the means among the two treatments for each storage form are presented in Tables 26, 27 and 28. When comparing the fumonisins contamination of maize in different storage forms after 3 months of storage, unshelled maize experienced significantly greater contamination level ($152.50 \pm 1.16 \mu\text{g/kg}$), than the shelled maize ($136.83 \pm 0.69 \mu\text{g/kg}$, $t(22) = -11.659$, $p < 0.05$). Assuming equal variances: $t = -11.659$, the $p = 0.000$ indicates that the null hypothesis ($H_0: \mu_1 = \mu_2$) should be rejected at 5% significance level. This means that there is significant difference between the mean quantities of fumonisins in maize samples stored in two different forms as shown in Table 26 and Fig. 7.

Table 26: Independent Samples t-tests: Investigating the differences between the mean quantities of fumonisins in the maize samples stored in shelled and unshelled form after 3 months of storage

Homogeneity test	Statistical test	Fumonisin concentration ($\mu\text{g/kg}$)		
		Equal variances assumed	Equal variances not assumed	
Levene's test for equality of variances		1.473		
	Sig	0.238		
t-test for equality of means	T	-11.659	-11.659	
	df	22	17.887	
	Sig(2-tailed)	0.000	0.000	
	Mean Difference	-15.66667	-15.66667	
	SED	1.34369	1.34369	
	95% CI of the Difference			
	Lower	-18.45331	-18.49094	
	Upper	-12.88002	-12.84240	

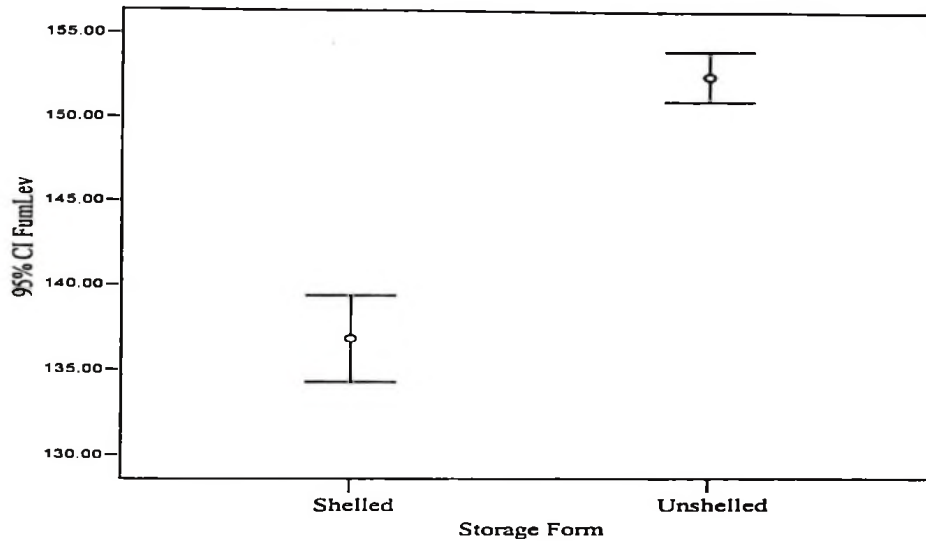


Figure 7: Level of fumonisin ($\mu\text{g}/\text{kg}$) contaminations in two (2) different forms of maize after 3 months of storage

Similarly, fumonisin contamination level after 6 months of storage were ($149.08 \pm 0.87 \mu\text{g}/\text{kg}$) for shelled maize and for unshelled maize were ($165.45 \pm 0.78 \mu\text{g}/\text{kg}$, $t(22) = -13.976$, $p < 0.05$) as shown in Table 27. Fumonisin contamination level after 9 months of storage for shelled maize were ($163.17 \pm 0.82 \mu\text{g}/\text{kg}$), and for unshelled maize were ($180.48 \pm 0.53 \mu\text{g}/\text{kg}$, $t(22) = -17.731$, $p < 0.05$) as shown in Table 28. The difference between the two forms were significant ($p < 0.05$). The results in this research are in contrast to what have been reported by other scholars. Mora and Lacey (1997) found higher levels of aflatoxigenic fungi in maize that was shelled immediately after harvest than maize kernels that were left on the cob through drying. Similarly, there are reports of higher development rates of insects on maize stored as loose grains which would have an aflatoxins and fumonisins-increasing effects (Sinha and Sinha 1992; Wright, 1992). There is also concern that maize cob storage promotes attack of maize by *Prostephanus truncatus*, which prefer grain on cobs rather than shelled grain (Infonet-biodivision, 2011) thus, attracting moulds infestation causing greater damage and loss to the unshelled maize

(FAO, 1994). Fig. 8 and 9 shows the error bar graph for the shelled and unshelled maize after 6 and 9 months of storage.

Table 27: Independent Samples t-tests: Investigating the differences between the mean quantities of fumonisins in the maize samples stored in shelled and unshelled form after 6 months of storage

Homogeneity test	Statistical test	Fumonisin concentration ($\mu\text{g}/\text{kg}$)	
		Equal variances assumed	Equal variances not assumed
Levene's test for equality of variances	F	0.112	
	Sig	0.741	
t-test for equality of means	T	-13.976	-13.976
	df	22	21.762
	Sig(2-tailed)	0.000	0.0000
	Mean Difference	-16.3750	-16.3750
	SED	1.17167	1.17167
	95% CI of the Difference		
	Lower	-18.80490	-18.80645
	Upper	-13.94510	-13.94355

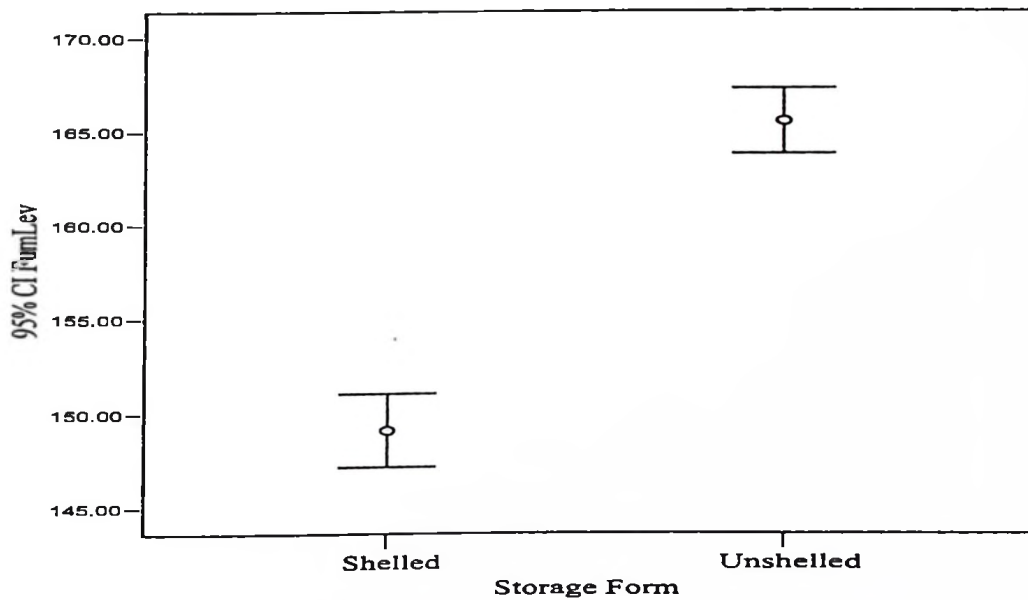


Figure 8: Level of fumonisins ($\mu\text{g}/\text{kg}$) contaminations in two (2) different forms of maize after 6 months of storage

Table 28: Independent Samples t-tests: Investigating the differences between the mean quantities of fumonisins in the maize samples stored in shelled and unshelled form after 9 months of storage

Homogeneity test	Statistical test	Fumonisin concentration ($\mu\text{g}/\text{kg}$)	
		Equal variances assumed	Equal variances not assumed
Levene's test for equality of variances	F	1.133	
	Sig	0.299	
t-test for equality of means	T	-17.731	-17.731
	Df	22	18.933
	Sig (2-tailed)	0.000	0.000
	Mean Difference	-17.31667	-17.31667
	SED	0.97663	0.97663
	95% CI of the Difference		
	Lower	-19.34208	-19.36127
	Upper	-15.29125	-15.27206

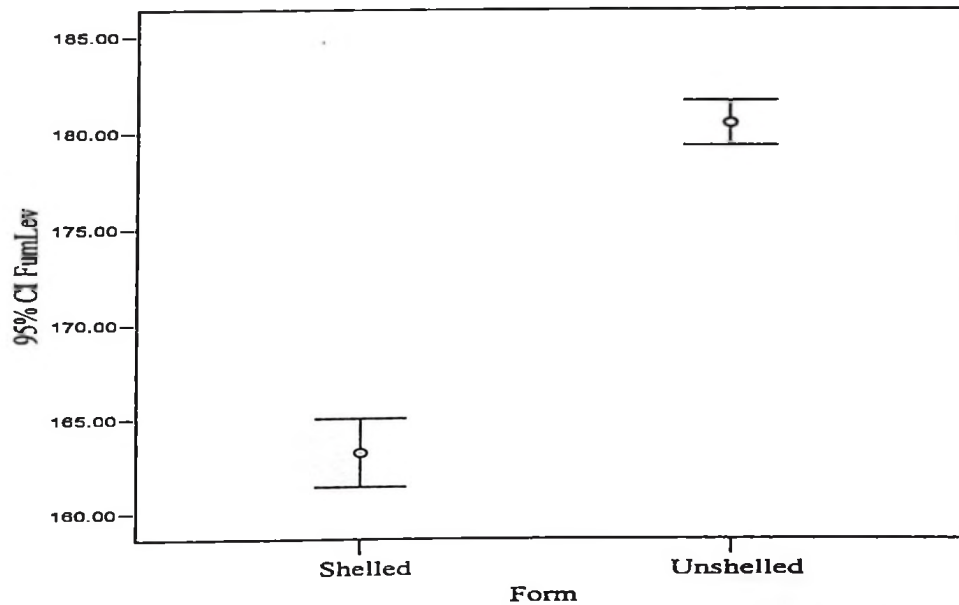


Figure 9: Level of fumonisins ($\mu\text{g}/\text{kg}$) contaminations in two (2) different forms of maize after 9 months of storage

When comparing the aflatoxins contamination of groundnuts in two different storage forms after 3 months of storage, no significant difference was observed between the mean quantities of each form (shelled versus unshelled) as shown in Table 29. The t-tests ($p > 0.05$) confirmed this claim. Assuming equal variances: $t=1.829$, the $p\text{-value}=0.081$

indicates that the null hypothesis ($H_0: \mu_1 = \mu_2$) should not be rejected at 5% significance level. This means that there is no significant difference between the mean quantities of aflatoxins in groundnuts samples stored in two different forms as shown in Fig. 10.

Table 29: Independent Samples t-tests: Investigating the differences between the mean quantities of aflatoxins in the groundnuts samples stored in shelled and unshelled form after 3 months of storage

Homogeneity test	Statistical test	Aflatoxin concentration ($\mu\text{g}/\text{kg}$)	
		Equal variances assumed	Equal variances not assumed
Levene's test for equality of variances	F	1.133	
	Sig	0.299	
t-test for equality of means	T	1.829	1.829
	df	22	15.485
	Sig(2-tailed)	0.081	0.081
	Mean Difference	8.07167	8.07167
	SED	4.41281	4.41281
	95% CI of the Difference		
	Lower	-1.07994	-1.30841
	Upper	-17.22327	-17.45174

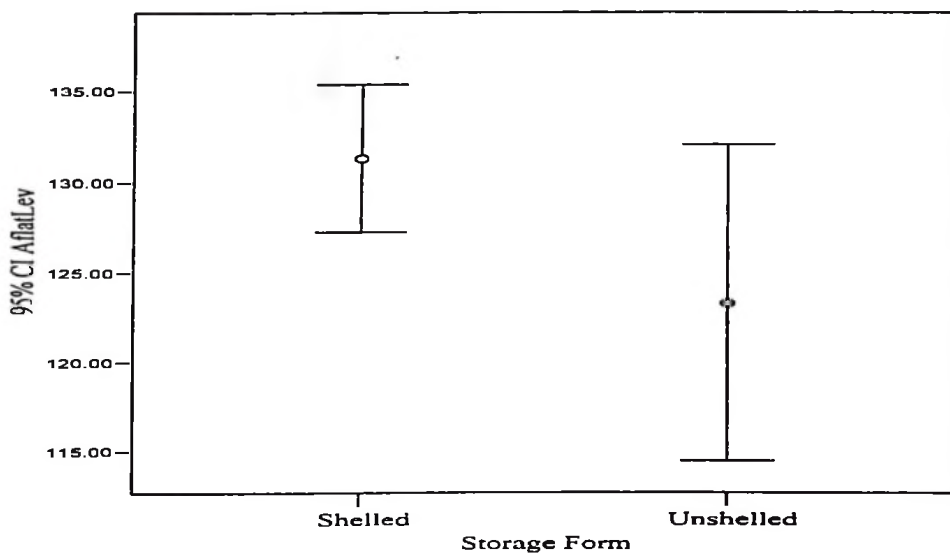


Figure 10: Level of aflatoxin ($\mu\text{g}/\text{kg}$) contaminations in two (2) different forms of groundnuts after 3 months of storage

A significant difference was observed after 6 months of storage and the mean concentration level were higher in shelled groundnuts ($149.89 \pm 1.07 \mu\text{g/kg}$), than in unshelled groundnuts ($136.22 \pm 2.11 \mu\text{g/kg}$, $t(22) = 5.79$, $p < 0.05$) and after 9 months of storage the mean concentration level were ($158.82 \pm 1.94 \mu\text{g/kg}$ and $149.14 \pm 2.16 \mu\text{g/kg}$, $t(22) = 3.34$, $p < 0.05$) for shelled and unshelled groundnuts respectively as shown in Table 30 and 31. The difference between the two forms were significant ($p < 0.05$). Fig. 11 and 12 show the results for the effect of shelled and unshelled groundnuts after 3, 6 and 9 months of storage.

Table 30: Independent Samples t-tests: Investigating the differences between the mean quantities of aflatoxins in the groundnuts samples stored in shelled and unshelled form after 6 months of storage

Homogeneity test	Statistical test	Aflatoxin concentration ($\mu\text{g/kg}$)	
		Equal variances assumed	Equal variances not assumed
Levene's test for equality of variances		5.810	
	Sig	0.025	
t-test for equality of means	T	5.790	5.7900
	df	22	16.318
	Sig(2-tailed)	0.0000	0.0000
	Mean Difference	13.66750	13.6675
	SED	2.36066	2.36066
	95% CI of the Difference		
	Lower	8.77180	8.671050
	Upper	18.56320	18.66395

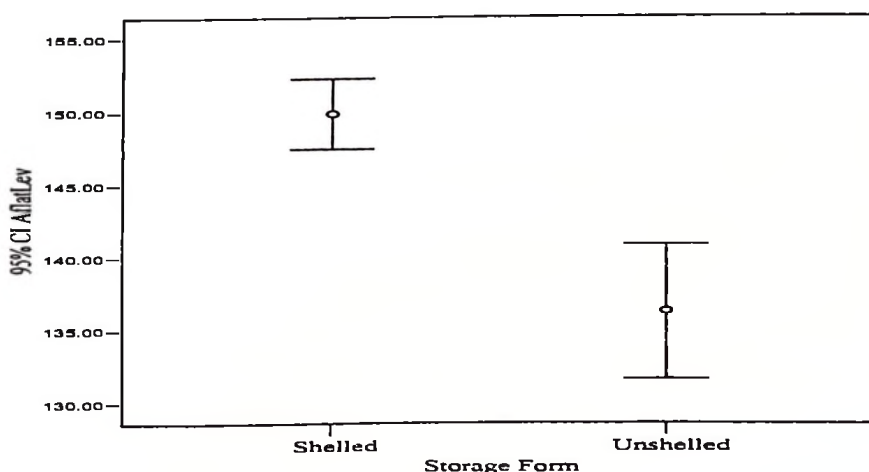


Figure 11: Level of aflatoxins ($\mu\text{g/kg}$) contaminations in two (2) different forms of groundnuts after 6 months of storage

Table 31: Independent Samples t-tests: Investigating the differences between the mean quantities of aflatoxins in the groundnuts samples stored in shelled and unshelled form after 9 months of storage

Homogeneity test	Statistical test	Aflatoxin concentration ($\mu\text{g}/\text{kg}$)	
		Equal variances assumed	Equal variances not assumed
Levene's test for Equality of variances		0.210	
t-test for Equality of Means	Sig	0.651	
	T	3.335	3.335
	Df	22	21.747
	Sig(2-tailed)	0.003	0.003
	Mean Difference	9.67833	9.67833
	SED	2.90177	2.90177
	95% CI of the Difference		
	Lower	3.65635	8.67105
Upper	3.66042	15.70031	

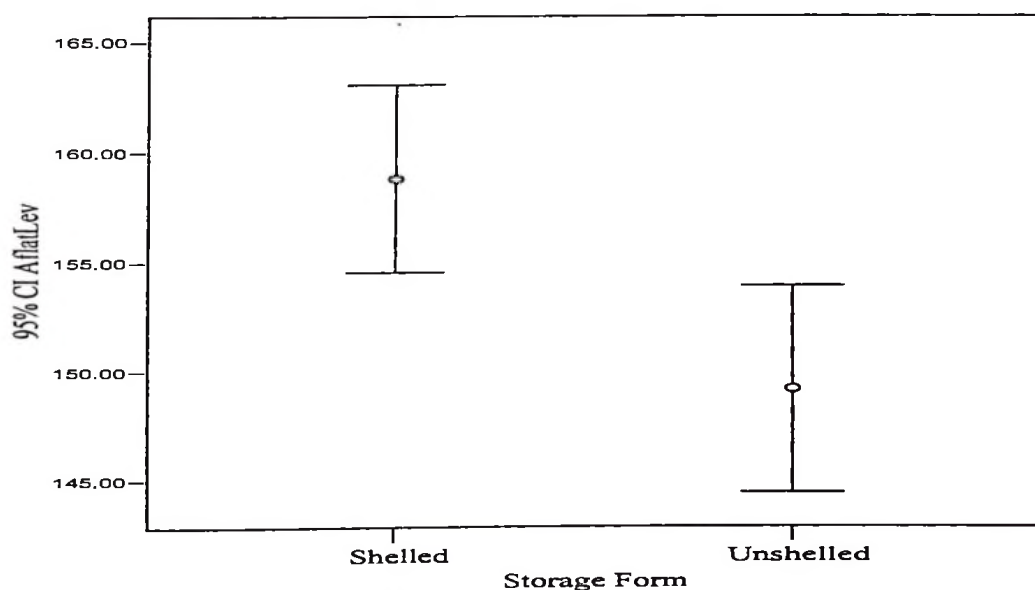


Figure 12: Level of aflatoxins ($\mu\text{g}/\text{kg}$) contaminations in two (2) different forms of groundnuts after 9 months of storage

The survey findings in Table 30 and 31 indicate that storage form of groundnuts has an effect on the levels of aflatoxin contamination after a given period. The shells impede penetration of moisture preventing germination of fungal growth inside the pod thereby keeping the nuts aflatoxin free. Groundnuts stored in pods had the lowest levels of aflatoxins (Mutegi *et al.*, 2013). The pods of nuts are likely to act as a protection against

fungi that penetrate the kernels (Mutegei *et al.*, 2013). Groundnuts in shelled form had the highest proportion of highly contaminated sample. The pieces of groundnuts are more likely to be substrate for aflatoxins production than the whole nuts in good conditions. Anything that damages the seed coat and allows the fungus access into the carbohydrate-rich endosperm increases the risk of fungal proliferation and aflatoxins production (Rosalind, 1999). Suleiman *et al.* (2007) indicated that raw shelled groundnuts and its products are the good substrate for the growth of aflatoxins by *A. flavus* and *A. parasiticus*. Generally, it can be said that, maize and groundnuts stored in different forms were not equally affected by mould infection. This suggests that the infection of maize and groundnuts by moulds in Kilosa District was influenced by the form in which the maize and groundnuts were stored.

4.2.4 Storage time and its relationships with mycotoxin contaminations

The research findings indicate that the mycotoxin concentrations of maize and groundnuts were significantly ($p=0.05$) affected by storage periods. They were increased from 144.7 to 171.7 $\mu\text{g}/\text{kg}$ (18.66%) in maize and from 127.3 to 154.0 $\mu\text{g}/\text{kg}$ (20.97%) in groundnuts by 9 months of storage. Generally, there was high correlation between storage time and the level of mycotoxin contamination obtained after 3, 6, and 9 months. This difference was significant ($p=0.05$). As the storage time increased, the level of mycotoxin concentration in the stored products increased. Mycotoxin incidence significantly increased in all storage systems throughout the storage period ($p=0.01$). These results findings are in consistent with those of Que- King *et al.* (1997), Bojle *et al.* (1998) and Mutegei *et al.* (2009). In another study, Hell *et al.* (2000) found that the percentage of maize samples with more than 5 $\mu\text{g}/\text{kg}$ aflatoxin levels was between 9.9% and 32.2% before storage in the different agro-ecological zones of Benin; but these levels increased to 15.0% and 32.2% after six months storage. Aflatoxin contamination of foods has

further been shown to increase with storage period (Kaaya and Kyamuhangire, 2006). It is compounded in Africa through excessive heat, high humidity, lack of aeration in the stores, and insect and rodent damage resulting in the proliferation and spread of fungal spores. The relationship between storage period and the total number of aflatoxins and fumonisins in groundnuts and maize is shown in Fig. 13.

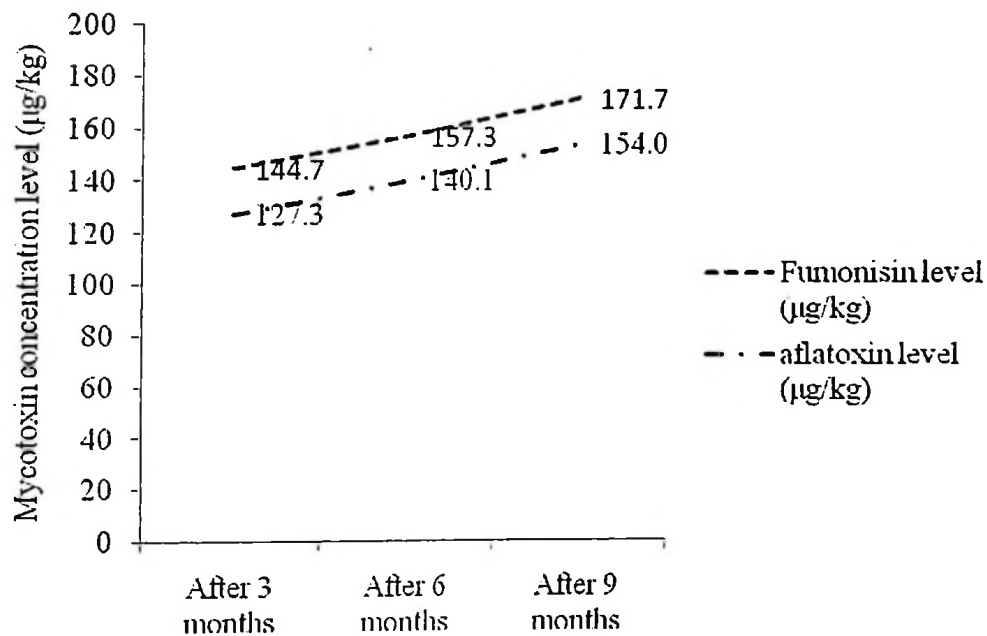


Figure 13: The relationship between storage period and mycotoxins (aflatoxins and fumonisins) concentrations in maize and groundnuts

4.3 Assessment of the Effect of Weather on Mycotoxin Incidences in Maize and Groundnuts

4.3.1 Effect of storage seasons on mycotoxin concentration

Results shown in Table 32 reveal that, storage seasons influenced mycotoxin contamination and was highly significant ($p=0.05$). The level of contamination was the highest during the month of December ($174.70 \pm 5.32 \mu\text{g/kg}$) followed by November and October (166.7 ± 7.45 and $156.4 \pm 8.18 \mu\text{g/kg}$, respectively). The lowest level of fumonisin contamination was found in the month of July that ranged from 118.80 to 136.40 $\mu\text{g/kg}$

with the mean of 125.30 ± 8.16 $\mu\text{g}/\text{kg}$. The level of FB_1 in stored maize in different seasons (months) is shown in Table 32.

Table 32: Levels of FB_1 ($\mu\text{g}/\text{kg}$) in stored maize samples during dry and rainy storage seasons in 2010 and 2011

Months	Average T ($^{\circ}\text{C}$)	Average RH (%)	Average RF (mm)	Mean \pm SD ($\mu\text{g}/\text{kg}$)	Range ($\mu\text{g}/\text{kg}$)
July ^{ds}	23.5	62.5	380	$125.3 \pm 8.16^{\text{ab}}$	118.8-136.40
August ^{ds}	24.6	68.6	510	$131.8 \pm 5.38^{\text{ab}}$	124.6-137.39
September ^{ds}	26.0	73.2	565	$144.8 \pm 9.46^{\text{ab}}$	133.6-156.40
October ^{rs}	27.8	80.0	660	$156.4 \pm 8.18^{\text{cd}}$	148.0-166.50
November ^{rs}	28.9	82.2	750	$166.7 \pm 7.45^{\text{cd}}$	158.8-176.80
December ^{rs}	31.5	91.8	800	$174.7 \pm 5.32^{\text{cd}}$	169.9-180.60

Values within same column followed by different superscript differ significantly at $p \leq 0.05$.

^{ds} = Dry season and ^{rs} = Rainy season, T = Temperature, RH = Relative Humidity and RF = Rainfall

The season has a major impact on fungal growth and fumonisins production. The fumonisins production is influenced by environmental conditions such as humidity and temperature (Hanif *et al.*, 2008; Sultana and Hanif, 2009). In the present study, high levels of fumonisin B_1 in the wet season of October through December and low concentrations during July to September indicate the effect of season. In Kilosa, humid conditions (hot season with heavy rainfall in which there is persistently high relative humidity and temperature) commence from October to December (Somboja, B. personal communication, 2010) which provide ideal conditions for the growth of fungi including *Fusarium* spp., ultimately resulting into increased production of fumonisins. There is evidence that weather conditions also influence the frequency of occurrence of fungi in dent maize and leaf axil debris (Dowd *et al.*, 2004). In Argentina, Hennigen *et al.* (2000) found fumonisin contamination to vary markedly during two consecutive growing seasons. Such yearly variation may, among others, be due to differences in environmental conditions. In this study for example, the mean rainfall during the period of the survey was higher in 2010 (900 mm) than in 2011 (700 mm).

4.3.2 Effects of weather variables (temperature, relative humidity and rainfall) on mycotoxin contaminations

The results in Table 33 reveal that temperature, relative humidity, and rainfall were found to significantly influence the incidence of mycotoxin contamination in stored maize and groundnuts ($p=0.05$). The two-way interaction [temperature x relative humidity] were found to be highly significant ($p=0.001$) and the interactions between [temperature x rainfall] and [relative humidity x rainfall] were also found to be significant at $p=0.018$, and $p=0.016$ respectively. Third-way interaction between temperature, relative humidity and rainfall were also found to be significant ($p=0.036$). Likewise, the results shown in Table 33 indicate that, temperature have the highest odds ratio ($\exp(1.977) = 7.22$), followed by relative humidity and rainfall with odds ratio of ($\exp(1.914) = 6.78$ and $\exp(1.653) = 5.22$, respectively which signifies the strong effect on influencing mycotoxin contaminations in the stored commodities. The parameter estimates for the log-linear model indicates the effects of variables on the log of the expected frequencies of the cross tabulation cells. Table 34 shows temperature ($^{\circ}\text{C}$), relative humidity (%) and rainfall (mm) recorded in Kilosa District for the 2010 and 2011 period.

Table 33: Output from a saturated log-linear model with Poisson distribution

Variable	Parameter estimate	Std Error	Z-value	p-value	95% CI		Odds Ratio(e^{β})
					Lower bound	Upper bound	
Constant	1.504	0.471	3.193	0.001	0.580	2.428	4.50
T	1.977	0.503	3.930	0.000	0.991	2.963	7.22
RH	1.914	0.505	3.790	0.000	0.924	2.903	6.78
RF	1.653	0.515	3.210	0.001	0.644	2.661	5.22
T x RH	-1.825	0.560	-3.259	0.001	-2.924	-0.727	0.16
T x RF	-1.339	0.564	-2.374	0.018	-2.444	-0.233	0.26
RH x RF	-1.369	0.568	-2.412	0.016	-2.482	-0.257	0.25
TxRHx RF	1.367	0.651	2.099	0.036	0.090	2.644	3.92

T=Average annual temperature, RH=Average annual relative humidity and RF=Average annual rainfall

Table 34: Temperature (⁰C), relative humidity (%) and rainfall (mm) recorded in Kilosa District for the 2010 and 2011 period

2010		Average			Minimum			Maximum		
Months	T (⁰ C)	RH (%)	RF (mm)	T(⁰ C)	RH (%)	RF (mm)	T(⁰ C)	RH (%)	RF (mm)	
July ^{ds}	20.4	60.5	400	20.1	50.3	380	20.7	70.7	420	
August ^{ds}	21.3	66.2	450	19.7	72.5	452	22.9	59.9	448	
September ^{ds}	23.4	69.9	550	20.5	76.0	590	26.3	63.8	510	
October ^{rs}	25.4	77.8	600	25.7	81.5	594	25.1	74.1	606	
November ^{rs}	25.9	79.8	750	29.1	81.1	712	22.7	78.5	788	
December ^{rs}	29.4	90.2	700	22.3	84.1	681	36.5	96.3	719	
2011		Average			Minimum			Maximum		
Months	T (⁰ C)	RH (%)	RF (mm)	T(⁰ C)	RH (%)	RF (mm)	T (⁰ C)	RH (%)	RF (mm)	
July ^{ds}	26.5	64.4	360	24.4	62.6	355	28.62	66.2	365	
August ^{ds}	27.8	71.0	570	25.2	63.9	478	30.4	78.1	662	
September ^{ds}	28.5	76.5	580	26.7	65.2	500	30.3	87.8	660	
October ^{rs}	30.1	82.1	720	28.1	70.5	611	32.1	93.7	829	
November ^{rs}	31.9	84.5	750	27.9	72.6	684	35.9	96.4	816	
December ^{rs}	33.6	93.3	900	28.4	76.2	723	38.8	110.4	1 077	

T=Temperature, RH=Relative humidity and RF=Rainfall
^{ds}= dry season and ^{rs} = rainy season

4.3.3 Interpretations of parameter estimates (β) from log-linear

Suppose, the dependent variable is log- transformed, and the regression is estimated as follows: $\ln(Y) = 1.504 + 1.977 \cdot T + 1.914 \cdot RH + 1.653 \cdot RF \dots\dots\dots(7)$

The estimated coefficients of temperature, relative humidity and rainfall variables are $\beta_1=1.977$, $\beta_2=1.914$, $\beta_3=1.653$, respectively as shown in Table 30. An increase of one unit in the temperature (T), would result in $(e^{\beta_1} - 1) \cdot 100$ percentage change in Y. Similary, an increase of one unit in relative humidity (RH) and rainfall (RF), the result would be $(e^{\beta_2} - 1) \cdot 100$ and $(e^{\beta_3} - 1) \cdot 100$ percentage change in Y respectively.

If the independent variable is log-transformed, the regression equation would be:

$Y = 1.504 + 1.977 \ln (T) + 1.914 \ln (RH) + 5.22 \ln (RF) \dots\dots\dots (8)$

Here, $\beta_1=1.977$, $\beta_2=1.914$ and $\beta_3=1.653$. It can be stated that, one percent change in temperature, relative humidity and rainfall are associated with $1.977 * \ln (101/100)$, $1.914 * \ln (101/100)$ and $1.653 \ln (101/100)$ respectively.

If both the dependent variable and independent variables are log-transformed, the fitted regression is: $\ln (Y) = 4.50 + 7.22 \ln (T) + 6.78 \ln (RH) + 5.22 \ln (RF)$(9)

Conclusion: One percentage change in temperature, relative humidity and rainfall results in $[(1.01)^{\beta_1} - 1] \cdot 100$, $[(1.14)^{\beta_2} - 1] \cdot 100$, $[(1.23)^{\beta_3} - 1] \cdot 100$ percentage change in Y, or around a 0.11%, 0.14% and 0.23% change in fungal and mycotoxin contamination in stored products. In short, it can be stated that, if $\beta_1=1.16$, we would say that one percent change in temperature is associated with $1.16 * \ln (101/100)$ equivalent to 0.116 change in Y. If $\beta_1=1.977$, Y has to be changed by $e^{1.977} - 1$ or 6.22% and if $\beta_2=1.914$, $\beta_3=1.653$, Y has to be changed by 5.78% and 4.22 % respectively. In this case, Y is mycotoxins production in stored products. Derivations of the three equations 7, 8 and 9 are shown in Appendix 5. It must be noted that; an odds ratio above 1 indicates a positive association among variables, while odds ratios smaller than one indicate a negative association. Odds ratios equal to 1 demonstrates that the variables have no association. If the coefficient is negative, $\exp(\beta) < 1$ and if the coefficient is positive, then $\exp(\beta) > 1$.

The role of humidity in fumonisin contamination is clearly important. Hennigen *et al.* (2000) found high levels of fumonisin in maize to be associated with high relative humidity in Argentina. Fumonisin contamination is likely to be strongly influenced by several environmental factors in different geo-areas, and among these, temperature, humidity, and rainfall during preharvest and harvest periods were very important

(FDA, 2001; Alptekin *et al.*, 2009). Similarly, the results presented in Fig. 14 and 15 indicate that variations in weather patterns noted through the 2010/11 seasons were the evidence on its influence on mycotoxin contaminations in the stored maize and groundnuts. Many researchers consistently found high temperature to be a major factor influencing aflatoxin contamination and fungal growth (Kaaya and Kyamuhangire, 2006). Alborch *et al.* (2011) revealed that temperature and water activity (a_w) influence not only rate of fungal spoilage, but also the production of mycotoxins. The differences in the prevalence and levels of contamination of fumonisins in stored maize depend on the number of factors such as the geographical area, climatic conditions (Dersjant-Li *et al.*, 2003), the type of raw material used, birds and insects damage to grains (Thompson and Henke, 2000), and storage conditions (Moss, 2002). To maintain high quality maize and groundnuts during storage, maize and groundnuts should be protected from weather (including relative humidity and temperature), growth of microorganisms, and insects (Oyekale *et al.*, 2012).

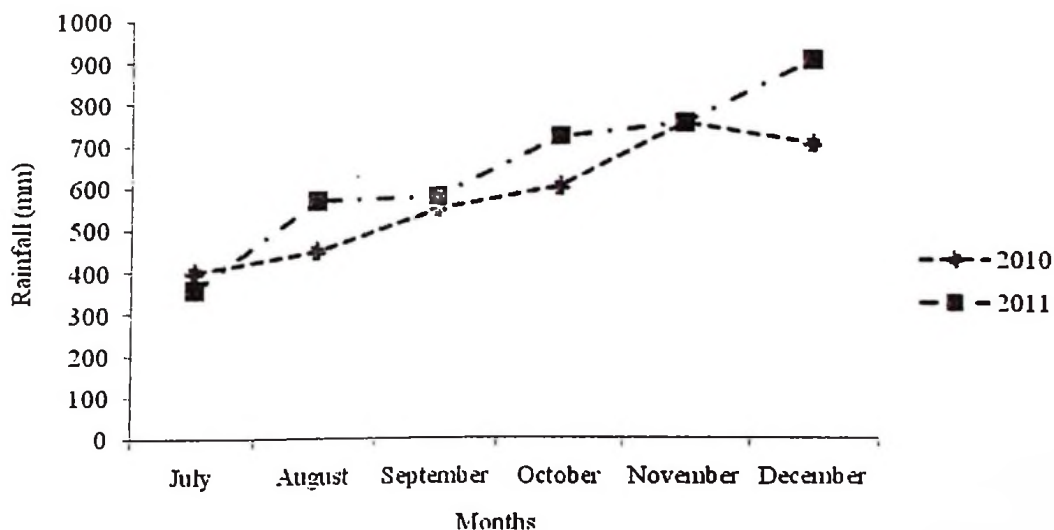


Figure 14: Average rainfall in the study villages in Kilosa District recorded during the 2010 and 2011 seasons/periods

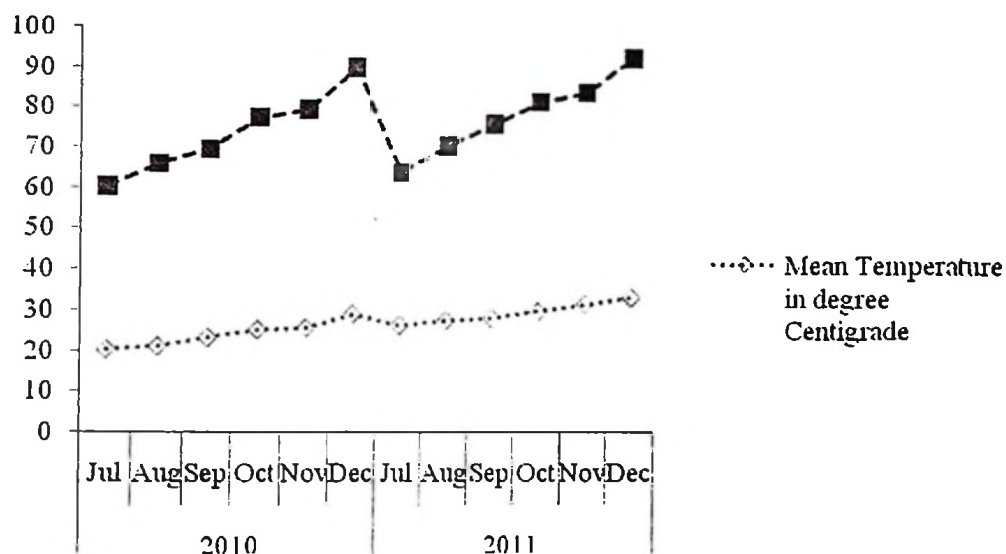


Figure 15: Temperature and Relative Humidity recorded during the 2010 and 2011 season

4.3.4 Correlation between levels of mycotoxins (fumonisins and aflatoxins) and weather data in 2010 and 2011

A significant positive correlation was observed between temperature data and levels of fumonisins and aflatoxins for the 2010 ($r = 0.9840$, $p = 0.0004$) and 2011 ($r = 0.9770$, $p = 0.0007$) season (Table 35 and 36). Similarly, a significant positive correlation was observed between relative humidity and the levels of fumonisins and aflatoxins for the 2010 ($r = 0.9840$, $p = 0.0004$) and 2011 ($r = 0.9650$, $p = 0.0079$) season and for the effect of rainfall on fumonisins and aflatoxins production. A significant positive correlation was observed for the 2010 ($r = 0.9540$, $p = 0.0031$) and 2011 ($r = 0.9460$, $p = 0.0150$) season (Table 35 and 36).

It is relevant to mention that, changes in weather results in large alterations in the quantity of aflatoxins producing fungi (Bock *et al.*, 2004). Indeed much of the organic matter in soils is colonized by aflatoxigenic fungi and hence, this colonization may occur when temperature become warmer (Miraglia *et al.*, 2009). Long, warm and humid periods

encourage cereal ears infection by *Fusarium* spp. (Jenkinson and Parry, 1994) and intense rainfall disperses fusaria to ears during anthesis.

When the weather conditions are favourable for the development of fungi, the fungus may produce aflatoxins at any stages of production and transformations (Alptekin *et al.*, 2009). The optimal conditions for *Fusarium* species, which causes maize ear rot, tend to be hot and dry weather (Doohan *et al.*, 2003). According to Miller (2001), the incidence of *Fusarium* kernel rot (*F. verticillioides* and *F. proliferatum*) is higher in warmer climates under dry conditions. In such environments, insect damage is well recognized as a collateral factor (Miller, 2001). There is no doubt that the climate is the principal factor triggering the fungal attacks, but several other factors may intervene and consequently affect the incidence of the fungal infection in cereal crops over the regions such as the influence of location, cultivar properties and agricultural practices (crop rotation and management) (Guerif *et al.*, 2001).

Table 35: Correlation between levels of mycotoxins in stored maize and groundnuts and weather data in 2010

		Temperature (°C)	Relative Humidity (%)	Rainfall (mm)	Mycotoxin ^{**} level (µg/kg)
Temperature (°C)	Correlation	1.0000	0.9940**	0.8930*	0.9840**
	Significance (2-tailed)		0.0000	0.0160	0.0004
	N	6	6	6	6
Relative Humidity (%)	Correlation	0.9940**	1.0000	0.8990*	0.9840**
	Significance (2-tailed)	0.0000		0.0150	0.0004
	N	6	6	6	6
Rainfall (mm)	Correlation	0.8930*	0.8990*	1.0000	0.9540**
	Significance (2-tailed)	0.0160	0.0150		0.0310
	N	6	6	6	6
Mycotoxin level (µg/kg)	Correlation	0.9840**	0.9840**	0.9540**	1.0000
	Significance (2-tailed)	0.0004	0.0004	0.0310	
	N	6	6	6	6

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

** Fumonisin B₁ and Aflatoxin B₁

Table 36: Correlation between levels of mycotoxins and weather data in 2011

		Temperature (°C)	Relative Humidity (%)	Rainfall (mm)	Mycotoxin ⁺⁺ level (µg/kg)
Temperature (°C)	Correlation	1.0000	0.9810**	0.9640**	0.9770**
	Significance (2-tailed)		0.001	0.002	0.0007
	N	6	6	6	6
Relative Humidity (%)	Correlation	0.9810**	1.0000	0.9840**	0.9650**
	Significance (2-tailed)	0.001		0.000	0.0079
	N	6	6	6	6
Rainfall (mm)	Correlation	0.9640**	0.984**	1.0000	0.9460**
	Significance (2-tailed)	0.002	0.0150		0.0150
	N	6	6	6	6
Mycotoxin level (µg/kg)	Correlation	0.9770**	0.9650**	0.9460**	1.0000
	Significance (2-tailed)	0.0007	0.0079	0.0150	
	N	6	6	6	6

** Correlation is significant at the 0.01 level (2-tailed)

⁺⁺ Fumonisin B₁ and Aflatoxin B₁

4.4 Assessment of Food Spoilage Loss due to Mycotoxins and its Effect on Household

Food Security in Kilosa District, Tanzania

4.4.1 Spoilage loss of maize due to fumonisin contaminations

The cumulative total loss due to mould damage in nine months of maize storage was about 20.8% of the total stored grains (Fig. 16). Incremental losses were observed in December to March, the period which coincides with high rainfall in the study area.

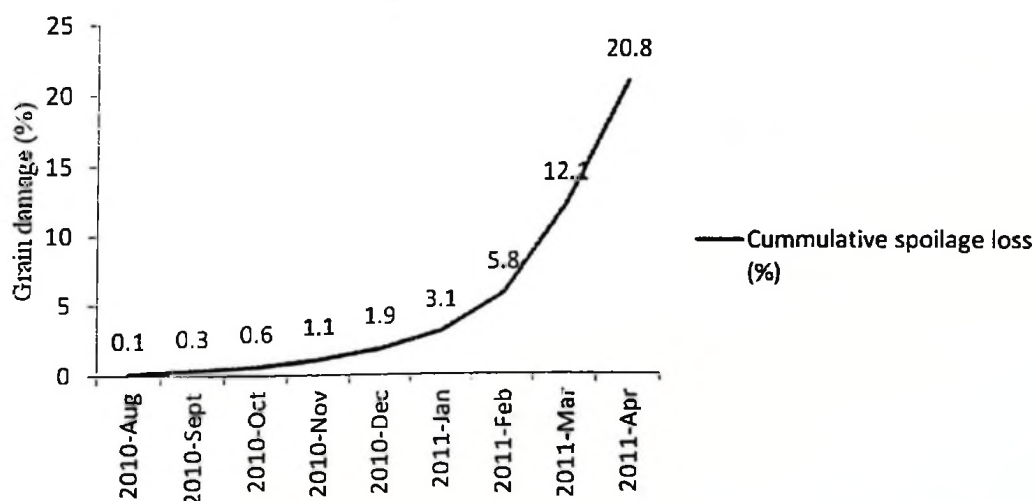


Figure 16: Cumulative spoilage loss (%) for the stored maize grain after 9 months of storage in Kilosa District, Tanzania

The results show that in Kilosa District, for every one percent damage of maize grains above 5% (damage referring to grains with fungal infection), the value decreases by one percent. So if undamaged grain is worth USD 0.33 per kg (=495 Tshs/kg), then the grain with 10% damage is worth only USD 0.285 per kg (=427.50 Tshs/kg), and with 20% damage it is worth only USD 0.275 per kg (=412.50 Tshs/Kg (Fig.17).

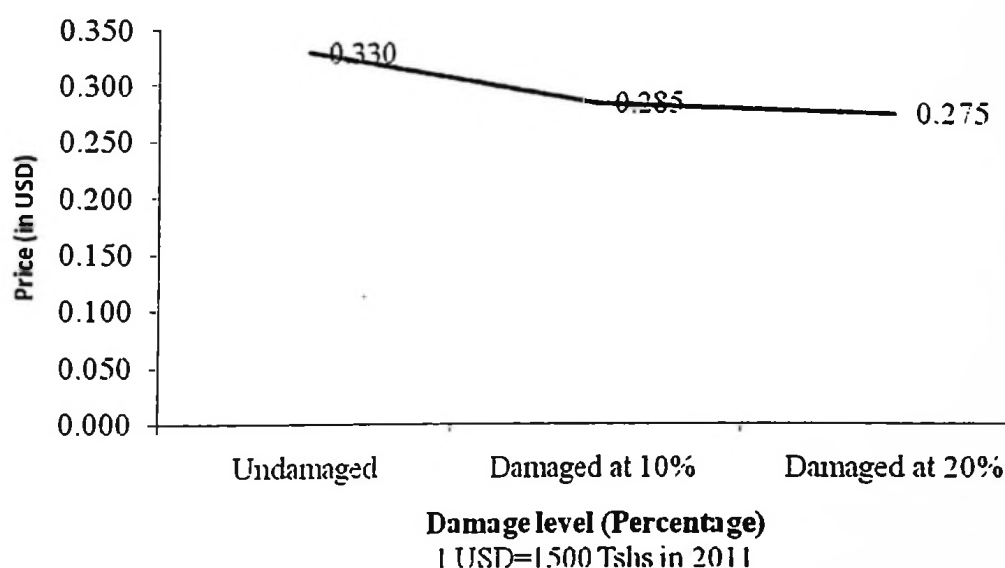


Figure 17: Relationship between fungal spoilage in stored maize and relative price

Tiongson and Gacilos (1990) observed an inverse relationship between the price of maize grits and aflatoxin content in the Philippines i.e. the lower the level of aflatoxin content the higher the price of maize grits. Accordingly, Cardino-Bermundo *et al.* (1991) concluded that moisture content and colour of the commodity determines the price of corn grain in the Philippines. Bottema and Altemeier (1990) and Wattanutchariya *et al.* (1991) indicated that moisture content and colour are the two most important factors in grain price formation in Indonesia and Thailand. In these countries the grain trader (middleman) measures the two factors through sensory evaluation and visual observation. Generally, local grain traders and processors do not use laboratory equipment, like

moisture testers, to measure grain attributes. The trader discounts wet or discoloured grain by deducting a certain percentage off the gross weight of grain. Alternatively, the trader deducts a percentage off the market price to get the price per unit weight of wet or discoloured grain. The discounts increase with the wetness of grain. As Cardino Bermundo *et al.* (1991) observed damaged grain in the Philippines; traders reduced the gross weight or the unit price of the produce by a factor ranging from 30% to 50%. These potential losses in value can make a substantial difference to a family's livelihood due to low value (carbohydrate) starch. Withdrawing contaminated crop without alternative uses may heighten economic losses and affect food security among the poor.

4.4.2 Spoilage loss of groundnuts due to aflatoxin contamination in the four surveyed villages in Kilosa District, 2010/2011 season

On average, 8.0 kg of groundnuts was rejected per farmer (rejecting rotten groundnuts, which were infected by fungi). On further examination of the rejects, the study discovered that 8.3% of the interviewed groundnut farmers had rejects of less than 2.0 kg. Further analysis indicated that about 22% of farmers lost up to 10 kg of groundnuts as a result of aflatoxin contamination. The highest volume of rejected groundnuts was above 10 kg (31.9%) (Table 37).

Table 37: Ranges of rejects of groundnuts due to aflatoxins from the 2010/2011 sample survey

Amount rejected (Kg)	Frequency (n)	Percent
<2.1	6	8.3
2.1-4	10	13.9
4.1-6	17	23.6
6.1-10	16	22.2
>10	23	31.9
Total	72	100.0

The estimated annual revenue loss in USD was about 179 116.37 (=2 686 745.60 Tshs). If Tanzania has to export groundnuts at a world market, a nation's total export loss of a particular food crop, given a particular internationally imposed mycotoxin standard, can be calculated as the product of the price of the food crop per unit volume on the world market, the total volume of that crop exported by a particular nation, and the fraction of that nation's food export crop that is rejected as a result of a worldwide mycotoxin standard. In a study carried out in 2004, Wu estimated a USD 450 million annual loss, mainly charged to the US, China, Argentina, and sub-Saharan African groundnuts markets, if the EU aflatoxin standard were adopted worldwide (Wu, 2004). Africa loses 670-750 million USD annually to EU restrictive standard for aflatoxins on cereals, dried fruits and nuts; and billions on the same issue the world over (Okello *et al.*, 2010). In Africa and Asia, Kazemi *et al.*(2014) revealed that *A. niger* and *A. flavus* have been implicated in the toxic deterioration of 30.97 million MT of peanuts and pistachios on annual basis. These fungal contaminants affect produce, quality, produce end-products and ultimately food safety (Kazemi *et al.*, 2009). Food safety is defined as the degree of confidence that food will not cause sickness or harm to the consumer when it is prepared, served and eaten according to its intended use (FAO/WHO, 2003).

Damaged kernels cause great concern among farmers due to the loss of maize quality and quantity. In this study, the spoilage loss due to mould infection in the stored maize amounted to 4330.62 tons of maize per year equivalent to one-fifth of the total maize production (20 622 tons per year). The amount of maize loss would be enough to feed 23 730 people for the whole year (at about 0.50 kg/day/person at an estimated value of 33.3 USD per 100 kg bag of maize) (Ng'unga, M. personal communication, 2010). It has been reported by Fandohan *et al.* (2003) that storage fungi contributes to loss of more than 50

% of maize grain in tropical countries, and ranks second after insects as the major cause of deterioration and loss of maize. According to Williams and McDonald (1983), when storage molds invade maize grain they cause rot, kernel discoloration, loss of viability, vivipary, mycotoxin contamination, and subsequent seedling blights. Kossou and Aho (1993) reported that fungi could cause about 50 to 80% of damage on farmer's maize during the storage period if conditions are favourable for their development. This is consistent with the typical range of maize storage losses described by the study earlier.

4.5 Farmers' Practices Contributing to Mycotoxins Contaminations in Stored Maize and Groundnuts

4.5.1 Drying techniques for maize and groundnuts

The methods used to dry maize and groundnuts in Kilosa District were: on bare ground (45.8%, 51.2%), over roof (25%, 0.01%), on polythene sheets (20.2%, 36.5%), on raised platforms (3.2%, 0.0%), on mats (5.8%, 10.6%) and on iron sheets (0.0%, 1.7%) respectively (Fig. 18). Drying of the harvested maize on bare ground was commonly practiced by most of the farmers although this method is not good since it results into contamination by soil borne microorganisms (Odogola, 1994).

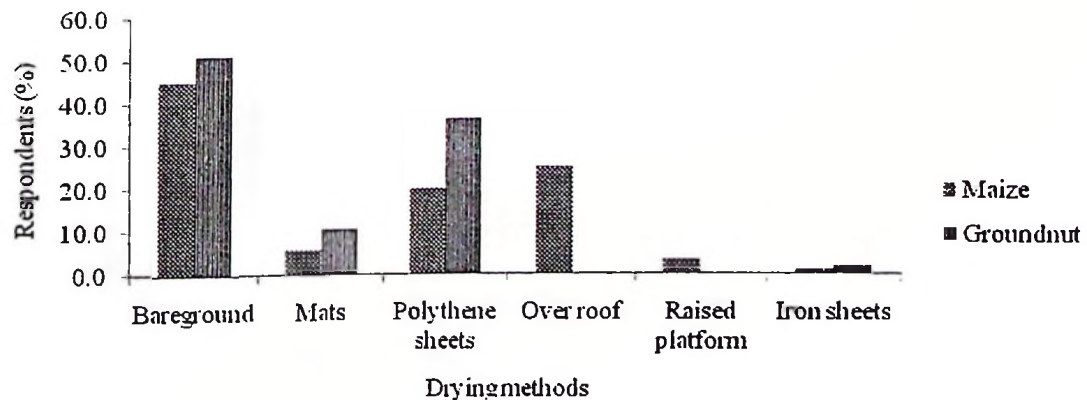


Figure 18: Drying methods for maize and groundnuts used by farmers in the four surveyed villages in Kilosa District, in 2010

4.5.2 Methods used for shelling maize and groundnuts

Three methods of shelling maize and groundnuts were reported by farmers in the surveyed villages (Fig. 19). Hand shelling was used by majority of the respondents (51.8%), followed by beating on the threshing floor (30.8%), while shelling by using machines was done by few respondents (17.4%). Shelling by hand was done to kernels intended for home consumption and for seeds. Beating cobs on threshing floor was reported to be done either on bareground or in polypropylene bags. Beating cobs in bags on threshing floor has been reported to inflict physical or mechanical damage to the kernels. According to Tuite *et al.* (1985), mechanical damage to maize kernels makes them much more vulnerable to invasion by storage moulds.

Similarly, shelling methods for groundnuts practiced by the respondents were as follows: hand shelling 64.2%, beating on the threshing floor (24.2%) and machinery shelling (11.7%). The conditions of the shells were found to be of importance in relation to fungal growth on grains and *A. flavus* was commonly associated with kernels from broken pods, and that most toxic samples came from this grade of pod (McDonald and Harkness, 1965).

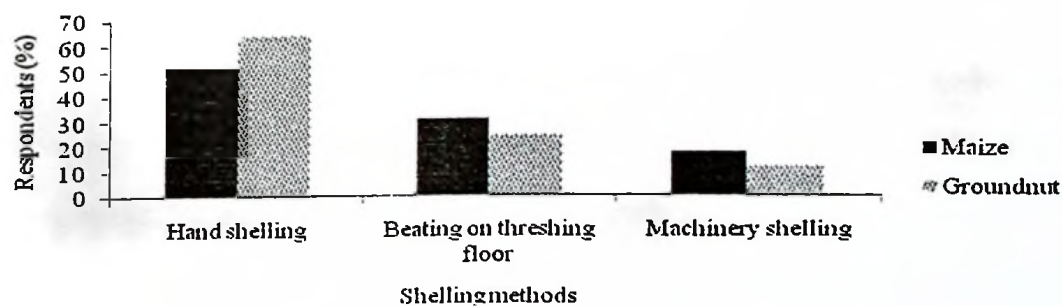


Figure 19: Maize and groundnuts shelling methods commonly practiced by farmers in the study villages in Kilosa District, in 2010/2011

4.5.3 Determination of safe storage moisture content in maize and groundnuts

It was important to find out how farmers determine the storage moisture content (MC). Moisture content is one of the factors responsible for deterioration during storage including mould infection (Sauer, 1988). The results indicated that all farmers interviewed in the four villages did not use modern methods of determining moisture content in the produce before storage. The different ways of estimating moisture content practiced by farmers in the study area included; biting kernels (16.7%), physical observation of the grains or husks/leaves (18.1%) and hand feeling (48.6%) while 16.7% did both (Fig. 20).

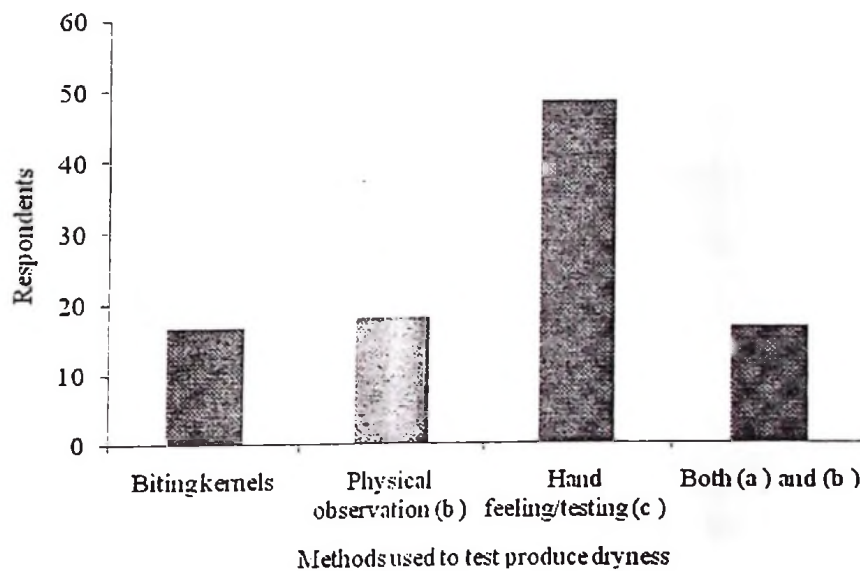


Figure 20: Estimation of maize and groundnuts dryness in the surveyed villages

These methods are however considered qualitative and depend on farmer's experience. It is therefore possible that maize and groundnuts in these villages were not being dried to the recommended safe storage moisture content levels of 12% to 14% and 6% to 8% respectively (FAO, 1993). This therefore, favoured microbial infection during storage. In literature, cereals dried to 12-14% moisture content were reported to be free from

fungal growth, but still can be very attractive to insects (FAO, 1993). Tuite and Foster (1979) also reported that insects in grain enhance mould development because they increase moisture content and temperature, and open areas of the grain for attack. In order to slow down insect development, the moisture content should be 9% or less according to FAO (1993) and Henderson (1985) recommendation (Appendix 6 and 7).

4.5.4 Maize and groundnuts sorting practices after shelling

During the survey, 37.5% of the respondents indicated that they sort their produce after shelling before storage. The parameters used for sorting include colour (26.4%), diseased grains (18.3%), discoloured/dirty grains (15.6%), and broken grains (29.7%) (Table 38). The fact that farmers sort maize to remove diseased grains implies a reduction in the initial microbial load. Sorting after shelling to remove foreign matter, diseased and discoloured grains and other grains (that are considered unfit) is one of the recommended activities for maintaining quality of the maize and groundnuts intended for storage and consumption. Cleaning the grain is reported by Vincelli *et al.* (1995) as the first initiative for reducing mycotoxin contamination. Cleaning of cereals or nuts, where mould-damaged kernels, seeds or nuts are removed from the intact commodity, may result in 40-80% reduction of aflatoxins (Park, 2002). Additionally, the removal of broken grains minimizes subsequent mould infection. Thus, maize sorting on-farm is strongly recommended for the management of mould and mycotoxin contamination.

Table 38: Basis for sorting maize before storage by farmers in Kilosa District (n=72)

Basis of sorting	Respondents (%)*
Colour	26.4
Diseased grain	18.3
Broken grain	29.7
Discoloured	15.6

* Multiple positive responses obtained

It has also been revealed that 18.1% of the respondents utilized the rejected grains rejects after sorting for feeding livestock/poultry, 34.7% threw them away, 6.9% used for making local brew (5.6%), 12.5% re-dried and consume, 13.9% sold them to the market, while 8.3% both did depending on the level of the damage (Fig. 21). The results indicated that some farmers are not aware of the effects of feeding diseased grains to their animals. Mycotoxins especially aflatoxins and fumonisins have adverse effects to animals (Munimbazi and Bullerman, 1996).

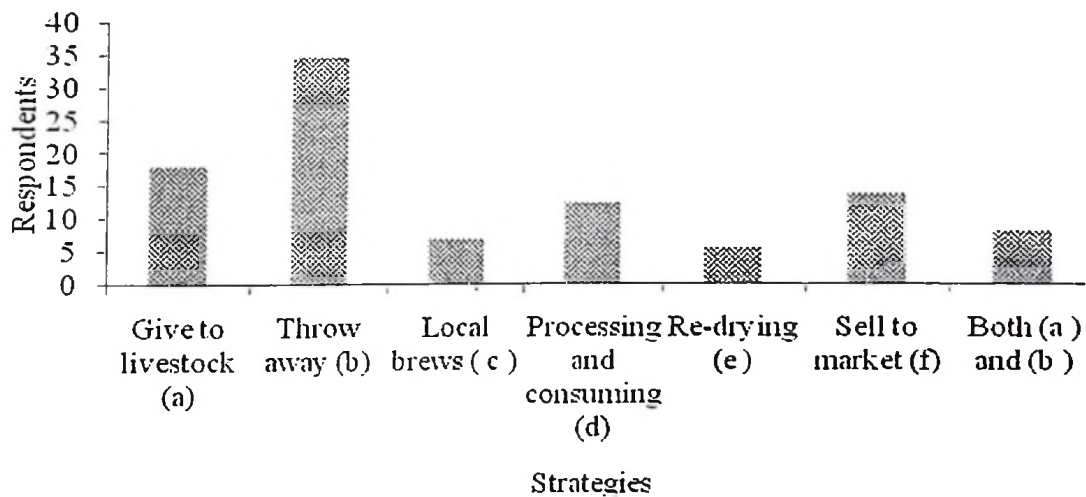


Figure 21: Methods of resolving storage problems adapted by farmers in the study villages in Kilosa District

Fig. 22 shows the multiple infections of maize ear rots with *F. moniliforme* and *A. flavus* used as poultry feed in one of the surveyed villages. High level of aflatoxins in feed is not only hazardous to poultry because of direct exposure but it also poses a high risk to human health with the possibility of indirect exposure through contaminated meat, eggs and other poultry products and by-products (Maqbool *et al.*, 2004; Bintvihok and Kositcharoenkul, 2006).



Figure 22: Multiple ear rot caused by *F. moniliforme* and *A. flavus* used as poultry feeds in one of the study village

4.5.5 Storage methods for maize and groundnuts

Three storage methods of maize used by farmers in the study villages as reported by respondents are presented in Table 39. These storage methods include the use of polythene bags (100 kg) (75%), the use of cribs (15.3%) and heaping of maize cobs on the floor in the house (9.7%). Similarly, three storage methods were used to store groundnuts include polythene bags (100 kg) (50%), old plastic buckets (20 litres) (18.1%), and nylon bags (31.9%). Some typical examples of storage structures used in the study area are shown in Fig. 23.

Table 39: Percentage of farmers using different storage methods for maize and groundnuts (n=72)

Method	Farmers (%)	
	Maize	Groundnuts
Polythene bags (100kg)	75.0	50.0
Crib	15.3	-
Heaping on the floor	9.7	-
Plastic buckets (20 litres)	-	18.1
Nylon bags	-	31.9



Figure 23: Storage methods encountered in the study villages in Kilosa, 2010/2011

- A: Traditional cribs constructed with tree poles
 B: Shelled maize grains in polythene bags,
 C: A pile of maize cobs heaped on floor in a house

Generally it can be stated that, storage methods used by farmers in the study area are of low quality, and commodities are stored without means of monitoring the temperature and humidity of such storage methods. Many farmers in the study area store their grains in bags especially polythene which are not airtight, but there is evidence that this method facilitates fungal contamination and aflatoxins development (Udoh *et al.*, 2000).

4.5.6 Storage problems and storage treatments

The farmers reported and ranked common storage problems, and these included insects, rodents, moulds and leaking roof during rainy season. Insects and rodents were ranked the highest. Table 40 shows the percentage of respondents giving various rankings to various problems.

Table 40: Respondents' ranking of various problems (n=72)

Ranking	Insects	Rodents	Mould	Leaking roof during rainy season in the store room
1 st	63.9	25.4	0.0	2.4
2 nd	35.6	27.8	15.4	3.6
3 rd	2.4	19.0	11.9	16.2
4 th	0.0	0.0	21.4	10.4

The results show that 89.3% of the respondents reported insects or rodents as the most troublesome storage pests, while 63.4% considered them as the 2nd most important. Only 15% of the respondents ranked mould and insects among the two most important storage pests. Farmers used traps, chemicals and ashes to control insect pests. Unfortunately, little or nothing was done to control the mould despite its severe consequential effects. The respondents faced several problems during storage. More than 76% of the respondents indicated storage problems, and these were mainly due to moulds (reported by 37.1%), insects (34.7%), rodents (11.1%) and leakage or re-wetting (5.6%). These problems have been reported to encourage mould infection and mycotoxin production (Cardwell *et al.*, 2000). Farmers used several strategies in managing pests, and these included the use of rodenticides and traps to control rodents, the use of synthetic pesticides and local storage protectants to control insect pests and moulds.

The results revealed a number of interesting features. First, it confirmed that farmers use pesticides and traditional methods for the prevention and control of storage pests.

Elsewhere it was reported that the combination of chemical and non-chemical methods of insect pest control has been recommended for farm stored grains since the early 1900s (Reed and Arthur, 2000). Secondly, the results showed that the use of pesticides is the most preferred storage pest control strategy. There are, however, a number of issues which must be addressed in line with farmers' desire to adopt a particular pest control strategy. This and other studies show that maize producers in the study villages do not follow recommended insect control techniques for farm-stored grains. It appears that lack of information on proper use of storage (synthetic) pesticides on the part of both extension staff and farmers is one of the most notorious problems facing pest management strategies in the study area. Among 72 farmers interviewed, about 85% complained that they never received any expert advice on proper grain storage methods and practices. This high percentage (85%) provides evidence of the magnitude of the problem. As Norgaard (1976) attested, lack of information resulted in sub-optimal decisions and uncertainty about outcomes. Decisions can be improved and certainty be increased by acquisition of better knowledge.

Another problem is the unavailability of commercial pesticides. Although more than half of the surveyed farmers were aware of the need for pesticides, failure of the distribution system was a major constraint. There appear to be four commonly employed means of distributing pesticides in the rural areas. About 61% of farmers using pesticides reported that they purchased the pesticides from private dealers. Others (12.5%) reported to have obtained pesticides from village shops, 11.7% from village agriculture extension officers (VAEO) and 5% cited government agents as a source of pesticides. The rest of the respondents obtained pesticides from a variety of other sources, including private traders (Fig. 24). Most of the subsistence farmers planted their fields with the seeds retained from

the previous harvest (Ncube, 2008) thereby increasing the risk of systemic infection by plant pathogens (Shephard *et al.*, 2007). This diversity of input sources increased the risk of using expired or non-recommended pesticides. Norgaard (1976) considered the misuse of pest control inputs as a social problem. The author argues that insufficient consideration is given to the economics and technological limitations of using pesticides and other pest control strategies in relation to the pest complex and the total post-harvest system. Investment in insect control for farm-stored grain may not be cost effective for many farmers. In addition, the Tropical Development and Research Institute (TDRI, 1984) stressed that proper supervision is necessary for safe and effective use of pesticides at farm level.

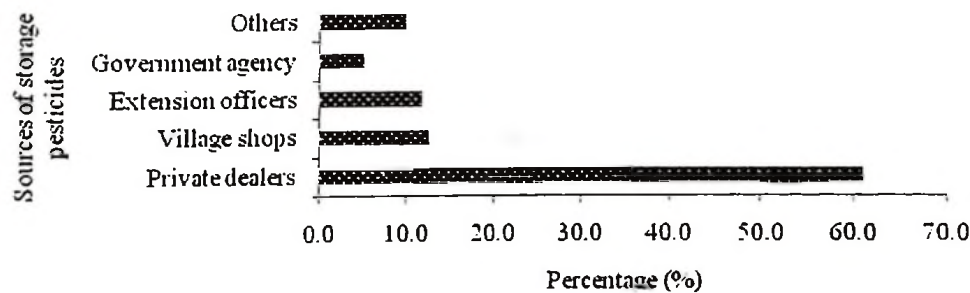


Figure 24: Different sources of storage pesticides reported in study villages in Kilosa District, Tanzania

4.5.7 Factors influencing mycotoxins production in maize and groundnuts in Kilosa District

Nine major factors affecting mycotoxin contamination of stored maize and groundnuts were identified (Table 41). A stepwise linear regression analysis of the factors gave a coefficient of determination (R^2) of 0.74 an indication that the model used was able to explain 74% of the variation in the dependent variable which were explained by independent variables and this value has a big explanatory power in the model. It appears

multicollinearity was not a concern because the VIF scores are less than 10 (Table 41). The VIF of an independent variable is the value of 1-minus-R-squared in a regression of itself on the other independent variables. The rule of thumb here is that *a VIF larger than 10 is an indicator of potentially significant multicollinearity* between that variable and one or more others. It is stated that, a VIF larger than 10 means that the regression of that independent variable on the others has an R-squared of greater than 90%. If this is observed, it means that the variable in question does not contain much independent information in the presence of all other variables, taken as a group. When this happens, it often happens for many variables at once, and it may take some trial and error to figure out which one(s) ought to be removed. However, like most other diagnostic tests, the VIF-greater-than-10 test is not a hard-and-fast rule, just an arbitrary threshold that indicates the possibility of a problem. In this case it indicates a possibility that the model could be simplified, perhaps by deleting variables or perhaps by redefining them in a way that better separates their contributions. Similarly, the results showed no standardized Dfbeta values of < -2 or > 2 and it can be concluded that the dataset did not include outliers or influential cases (Table 42).

Table 41: Multiple regression analysis of factors influencing mycotoxins productions in the study area

Regression variables	Standardized β -coefficients	T-value	p-value	Tolerance	VIF
(Constant)	2.109	4.739	0.000***		
x_1 = Sorting before storage	-0.178	2.337	0.023*	0.631	1.585
x_2 = Leaving to dry after 3 weeks	-0.297	3.958	0.000***	0.651	1.536
x_3 =Shelling using machinery	0.195	2.342	0.022*	0.528	1.893
x_4 =Insect damage	0.227	2.587	0.012**	0.475	2.105
x_5 = Storing produce in the same room year to year	0.231	2.853	0.006**	0.560	1.786
x_6 = Heaping produce on the floor	0.250	3.003	0.004**	0.527	1.898
x_7 = Storing the produce in shelled form	0.142	2.080	0.042*	0.784	1.275
x_8 = Farmers awareness of insect damage	-0.172	2.598	0.012**	0.835	1.198
x_9 = Use of traditional protectants (biocides)	-0.141	2.095	0.040*	0.811	1.234

Adjusted $R^2=0.74$, β =Regression coefficients, $\beta_1 \dots \beta_9$ =Regression coefficients for x_1 to x_9

* Significant at $p<0.05$, ** significant at $p<0.01$ and *** significant at $p<0.001$

Table 42: Descriptive Statistics of factors influencing mycotoxins contamination in stored maize and groundnuts

	n	Minimum	Maximum	Mean	Std.Deviation
Standardized DfBeta SORT	72	0.0000	1.0000	0.1389	0.34826
Standardized DfBeta LAT	72	1.0000	2.0000	1.3889	0.49092
Standardized DfBeta SUM	72	1.0000	2.0000	1.3056	0.46387
Standardized DfBeta IND	72	1.0000	2.0000	1.6389	0.48369
Standardized DfBeta SSR	72	0.0000	1.0000	0.9028	0.29834
Standardized DfBeta HOF	72	1.0000	2.0000	1.1389	0.34826
Standardized DfBeta SPS	72	0.0000	1.0000	0.6389	0.48369
Standardized DfBeta FAI	72	0.0000	1.0000	0.1944	0.39855
Standardized DfBeta UTP	72	0.0000	1.0000	0.3889	0.49092
Valid N (listwise)	72				

The factors associated with decreased mycotoxin levels in maize and groundnuts were sorting the produce before storage and was significant ($p=0.023$), leaving to dry after three weeks ($p<0.0001$), farmers' awareness of the insects problems in storage ($p=0.012$), and the use of traditional protectants ($p=0.040$). Mycotoxin development in maize and groundnuts was positively related to shelling of the stored produces by using machinery ($p=0.022$), insect damage ($p=0.012$), storing maize and groundnuts in the same room from year to year ($p=0.006$), heaping of maize on the floor in a house ($p=0.004$) and storing the produce in shelled form ($p=0.042$). All of these factors increased mycotoxin development in maize and groundnuts.

Leaving to dry after three (3) weeks had significant effects on the contamination of maize grain by fumonisin. As Tanboon-ek (1989) proposed, field drying on the stalk before harvest, followed by mechanical drying after shelling, were the most effective ways of reducing fumonisin contamination of maize. Siriacha *et al.* (1989) found that if shelled grain was immediately sun-dried the chance of contamination was reduced as compared with that of undried shelled maize.

Sorting before storage such as sorting out diseased, damaged and discoloured maize and groundnuts kernels as well as cleaning before storage were associated with reduced

aflatoxin and fumonisin levels. Similar results were reported by Hell *et al.* (2000) and Udoh *et al.* (2000) in Benin and Nigeria, respectively. Udoh (1995) and Hell (1997) found that sorting bad grains reduced losses due to insect attack and aflatoxin contamination after harvest. Sorting out physically damaged and infected grains from produce can result in 40-80% reduction in aflatoxin levels (Park, 2002). Therefore, the removal of mouldy cobs increases the chances of preserving good quality grains in storage. Such practices help to reduce the fungal inocula load and infected substrates. This reduces chances of mould proliferation by infecting health kernels and subsequent production of mycotoxins as confirmed by Martin *et al.* (1999).

There is a close relationship between storage fungi and insect infestation. Jian and Jayas (2012) reported that some storage fungi attract insects and promote their growth, but other prevent through secretion of toxic metabolites. In connection to this, Bruns (2003) found direct association between insect feeding activity, fungal growth and mycotoxin production. Likewise, Setamou *et al.* (1997), detected low levels of mycotoxin for less damaged maize (2%) than in higher damaged maize.

In this study, the application of traditional storage protectants was negatively related to fumonisin concentrations in the stored maize samples. This is similar to other studies in which plant substances were used *in vitro* to control growth of *Aspergillus* fungi (Cardwell and Dongo, 1994). It was reported that *Aspergillus* fungi would not grow on medicinal plants, and could not lead to aflatoxins and fumonisins on them (Roy and Kumari 1991). Thus, the mixing of plant substances with stored grains may actually reduce the risk of aflatoxin development and controlling it. Also, plant materials may reduce relative humidity inside the grain store through their biomasses, and consequently reduce fungal growth. Ash is used both as inert filler and for its other negative effects on

insects. As inert filler, ash works by filling up the space around the seed and impeding the movement of insects as well as in sealed containers, reducing the volume of air available to the insects for respiration. Ash has been reported to damage the cuticle of insects causing them to dehydrate and to have detrimental effect on egg development (Almekinder, 1999).

Education is positively related to awareness, knowledge and perceived benefits (Jolly *et al.*, 2009). It is understood that people with higher education level are likely to be better informed, and therefore, may be more aware of some types of risk of food additives or pesticides in foods than those with less education. It is thus believed that educated individuals are more likely to seek information about mycotoxin and consequently develop an action plan to prevent them from being exposed to mycotoxin. Nevertheless, a particular attention should be given to those with low education level in order to raise their awareness and knowledge on mycotoxins contamination in the food commodities. It is believed that through comprehensive educational program such as campaign and advertisement, the public generally can be educated and made aware of the presence of mycotoxins in the diets and its detrimental health effects.

Beating cobs on a threshing floor also inflicts physical or mechanical damage to the grain making them prone to fungal invasion and therefore mycotoxin production (Tuite *et al.*, 1985; Bankole and Adebajo, 2003). Similarly, Dharmaputra *et al.* (1994) reported that mechanical damage during or after harvesting on maize grains can provide entry points to fungal spores. Likewise, Fandohan *et al.* (2006) reported that increases in grain damage and cracking create an opportunity for fungi to grow and penetrate the maize grain. Possibly, the use of hand shellers should be promoted in Kilosa District since grain

shelled by this equipment is often clean with no mechanical damage (Kaaya *et al.*, 2006).

Fig. 25 shows the use of hand shellers by farmers in Mkalama village.



Figure 25: Hand operated sheller used to shell groundnuts in Mkalama village

Insect damage was observed to increase mycotoxin contamination in the storage. This is substantiated with a study by Mutiro *et al.* (1992) who evaluated insect damage and aflatoxin development on maize in traditional and improved storage structures in Zimbabwe. The grain damage by insects and rodents, as well as birds predisposes the crop to colonization by the fungus and aflatoxin contamination and lead to aflatoxin occurrence in groundnuts and maize (Williams *et al.*, 2004). It is well documented that insects propagate *Aspergillus* spores in the stores (Lynch and Wilson, 1991).

As Wright (1992) revealed, *A. flavus* contamination was strongly correlated with high densities of weevils. Insects play a big role in the vectoring of fungal spores and also provide entry holes to fungal organisms through their tunnelling activity, both prior to and after harvest (Hell *et al.*, 2000).

4.5.8 Farmers awareness of mouldy infection in stored maize and groundnuts

The Cronbach's alpha of 0.745 from the 8-item questionnaire completed by respondents indicated the consistency of the questionnaire. Besides, the output of factor analysis; the Kaiser-Meyer-Olkin (KMO) value of 0.688 and the significance of Bartlett's Test ($p < 0.000$) confirmed the suitability of data. Two factors (with Eigenvalues exceeding 1 and factor loading above 0.4) were identified as underlying factors of the eight (8) questionnaire items. In total, these factors accounted for 68.5% of the variance in the questionnaire data. The measures of overall goodness of fit of the model were acceptable. The χ^2 -statistic was 84.908 for 28 degrees of freedom ($p < 0.005$). All factor loadings were statistically significant at the 95% confidence level. The final items defining the scales of individuals' awareness of mouldy infection are presented in Table 43.

Table 43: Varimax rotated factor structure of the eight items of awareness and knowledge of mouldy infection

Statements	Factor Loadings	Cronbach's alpha
1. Can you identify spoilt maize/groundnuts?	0.531	0.745
2. Do you sort your crops after harvest?	0.548	
3. Do you know how to identify well –dried pods/ grain?	0.661	
4. Do you clean the storehouse before storage?	0.527	
5. Aflatoxins and fumonisins cause liver cancer in human being	0.696	
6. Have you heard of the word mycotoxins before?	0.783	
7. Do you know the right time to harvest your crops?	0.789	
8. Do you know the measures for controlling fungal spoilage in the store?	0.506	

Note: All factor loadings had value above 0.4.

About 97% of the respondents were found not to be aware of mouldy infection. Even though this is the status, most of the respondents identified aflatoxins-infested nuts and fumonisin-infested kernels as the only ones which were rotten. This poses a great threat to the district as shrivels and mechanically damaged nuts are considered suitable for consumption. Those who had heard, of mycotoxins thought that it was a “poison found in spoiled maize and also they felt that it was caused by wet or rotten maize. 48.6% of respondents agreed that, it was a “mould that attacks maize and groundnuts.” These insights reveal a limited understanding of what aflatoxins and fumonisins are and how they are formed. When asked specifically about their causes, 50% of farmers identified poorly dried and or wet maize as the cause, followed by heaping maize on floor (38.9%). 11.1% of respondents identified the use of expired pesticides on the grain as a causing problem. Farmers in Kilosa District, clearly don’t have some recognition of the connection between postharvest handling and aflatoxins and fumonisin contamination. In terms of threats to human health, large percentages (66.7%) of respondents were not aware of the problem.

With respect to the low level of awareness, 63.9% of respondents reported that they did not know how to tell if maize and groundnuts were affected by fumonisins or aflatoxins, while 36.1% answered that they could tell, identifying the “discoloration of the maize” as a key tell-tale sign even though the presence of aflatoxins cannot be detected visually. Mouldiness and wetness were other indicators listed by all farmers. 15.3% of respondents also identified finding insects in their maize as an indicator of fumonisin and aflatoxin contamination. With regard to mould infections, 47.2% of the respondents took precautions to prevent it from affecting their maize stores by drying their grain before putting it into storage either as cobs or grain. Once mold was detected, farmers dealt with

the problem in various ways. Airing the maize was a dominant method. It has also been revealed that 18.1% of the respondents utilized the rejected grains rejects after sorting for feeding livestock/poultry, 34.7% threw them away, 6.9% used for making local brew 18.1% re-dried and consume, 13.9% sold them to the market, while 8.3% both throw and gave them to livestock/poultry depending on the level of the damage (Table 44).

Table 44: Identification of awareness and knowledge on mouldy infection in maize and groundnuts)

Statements	Response	N	Percent
Can you identify spoilt maize/groundnuts?	Yes	32	44.4
	No	40	55.6
How do you identify spoilage?	Insect rot	11	15.3
	Rot	35	48.6
	Discoloration	7	9.7
	Insects	15	20.8
	Visible water vapour on grains	4	5.6
Do you sort your crops after harvest?	Yes	36	50.0
	No	36	50.0
What do you do with spoilt grains?	Throw them away	25	34.7
	Feeding livestock/poultry	13	18.1
	Making local brew	5	6.9
	Re-dry and consume	13	18.1
	Sell to the market	10	13.9
	Throw and gave them to livestock/poultry	6	8.3
What causes spoilage in maize and groundnuts	Poor drying	36	50.0
	Heaping maize on floor	28	38.9
	Use of expired pesticides	8	11.1
Do you know how to identify well -dried pods/ grain?	Yes	31	43.1
	No	41	56.9
Do you clean the storehouse before storage?	Yes	34	47.2
	No	38	52.8
Aflatoxins and fumonisins cause liver cancer in human being	Yes	24	66.7
	No	48	33.3
Have you heard of the word mycotoxins before?	Yes	2	97.2
	No	70	2.8
Do you know the measures to be taken for controlling fungi and spoilage in store	Yes	26	36.1
	No	46	63.9
Do you know the right time to harvest your crop?	Yes	31	43.1
	No	41	56.9

The results indicated that some farmers are not aware of the effects of feeding diseased grains to their animals. In Malaysia, a recent survey reported low awareness and knowledge among the public on the problems associated with fungal and aflatoxin contamination in the diets (Mohd Redzwan *et al.*, 2012). Awuah *et al.* (2009) showed that up to 90% of surveyed farmers, processors, and consumers were unaware of aflatoxin, while 92% of farmers in the Ejura Sekyeredumase District of Ashanti Region had never heard of aflatoxin (Jolly *et al.*, 2009). Furthermore, the similar observations were also reported by several studies in African countries (Jolly *et al.*, 2009; Ilesanmi and Ilesanmi, 2011). The presence of aflatoxin in the food chain is a serious matter but not knowing its impact to the health is a big problem and should be a public concern.

The responses given by participants in this survey show clearly that most participants do not identify fungal contamination of grains until there are obvious signs of spoilage such as discoloration, insect infestation or rotting. Further, unwholesome grains may be eaten (boiled, roasted), or processed into other products such as local beers and be consumed (Table 44). The overwhelming majority of participants did not know economic effects of aflatoxin and fumonisin let alone its harmful health effects. Given the lack of awareness of the health effects of consuming aflatoxin-contaminated foods, the amounts of contaminated groundnuts and maize consumed are likely to be very high in the study area, therefore further studies should be undertaken. There is a need to increase dissemination of information on the effects of mycotoxin due to its fatal effects especially bearing in mind that at least 97% of the respondents in the surveyed villages indicated that they were not aware of mouldy infection. The respondents who said to be aware thought that their produces was only contaminated when they could see fungal growth and rotting. Agricultural extension workers have a duty to increase awareness on how to identify mouldy infection in the study area.

4.5.9 Association between socio-demographic and socioeconomic variables and knowledge on fungal contamination in food crops

The Ordinal logistic regression analysis showed that there were correlations between age, education level, gender, marital status and household income compared with mouldy infections awareness score (Table 45). The odds ratio of 1.805 indicated that respondents with low level of education below secondary level were 1.805 times more likely to have low level of awareness and knowledge than those who had higher education. Significant different were also observed between income groups and marital status (Table 45). Being single, divorced or widow had significant odds ratio of 3.665 as predictor of awareness and level of knowledge among the respondents. Similarly, the odds ratio of 0.104 indicated that respondents with age < 35 years were 0.104 times more likely to have low level of awareness on mouldy infection than those who had age of 35 years and above.

Table 45: Odds ratios (95% CI) from ordinal logistic regression (OLR) of awareness and knowledge level as a function of sociodemographic and socio-economic variables

Variable ^b	Odds Ratio (95% CI)	p-value
Age	0.104 (0.029 – 0.178)	0.006**
Education	1.805 (0.090 – 3.521)	0.039*
Marital Status	3.665 (0.791 – 6.539)	0.012*
Gender	1.869 (0.112 – 3.626)	0.037*
Income	0.936 (0.023 – 1.848)	0.044*
Chi-square (χ^2)=61.068, Sig.< 0.0001		
Pseudo R-Square ^c		
Cox and Snell	0.572	
Nagerkelke	0.767	
McFadden	0.620	

*Significant different at $p < 0.05$ and ** Significant different at $p < 0.01$

^b Variables are coded either 1 or 0. Age: less than 35 years = 0, 35 years and above = 1; Gender: Male = 0, Female = 1; Education status: Less than high school = 0, High school and above = 1; Marital status: Single, divorced or widow = 0, Married = 1; Household annual income: Below USD 66.7 (below 100 000 Tshs) = 0, Above USD 66.7 (above 100 000 Tshs) = 1.

^c Pseudo R-Square measures the strength of associations between variables.

Results presented in Table 46 also shows similar findings. The mean score of moldy awareness of females (1.7273±0.45055) is significantly higher than that of males

(1.5714± 13.7). Similarly, the mean score of more educated people (1.9231.3 ±0.31470) is significantly higher than that of less educated people (1.8913 ± 0.27175). Significant different were also observed between income groups and marital status. Table 46 shows the Independent t-test on sociodemographic and socio-economic characteristics on knowledge of moldy infections in stored maize and groundnuts.

Table 46: Independent t-test on sociodemographic and socio-economic characteristics on knowledge of mouldy infections in stored maize and groundnuts

Variable	n	Mean ± SD	T	p-value
Gender			1.366	0.024*
Female	28	1.7273 ± 0.4506		
Male	44	1.5714 ± 0.5040		
Education level			-6.295	0.000***
> high school	26	1.9231 ±0.3147		
< high school	46	1.8913 ±0.2718		
Age			1.993	0.050*
< 35 years	23	1.9565 ± 0.2085		
> 35 years	49	1.9796 ± 1.9796		
Marital Status			-3.949	0.001**
Single,divorced, or widow	25	2.5600 ±1.8726		
Married	47	2.9787 ±1.6874		
Household income			2.802	0.008*
< 66.7 USD	51	1.6275 ±0.48829		
> 66.7 USD	21	1.2857 ± 0.46291		

* Significant different at $p < 0.05$, ** Significant different at $p < 0.01$, *** Significant different at $p < 0.0001$

Several important findings were obtained with regard to the effects of socio-demographics and socio economic variables on awareness and knowledge of mouldy infection in food crops. The level of education, age, gender, marital status and income level of the farmers had significant ($p < 0.05$) positive impact on awareness and knowledge of mouldy infection in food crops. Dosman *et al.* (2001) found that people with higher levels of education are likely to be better informed, and therefore, may be more aware of some types of risk of food additives in food than people with less education. Similarly, Baker (2003) found that those with the highest levels of education were more willing to pay for food safety. This is clearly a significant finding because the higher an individual's education the more likely he/she is to develop an interest in searching for information on

a particular problem. They also have greater access to information sources on food safety. Therefore, they are more likely to hear about mycotoxins, and to be cognizant of its consequences on humans and animals in particular.

The significant difference between men and women with respect to the awareness of mouldy infection in food crops and how to manage mycotoxin in spoilt food were noted. Male seemed not much engaged in the postharvest operations and the whole burden was left to female. In Tanzania, women in rural areas are responsible for 87% of the labour used for growing food consumed by households (Ogunlela and Mukhtar, 2009). In this regard, female respondents showed greater awareness on mouldy infection because they were responsible to care of the produce after harvests. Lin (1995) found that those most concerned with food safety tended to be women, and full-time homemakers. Baker (2003) also indicted that women had the strongest reaction to low-visibility food safety risk.

Persons with age >35 years who had been farming maize and groundnuts in the past 20 years are more likely to perceive that mouldy infections is a problem than people with age < 35 years ($p=0.006$). Older individuals are more likely to rate and perceive risks higher than young individuals (Krewski *et al.*, 1994). Several explanations for this finding have been posited by Dosman *et al.* (2001).

Another notable finding is the significant determinant of personal income status with the knowledge level of mouldy infection in stored crops. Respondents with higher income were knowledgeable compared to lower income respondents. Inevitably money appears to matter because it is a marker of something else. In the case of the effects of aflatoxins on human health, those who had better income are more likely to have more access to

knowledge compared to those with low income status. Furthermore, fearing the lack of information and knowledge about the adverse effects of aflatoxin allows the people to try to access knowledge about food safety from the available experts, which can be expensive and costly. To some extent, the option might be unbearable for individuals with low income status. Gender analysis also showed that being single, divorced or widow may contribute to low level of knowledge among the respondents. Once an individual is in a relationship or married, there is a possibility that knowledge of some particular mouldy spoilage issues is exchanged between partners, in which lead to the increasing level of awareness.

Results of the study have strong implications for strategies aimed at managing mycotoxin contamination of maize and groundnuts in Kilosa and Tanzania as a whole. As Strosnider *et al.* (2006) have indicated education and awareness are key factors in mitigating the problems of mycotoxins in developing economies. Similarly, the above results have important implications for food safety education programmes and government policies. Educating consumers about preventive methods to reduce food safety threats will lead to reduced concerns and changes in food consumption habits. Resource allocation toward the provision of safe foods should therefore include line items for the creation and maintenance of awareness campaigns to constantly inform the public of the risks associated to consuming fumonisin and aflatoxin-contaminated maize and other grains, and since mycotoxins is ubiquitous, steps should be undertaken to keep the levels below the minimum acceptable in the more developed economies.

The information generated in this study is vital to both developing and developed countries as they can formulate strategies to combat the risks associated with ingestion of

aflatoxin-contaminated foods. Policy makers must determine whether they allocate funds for the prevention of mycotoxin contamination or to the cure of individuals who have been chronically ill from the long term ingestion of aflatoxin infested foods. Although the results indicated that higher education level, age and gender were positively related to awareness and knowledge on mouldy infection, it is important to make all categories of individuals aware of the problems. The data presented so far informs us of the need for urgent intervention strategies and public awareness campaigns focused at citizens from a low socio-economic background considering the low average level of education. A collaborative, informed approach between policy makers, governments and individuals is needed in order to develop a preventive strategy for mycotoxins contamination in food crops. The literature clearly states that food safety should be a collaborative approach between the government, food industry, and the consumers (Knabel, 1995).

4.5.10 The relationship between maize loss and maize variety in the surveyed villages in Kilosa District

Both the farm households that grew new varieties of maize and the farm households who grew local varieties of maize almost equally experienced maize loss due to moulds, rodents, and insect pests. A Chi-Square test $\chi^2_{0.05(3)} = 5.689$; $p = 0.128$ confirmed that there was no significant association between specific maize variety and the farm households' losing maize due to moulds, insect pests, or rodents as shown in Table 47.

Table 47: Chi-Square tests showing the relationship between maize loss and maize variety

Measures of association/relationship	Value	DF	Asymp.Sig. (2-sided)
Pearson Chi-Square	5.689	3	0.128
Likelihood Ratio	6.899	3	0.075
Linear-by-Linear Association	3.473	1	0.062

Pearson Chi-Square = 5.689, $p = 0.128$; Likelihood Ratio = 6.899, $p = 0.075$

The results similar to these are reported in other studies done elsewhere. Hennigen *et al.* (2000) compared contamination of maize varieties on flint endosperm to that of dent type and did not find significant differences. Shelby *et al.* (1994) tested fifteen maize hybrids and found no significant correlation between starch, lipid, fibre and protein contents and fumonisins production in maize. Thus, the plea has to be made for a careful consideration of the suitability of new crop varieties before they are forced onto the farmer. With increasing pressure on farmers to adopt new varieties, their problems are likely to increase rather than decrease. Therefore, great care must be taken to ensure that the farmer is not put at a disadvantage by encouraging them to use improved varieties which are more resistant to mould attacks.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

According to WHO/FDA standards, the majority of the samples in the study villages are not safe for consumption because they exceed a 20 ppb (20 μ g/kg). The high contamination of maize and groundnuts by mycotoxins represents a serious health problem for humans and animals as well as a considerable economic obstacle in Kilosa District. This evidence creates a need for more sensitization of key stakeholders about the mycotoxin problem in the study area.

This study showed that the use of neem tree leaves as a grain storage protectant in maize has significantly less toxin production in maize as compared to other grain protectants (actellic super dust, wood ash and untreated control). Therefore, use of neem tree leaves in grain is a promising treatment to reduce fumonisins contamination in maize, but further studies are required, including determining its active ingredients to be used as a storage protectant in other food crops.

Temperature, relative humidity and rainfall fluctuations cause significant proliferations of fungi which play a role in reducing both quality and quantity of the products and significantly diminishing market value. For proper storage of maize and groundnuts, weather factors such as temperature, relative humidity and rainfall must be controlled. These factors are the major influences of maize and groundnuts deterioration, because they affect moulds, insects, and other pest, which can result in huge losses of maize grain and groundnuts in a very short time.

One-fifth of maize produced for human consumption was lost through fungal spoilage equivalent to 4331 tons/year. This connotes a waste of productive resources as well as a significant reduction in expected income and consequently welfare of the farmers.

Farmers' awareness on mouldy infections in stored maize and groundnuts was very low and farmers were not informed on the implications of using fungal infected maize and groundnuts for food and feed, and this rendered both farm households and livestock vulnerable.

Socio-economic and demographic factors such as age, gender, education, marital status, and household income have been shown to influence farmers' awareness on mouldy infections in stored maize and groundnuts. Factors which increased mycotoxin development in maize and groundnuts were: shelling of the stored produces by using machinery, insect damage, storing maize and groundnuts in the same room from year to year, heaping of maize on the floor in a house, and storing the produce in shelled form.

5.2 Recommendations

- i. Human beings should learn to consume good quality maize grains and groundnuts as well as feeding their livestock and poultry with uncontaminated feeds. Similarly, hazard analysis critical control point (HACCP) should be employed in the agricultural production channel to minimize mycotoxin contamination in foods.

- ii. Heaping maize grains/cobs on floor in a house or storage house must be avoided. This practice promotes mould and insect proliferation especially when the grains are inadequately dry due to heat build-up. For bagged commodities, ensure that

bags are clean, dry and stacked on pallets or incorporate a water impermeable layer between the bags and the floor.

- iii. Proper monitoring of temperature and relative humidity of maize grain and groundnut and surrounding atmosphere on storage especially in the initial stage of storage to maintain the highest possible quality of stored grain; in general, the lower the temperature and moisture content the longer it can be stored without being infected by mould and insects.
- iv. In the context of food security and safety, the study recommends that, waste can be converted into products for further use (for example compost manure) or sources of energy (for example biogas in anaerobic digestion, bioethanol in refineries, or burned directly in power plants). Furthermore, to avert channeling of spoilt maize and groundnuts toward food uses, alternative uses of low grade maize and groundnuts should be explored.
- v. There is a clear need for awareness-raising for all stakeholders, as well as comprehensive capacity building along agricultural commodity value chains, including for consumers, health professionals and policy makers. The fact that, aflatoxins and fumonisins contamination is invisible adds to the challenge, as even foods that do not appear mouldy to the naked eye may still contain aflatoxins and fumonisins.
- vi. Awareness of what mycotoxins are and the dangers that they pose to human and animal health could be done through government bodies, private organizations, non- governmental organizations, national media networks such as radios and

television programs as well as features in newspapers and magazines. Awareness should target best agricultural practices aimed at the reduction of fungal infestation of groundnuts and maize seeds in the field and store. There is the need to train farmers through awareness campaign on mycotoxin, analyse the health impact and analyse economic impact.

- vii. The role of other socio-economic factors needs to be explored further in order to obtain a better understanding of the determinants of perceptions of aflatoxins and fumonisins in maize and groundnuts in Tanzania and developing economies. Policies designed to reduce aflatoxins in stored groundnuts and staples must stress the overall economic and health net benefits derivable from a better quality maize and groundnut.
- viii. Control of moulds through sorting out damaged cobs, early harvesting, use of improved cribs (vihenge/vidonga), control of moisture in the storage room and leaving to dry the harvested products for more than 3 weeks (if drying facilities are working at full capacity) must be encouraged.
- ix. Above all, it is highly recommended that the Tanzanian Government should encourage agricultural engineers to design driers such as solar driers that can be used for rapid drying of maize and other food crops prior to storage by village households in humid and warm places such as in Mamoyo and Rudewa-Batini villages.

- x. Epidemiological studies to determine the incidence of human esophageal cancer in Kilosa District and the possible link between this disease and fumonisins intake are needed. However, it should be emphasized that continuous study, with a greater number and variety of food commodities from different locations, should be performed to evaluate the natural occurrence of mycotoxins countrywide. Also, the study recommends conducting multi sectorial behavioural change campaigns for food safety against aflatoxins, especially among mothers, pregnant and lactating women, caregivers of infants, and immune compromised individuals.

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APPENDICES

Appendix 1: Geographical position (GPS) coordinates recorded for 72 households in four villages in Kilosa District in 2010-2011

Latitude (South)	Longitude (East)	Altitude (m)	Village Name
S06 ⁰ .83.447'	E037 ⁰ .04.475'	506.4	Mamoyo
S06 ⁰ .83.325'	E037 ⁰ .04.588'	485.4	Mamoyo
S06 ⁰ .83.249'	E037 ⁰ .04.807'	468.9	Mamoyo
S06 ⁰ .83.206'	E037 ⁰ .04.826'	478.0	Mamoyo
S06 ⁰ .83.354'	E037 ⁰ .04.165'	471.3	Mamoyo
S06 ⁰ .83.296'	E037 ⁰ .04.700'	476.5	Mamoyo
S06 ⁰ .83.222'	E037 ⁰ .04.816'	471.0	Mamoyo
S06 ⁰ .83.298'	E037 ⁰ .04.546'	485.0	Mamoyo
S06 ⁰ .83.174'	E037 ⁰ .05.186'	459.7	Mamoyo
S06 ⁰ .82.865'	E037 ⁰ .05.284'	466.7	Mamoyo
S06 ⁰ .83.487'	E037 ⁰ .03495'	469.4	Mamoyo
S06 ⁰ .83.613'	E037 ⁰ .03.253°	478.8	Mamoyo
S06 ⁰ .83.515'	E037 ⁰ .03.559'	477.8	Mamoyo
S06 ⁰ .83.474'	E037 ⁰ .03.416'	495.3	Mamoyo
S06 ⁰ .83.468'	E037 ⁰ .03.466'	471.6	Mamoyo
S06 ⁰ .83.030'	E037 ⁰ .02.565'	523.0	Mamoyo
S06 ⁰ .83.001'	E037 ⁰ .02.422'	477.6	Mamoyo
S06 ⁰ .83.053'	E037 ⁰ .02.560'	463.0	Mamoyo
S06 ⁰ .69.351'	E037 ⁰ .12.182'	428.1	Rudewa- Batini
S06 ⁰ .69.654'	E037 ⁰ .12.259'	449.0	Rudewa- Batini
S06 ⁰ .69.629'	E037 ⁰ .12.133'	470.6	Rudewa- Batini
S06 ⁰ .69.830'	E037 ⁰ .12.412'	475.9	Rudewa- Batini
S06 ⁰ .69.547'	E037 ⁰ .12.149'	442.4	Rudewa- Batini
S06 ⁰ .69.750'	E037 ⁰ .12.181'	454.5	Rudewa- Batini
S06 ⁰ .69.099'	E037 ⁰ .11.963'	443.9	Rudewa- Batini
S06 ⁰ .69.478'	E037 ⁰ .12.024'	460.5	Rudewa- Batini
S06 ⁰ .69.784'	E037 ⁰ .12.207'	446.2	Rudewa- Batini
S06 ⁰ .69.654'	E037 ⁰ .12.116'	451.0	Rudewa- Batini
S06 ⁰ .69.336'	E037 ⁰ .12.186'	426.9	Rudewa- Batini
S06 ⁰ .69.548'	E037 ⁰ .10.739'	439.6	Rudewa- Batini
S06 ⁰ .69.552'	E037 ⁰ .10.925'	460.1	Rudewa- Batini
S06 ⁰ .69.411'	E037 ⁰ .12.190'	462.1	Rudewa- Batini
S06 ⁰ .69.587'	E037 ⁰ .11.002'	439.8	Rudewa- Batini
S06 ⁰ .69.796'	E037 ⁰ .11.021'	427.1	Rudewa- Batini
S06 ⁰ .69.680'	E037 ⁰ .10.995'	463.5	Rudewa- Batini
S06 ⁰ .69.186'	E037 ⁰ .11.055'	474.3	Rudewa- Batini
S06 ⁰ .21.118'	E036 ⁰ .86.910'	1339	Msingisi
S06 ⁰ .20.229'	E036 ⁰ .86.934'	1343	Msingisi
S06 ⁰ .21.064'	E036 ⁰ .86.698'	1336	Msingisi
S06 ⁰ .21.010'	E036 ⁰ .86.809'	1347	Msingisi

Latitude (South)	Longitude (East)	Altitude (m)	Village Name
S06 ⁰ .21.324°	E036 ⁰ .86.646'	1357	Msingisi
S06 ⁰ .21.169°	E036 ⁰ .86.680'	1343	Msingisi
S06 ⁰ .20.534°	E036 ⁰ .86.781'	1340	Msingisi
S06 ⁰ .21.417°	E036 ⁰ .86.672'	1339	Msingisi
S06 ⁰ .21.189°	E036 ⁰ .86.644'	1339	Msingisi
S06.21.157°	E036 ⁰ .86.742'	1348	Msingisi
S06 ⁰ .20.490°	E036 ⁰ .86.914'	1344	Msingisi
S06.20.493'	E036 ⁰ .86.864'	1351	Msingisi
S06 ⁰ .20.494'	E036 ⁰ .86.868'	1348	Msingisi
S06 ⁰ .20.386'	E036 ⁰ .86.686'	1341	Msingisi
S06 ⁰ .20.395'	E036 ⁰ .86.752'	1349	Msingisi
S06 ⁰ .20.430'	E036 ⁰ .86.816'	1349	Msingisi
S06 ⁰ .20.407'	E036 ⁰ .86.824'	1339	Msingisi
S06 ⁰ .20.470'	E036 ⁰ .86.753'	1340	Msingisi
S06 ⁰ .08.692'	E036 ⁰ .85.160'	1270	Mkalama
S06 ⁰ .08.728'	E036 ⁰ .85.159'	1263	Mkalama
S06 ⁰ .08.778'	E036 ⁰ .85.233'	1258	Mkalama
S06 ⁰ .08.796'	E036 ⁰ .85.237'	1260	Mkalama
S06 ⁰ .08.596'	E036 ⁰ .85.138'	1283	Mkalama
S06 ⁰ .08.840'	E036 ⁰ .85.285'	1266	Mkalama
S06 ⁰ .08.701'	E036 ⁰ .85.104'	1260	Mkalama
S06 ⁰ .08.569'	E036 ⁰ .85.166'	1270	Mkalama
S06 ⁰ .08.529'	E036 ⁰ .85.105'	1260	Mkalama
S06 ⁰ .08.016'	E036 ⁰ .84.880'	1263	Mkalama
S06 ⁰ .07.974'	E036 ⁰ .84.925'	1273	Mkalama
S06 ⁰ .07.882'	E036 ⁰ .84.787'	1292	Mkalama
S06 ⁰ .08.337'	E036 ⁰ .85.218'	1254	Mkalama
S06 ⁰ .08.409'	E036 ⁰ .85.320'	1267	Mkalama
S06 ⁰ .08.364'	E036 ⁰ .85.300'	1260	Mkalama
S06.08.326'	E036 ⁰ .85.522'	1268	Mkalama
S06 ⁰ .08.348'	E036 ⁰ .85.658'	1274	Mkalama
S06 ⁰ .08.358'	E036 ⁰ .85.688'	1264	Mkalama

Appendix 2: Randomized complete block design (RCBD)

1	2	4	3	2	4	3	1	3	4	2	1	4	2	3	1
Rep 1				Rep 2				Rep 3				Rep 4			

Appendix 3: Layout of the experiment in split-plot design

BL ₄	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="border: 1px solid black; padding: 2px;">T₂</td> <td style="border: 1px solid black; padding: 2px;">T₃</td> <td style="border: 1px solid black; padding: 2px;">T₄</td> <td style="border: 1px solid black; padding: 2px;">T₁</td> </tr> </table> <p>3 months</p>	T ₂	T ₃	T ₄	T ₁	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="border: 1px solid black; padding: 2px;">T₂</td> <td style="border: 1px solid black; padding: 2px;">T₁</td> <td style="border: 1px solid black; padding: 2px;">T₃</td> <td style="border: 1px solid black; padding: 2px;">T₄</td> </tr> </table> <p>9 months</p>	T ₂	T ₁	T ₃	T ₄	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="border: 1px solid black; padding: 2px;">T₃</td> <td style="border: 1px solid black; padding: 2px;">T₁</td> <td style="border: 1px solid black; padding: 2px;">T₂</td> <td style="border: 1px solid black; padding: 2px;">T₄</td> </tr> </table> <p>6 months</p>	T ₃	T ₁	T ₂	T ₄
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T ₃	T ₄	T ₁	T ₂												
T ₂	T ₁	T ₃	T ₄												
BL ₁	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="border: 1px solid black; padding: 2px;">T₁</td> <td style="border: 1px solid black; padding: 2px;">T₂</td> <td style="border: 1px solid black; padding: 2px;">T₃</td> <td style="border: 1px solid black; padding: 2px;">T₄</td> </tr> </table> <p>3 months</p>	T ₁	T ₂	T ₃	T ₄	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="border: 1px solid black; padding: 2px;">T₄</td> <td style="border: 1px solid black; padding: 2px;">T₃</td> <td style="border: 1px solid black; padding: 2px;">T₁</td> <td style="border: 1px solid black; padding: 2px;">T₂</td> </tr> </table> <p>6 months</p>	T ₄	T ₃	T ₁	T ₂	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="border: 1px solid black; padding: 2px;">T₁</td> <td style="border: 1px solid black; padding: 2px;">T₂</td> <td style="border: 1px solid black; padding: 2px;">T₄</td> <td style="border: 1px solid black; padding: 2px;">T₃</td> </tr> </table> <p>9 months</p>	T ₁	T ₂	T ₄	T ₃
T ₁	T ₂	T ₃	T ₄												
T ₄	T ₃	T ₁	T ₂												
T ₁	T ₂	T ₄	T ₃												

T₁, T₂, T₃ and T₄=Grain treatments representing Actellic Super Dust (2 gm/2 kg), Kitchen Wood Ash (30 gm/2 kg), Dried Neem Tree Leaves (80 gm/2 kg) and Control (No treatment) respectively where; BL₁=Block 1, BL₂=Block 2, BL₃=Block 3 and BL₄= Block 4.

Appendix 4: Questionnaire for household survey

I. General background of respondents

District..... Village... Agroecological zone: ...GPS Ref..... Date of interview...
Household name.... (Contact No. if agreed)..... Interviewer's name.....

1. Age.....
2. Sex: Male..... Female.....
3. Marital status: Single...Married...Widow...Widower...Divorced...Separated.....
4. Number of children.....
5. Educational background: No formal education..... Primary..... Secondary.....
College.....University.....Other(s).....
6. Primary occupation.....
7. Secondary occupation.....

II Background in maize and groundnuts farming

8. What is your main and financially most important occupation? 1..... 2..... 3.....
9. What is your second most important occupation (financially).....
10. How many years have you been a maize farmer?
11. Do you grow maize during the rainy season? 1.No 2.Yes
12. Do you grow maize during the dry season? 1.No 2.Yes
13. What type of maize seed do you use in your farm? 1.Improved 2.Local 3.Both 1 and 2
14. Where do you get the seed? 1. Own seed 2. Bought 3.Gift
15. If bought, from whom did you get the seed? 1. Private dealer 2.Government agency
(specify).....
16. Give the variety name for maize you usually use in your farm 1....2.....3.....
17. Since which year have you grown this variety?
18. Do you treat the seed with insecticide before planting? 1.No 2.Yes
19. If Yes, give the name of the product used(show bag of product used)
20. Do you ever grow maize in the same field year after year? 1.No 2.Yes
21. If yes, how many years is it to date?.....
22. Sources of pesticides 1.Private dealers 2.Village shops 3.Agriculture extension officer
4. Government agent 5. Others (specify).....
23. How many years have you been a groundnuts farmer?.....
24. Do you grow groundnuts during the rainy season? 1.No 2.Yes
25. Do you grow groundnuts during the dry season? 1.No 2.Yes
26. What type of groundnuts seed do you use in your farm?
1. Improved 2. Local 3. Both 1 and 2
27. Where do you get the seed? 1. Own seed 2. Bought 3. Gift
28. If bought, from whom did you get the seed? 1. Private dealer 2.Government agency
29. Give the variety name for groundnuts you usually use in your farm
1.....2.....3.....
30. Since which year have you grown this variety?
- 31a. Do you treat the seed with insecticide before planting? 1. No 2.Yes
- 31b. If yes, give the name of the product used (show bag of product used).....
32. Do you ever grow maize in the same field year after year? 1. No 2.Yes
33. If yes, how many years is it to date?

34. Give/estimate the production trends of maize and groundnuts in the last 5 years

Year	Crop Maize	Mt/Ha	Grounsnuts	Mt/Ha
2009/2010				
2008/2009				
2007/2008				
2006/2007				
2005/2006				

35. Which of the following do you consider as the causes for low yields in maize and groundnuts? 1. Drought 2.Floods 3.Lack of inputs (seeds, fertilizer) 4.Poor soils 5.Insect/pest 6.Molds/fungi 7. Income 8.Others (specify).....
- 36 Of those ticked above, what are the two main causes? 1.....2.....
- 37 Which of the following pests attack maize and groundnuts in the field?
(Insects, mice/rats, birds moulds, others....)
38. Indicate the remedial action employed against the field pest attacks
1. Insects.....2. Mice/rats.....3. Birds.....4 Moulds.....5. Others.....

III. Harvesting Practices

39. How do you know that maize is ready for harvesting?
1.Cobs and husks are completely dry 2. Cobs fall down 3.Silk falls down 4. Duration from planting 5.Grain can't be scratched with the fingernail 6.Others (specify).....
40. Are you able to harvest as soon as the crop is mature? 1. No 2. Yes
41. If No, why? 1. No labour available 2. Other activities in this period 3. Others.....
42. How do you harvest maize? 1. Cut the whole stalk 2. Collect the ears 3. Bend the stalk before harvest to let it dry then harvest 4. Others
43. Why do you particularly use the harvest procedure indicated in Q.42 above?
44. Do you leave the cobs on the plant to dry before harvesting? 1. No 2. Yes
45. What is the best time for harvesting maize? 1. March 2. April 3. May 4. June 5. July
46. How do you harvest groundnuts? Mention 1.....2.....3.....
47. What is the best time for harvesting groundnuts? 1. February 2. March 3. April 4.May

IV. Drying Practices

48. How do you dry your grain after harvesting?
1. Sun drying 2.Smoking3.Others.....
49. In what form do you dry your grain after harvesting?
1. Ear with shealth 2.Ear only 3. Shelled
50. If shelled maize, how did you shell it? 1. Hand shelling 2.Beating cobs on threshing floor 3.Machinery shelling
51. Which method do you use in drying your grains/groundnuts after harvesting?
1. Bareground 2.Mats 3.Over roof 4. By using sieves 5. Others (specify)
52. In what form did you store your maize? 1. Shelled (grains) 2.Unshelled (cobs) 3.With husks 4.Others (specify).....
- 53a. In what form do you dry groundnuts after harvesting? 1. Plants with pods 2.Nuts in pods 3. Others (specify).....
- 53b. If nuts in pods how did you shell it? 1. Hand shelling 2.Beating nuts on threshing floor 3. Machinery shelling
54. In what form did you store your groundnuts? 1. Shelled 2.Unshelled

V. Storage Practices

55. After harvesting, do you put maize directly into storage or you keep it elsewhere before? 1. Directly into storage 2. Kept elsewhere for a while
56. After harvesting do you sort out damaged cobs? 1. No 2. Yes
57. If yes, on what basis did you sort the "good maize" and "bad maize"?
58. If no, why? 1. Time is not enough 2. Tedious work 3. No money to hire for the work
59. Where do you sort the maize after harvesting?
 1. On farm 2. At the village 3. At your residence 4. At the site of the storage facility
60. Indicate the criteria for sorting and the reason 1. Colour 2. Cob size 3. Grain size 4. Damaged ears 5. Cobs protruding out of husk 6. Others (specify).....
61. After sorting, what do you do with the damaged cobs?
 1. Throw them away 2. Feed them to animals (which animal?) 3. Domestic consumption 4. Sell them at low price 5. Other (specify).....
62. Indicate the types of damage you usually sort out insect/rodents/birds/mould (discolouration) /Others
63. Do you use storage pesticides in your produce? 1. No 2. Yes 3. Only in maize 4. Only in groundnuts
64. (If yes) which one did you use last year? 1.....2.....3.....
65. Where did you obtain the chemicals?
 1. From private dealers 2. From village shops 3. Agriculture extension officer 4. Government agency (specify) 5. Others (specify).....
66. How effective was the treatment? 1. Not effective 2. Slightly effective 3. Very effective
67. What storage containers do you use when storing maize and groundnuts?
 1. Polythene bags (viroba) 2. Traditional cribs (kihenge) 3. Plastic tins 4. Drums 5. Heaping on the floor in a house 6. Sisal/jute bags (magunia) 7. Others (specify)...
68. Where is the storage facility located?
69. Over the past 5 years have you been storing maize in the facility every year? 1. No 2. Yes
70. If no, give reason(s).....
71. Do you use the facility to store any other foodstuffs? 1. No 2. Yes
72. If yes, what other food stuffs?
73. The maize is stored in bags 1. On a raised platforms 2. On the floor 3. Others (specify).....
74. Do you store other other products in the store room together with maize? 1. No 2. Yes
75. What is the size of the store room?.....(Take measure)
76. When is harvested maize stored? 1. Direct after harvest 2. Late after harvest
- 77a. Do you pre-store after harvesting? 1. No 2. Yes
- 77b. If yes, why do you pre-store?
78. Where do you pre-store? 1. Field 2. In the house
79. For how many days do you pre-store? 1. One week 2. Two weeks 3. Three weeks 4. One month
80. In what state do you store your maize? 1. Husked 2. Dehusked 3. Shelled
81. Do you use chemicals to treat your maize in storage? 1. No 2. Yes
82. If yes, what chemicals do you use? 1.....2.....3.....
83. How many months after storage you applied chemicals? 1. One month 2. Two months 3. Three months
84. By what means or method do you apply the chemical? 1.....2.....
85. Are there any other things you do during storage to ensure your maize keeps well?.....

86. How often do you inspect your produce in the store? 1. Never 2. Daily 3. Weekly 4. Monthly
87. Do you clean the storehouse before storage? 1. No 2. Yes
88. Estimate the cost (Tshs) you used in treating your stored maize and groundnuts (maize)Tshs and (groundnuts).....Tshs.

VI. Storage problems

89. Do you have storage problems in maize and groundnuts?
1. No 2. Yes 3. Only in maize 4. Only in groundnuts
90. Which storage problems are the most important in maize and groundnuts?
1. Insects/ pests 2. Rodents 3. Fungi/mould 3. Leaking roof during rain season
4. Others (specify).....
91. When did you observe this problem?
1. At the beginning of storage 2. At the end of storage 3. Soon after storage
92. What did you do to solve this problem? 1.....2.....3.....
93. How are insects found in maize and groundnuts? 1. Often 2. Sometimes
94. How are insects controlled? 1. Pesticides 2. Others.....
95. How are rodents found in maize and groundnuts storage area? 1. Often 2. Sometimes
96. How are rodents controlled? 1. Traps 2. Poison 3. Nothing

VII. Storage condition (Tick if present)

The store room

97. Walls 1. Cracks 2. Cobwebs 3. Mildew 4. Dampness
98. Roof (choose more than one)
1. Leaking 2. Not leaking 3. Rat proof 4. Not rat proof 5. Thatched with grass
6. Made of iron corrugated sheets with ceiling boards 7. Made of iron corrugated sheets without ceiling boards
99. Floors 1. Swept 2. Spilled water 3. Cracks
100. How often is cleaning done in storage area? 1. Daily 2. Weekly 3. Monthly 4. Never
101. Which method(s) is used in cleaning the store?
1. Fumigation before storage 2. Removed previous crop remains 3. Swept over to remove dust/dirtiness

VIII: Farmers awareness on mould infection in stored maize and groundnuts

102. State whether you are aware of the following or not aware

Statements	Farmers awareness		
	KN	NS	DK
1. Can you identify spoilt maize/groundnuts?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
2. Do you sort your crops after harvest?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
3. Do you know how to identify well-dried pods/ grain?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
4. Do you clean the storehouse before storage?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
5. Aflatoxins and fumonisins cause liver cancer in human being	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
6. Have you heard of the word mycotoxins before?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
7. Do you know the right time to harvest your crops?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
8. Do you know the measures for controlling fungal spoilage in the store?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
9. Insect-attacked groundnuts promote aflatoxins	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
10. What do you do with the damaged cobs?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
11. Shriveled groundnuts usually results in high levels of proliferation	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
12. Fungal spoilage in stored maize and groundnuts is a problem	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

KN=Know NS=Not Sure DK=Don't Know

103. Did any member of this household receive agricultural training or had contact with extension agents concerning mouldy contaminations in stored maize and groundnuts? 1. No 2. Yes.

104. If yes, fill the table provided

Name of person attended	Provider of Training/Extension	In which year?	Course contents
1.	1.FFS		1.Seed selection
2.	2.ARI		2.Post-harvest management
3.	3.MOAFS		3.Mycotoxins management
4.	4.NGOs		4.Others(specify)
5.	5.Fellow farmers		
6.	6.Others (specify)		

IX: Food spoilage and disposal patterns and the linkage to household food safety

105. Did the maize and groundnuts you stored show any sign of mold growth?

1. No 2. Yes 3. Only in maize 4. Only in groundnuts

106 If yes, how do you describe your infected grains?

1. Rotting/decaying 2. Visible water vapour on them 3. Off odors

4. Discoloration 5. Crusting 6. Sprouting grain 7. Condensation 8. Fecal matter (rodent and insect)

107. At what time of the year did most of this damage occur?....(probe for months after storage)

108. Indicate the spoilage loss (kg) incurred and its amount related to:

1. Mould (discoloration)2. Rodent infestation.....3. Insect infestation.....

X. Maize and groundnuts disposal patterns

109. Explain what you did with your maize and groundnuts soon after spoilage

1. Consumption after processing 2. Gave to livestock 3. Throw away 4. Brewing 5.Others (specify).....

110. If contaminated maize and groundnuts were taken for sale, explain (i) to whom sold?

1. Individual 2. Market 3. Retailers 4. Wholesalers 5.Others (specify)...

111a. When did you sell the affected/contaminated maize and groundnuts...(probe for months)

111b. Why did you sell the maize at that time? 1. Food deficit in the village 2. Disposal for preparing store for next crop 3. Procurement 4. To avoid loss 5. Others (specify).....

111c. Did you purchase any maize last season for consumption or seed as a result of food spoilage in the store? 1. No 2. Yes

112. If yes, mention (i) What quantity was bought... (ii) From whom did you buy?.....(iii) What price did you pay?.....

XI. Linkage between moldy spoilage in maize and groundnuts and household food safety

(Please let's now talk about linkages between mould spoilage and household's food security)

113. How much is each of the following problems to your household as a result of food spoilage due to fungi/muold contamination in maize and groundnuts?

Problems	Magnitude of problem		
	Not a problem	Somewhat a problem	A serious problem
1.			
2.			
3.			

Code: 1. Poor access to food security 2. Reduced income 3. Poor social network participation 4. Lower prices for inferior quality 5. Lower selling prices for contaminated food

114. Propose livelihood strategies related to mould/fungal infection problem in maize and groundnuts in this village for improving food safety.

1.....2.....3.....4.....

THANK YOU FOR YOUR COOPERATION

Appendix 5: Interpreting log-transformed parameter estimates in regression model

(1) If dependent variable is log- transformed

A linear regression model with a log-transformed dependent variable and two predictor variables can be expressed with the following equation.

$$\ln(Y) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \dots\dots\dots(i)$$

$$\text{Suppose, } \ln(Y_1) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 \dots\dots\dots(ii)$$

Now let's keep the same Y_1 value and increase X_1 by 1 unit so we will get a new value Y_2

$$\ln(Y_2) = \beta_0 + \beta_1(X_1 + 1) + \beta_2 X_2 \dots\dots\dots (iii)$$

We have a system of two equations; we can subtract the first from the second, and obtain $\ln(Y_2) - \ln(Y_1) = \beta_1$. Using the well known property about the difference of logs, we then write, $\ln(Y_2/Y_1) = \beta_1$ and by applying log both sides of the equation we get,

$$Y_2/Y_1 = e^{\beta_1} \text{ and } Y_2 = e^{\beta_1} \cdot Y_1 \text{ or } e^{\beta_1} = 1 + (Y_2 - Y_1)/Y_1 \text{ so that } Y \text{ has changed by } (e^{\beta_1} - 1) * 100.$$

The interpretation is that, the dependent variable changes by $100(e^{\beta_1} - 1)$ equivalent to $100\beta_1$ percent for a one unit increase in X_1 while holding all other predictor constant. Here the simple approximation works only when β_1 is small eg less than 0.1 (here all of the approximation can only happen using the natural log transformations).

(2) Independent variable is log transformed

A linear regression model with one log-transformed predictor variable can be expressed with the following equation

$$Y = \beta_0 + \beta_1 \ln(X_1) + \beta_2 X_2 \dots\dots\dots (iv)$$

$$\text{Suppose, } Y_1 = \beta_0 + \beta_1 \ln(X_1) + \beta_2 X_2 \dots\dots\dots (v)$$

$$Y_2 = \beta_0 + \beta_1 \ln[(X_1 * (1 + 1\%))] + \beta_2 X_2 \dots\dots\dots (vi)$$

$$Y_2 - Y_1 = \beta_1 \ln(1 + 1\%) = \beta_1 \ln(101/100) \text{ equivalent to } \beta_1/100.$$

The interpretation is that, one percentage in the independent variable is associated with $\beta_1 \ln(1.01) = \beta_1/100$ change in the dependent variable while all other variables in the model are held constant.

(3) Both dependent and independent variables are log transformed

When both dependent and independent variables are log transformed, the model can be expressed with the following equation.

$$\ln(Y) = \beta_0 + \beta_1 \ln(X_1) + \beta_2 X_2 \dots\dots\dots (vii)$$

$$\text{Suppose, } \ln(Y_1) = \beta_0 + \beta_1 \ln(X_1) + \beta_2 X_2 \dots\dots\dots (viii)$$

$$\ln(Y_2) = \beta_0 + \beta_1 \ln[(X_1 * (1 + 1\%))] + \beta_2 X_2 \dots\dots\dots (ix) \text{ subtracting (viii) from (ix)}$$

we get;

$$\ln(1 + Y_2 - Y_1/Y_1) = \beta_1 \ln(101/100). \text{ Then we have the \%ge change in } Y = [(1.01)^{\beta_1} - 1] * 100$$

So, we can interpret as one percentage change in X results in $100(1.01)^{\beta_1} - 1$ percentage change in Y while holding all other variables constant. For β_1 less than 10; $100(1.01)^{\beta_1} - 1$ can be approximated by β_1 . It is easy to get confused when interpreting percentage of change. Here is an example of the correct way to think about: a change of 70 percentage means the final value is $(1 + 70/100)$ or 1.7 times the initial value. A change of -40 percentage means the final value is $(1 - 40/100)$ or 0.6 times the initial value.

Appendix 6: Maximum moisture content (MC) for storage of selected cereals and legumes

Commodity	Moisture Content (% Wet basis)
Cereals	
Maize (shelled)	13.0
Maize (white)	13.5
Maize meal	11.5
Paddy	14.0
Milled rice	12.0
Millet	15.0
Sorghum	13.5
Wheat	13.5
Bulgur wheat	13.5
Wheat flour	12.0
Legumes	
Beans (haricot)	14.0
Lentils	14.0
Cowpeas	14.0
Pigeon peas	14.0
Field peas	14.0
Green grams	14.0
Soybeans	11.0
Groundnuts (unshelled)	9.0
Groundnuts (shelled)	7.0

Source: FAO, 1993

Appendix 7: Equilibrium moisture content and percentage wet basis of grains and other materials

Material	Temperature (°C)	Relative humidity (%)						
		40	50	60	70	80	90	100
Grains								
Barley	25	9.7	10.8	12.1	13.5	15.8	19.5	26.8
Buckwheat	25	10.2	11.4	12.7	14.2	16.1	19.1	24.5
Cottonseed	25	6.9	7.8	9.1	10.1	12.9	19.6	--
Field beans - flat small white	25	9.6	11.0	12.6	15.0	18.1*	--	--
Field beans - dark red kidney	25	9.6	10.7	12.5	15.0	18.6*	--	--
Flaxseed	25	6.1	6.8	7.9	9.3	11.4	15.2	21.4
Oats	25	9.1	10.3	11.8	13.0	14.9	18.5	24.1
Peas (green)	25-35	9.7	11.3	13.1	15.3	19.3	27.2	--
Poppy (opium)	25-35	5.9	6.9	8.0	9.5	11.7	17.0	--
Rice (whole grain)	25	10.9	12.2	13.3	14.1	15.2	19.1	--
Rye	25	9.9	10.9	12.2	13.5	15.7	20.6	26.7
Shelled maize	25	9.8	11.2	12.9	14.0	15.6	19.6	23.8
Sorghum	25	9.8	11.0	12.0	13.8	15.8	18.8	21.9
Soybean	25	7.1	8.0	9.3	11.5	14.8	18.8	--
Wheat - soft red winter	25	9.7	10.9	11.9	13.6	15.7	19.7	25.6
Wheat - hard red winter	25	9.7	10.9	12.5	13.9	15.8	19.7	25.0
Wheat - hard red spring	25	9.8	11.1	12.5	13.9	15.9	19.7	25.0
Wheat - durum	25	9.4	10.5	11.8	13.7	16.0	19.7	26.3
Other materials								
Alfalfa hay	25	6.6	8.3	10.0	13.0	14.5	--	--
Bran	21-27	--	--	--	14.0	18.0	22.7	38.0
Linseed cake	21-27	--	--	--	13.5	17.5	23.5	40.5
Oat straw	29	7.6	8.5	10.9	11.5	14.5	--	--
Pig feed pellets	25	9.4	10.6	12.2	14.0	17.0	22.7	--
Broiler pellets	25	--	--	--	13.0	--	--	--
Dairy cattle pellets	25	--	--	--	13.0	--	--	--

Source: Henderson 1985.

* Unreliable because of mould growth

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