

**FACTORS INFLUENCING AFLATOXIN CONTAMINATION IN MAIZE AT
HARVEST AND DURING STORAGE IN KONGWA DISTRICT, TANZANIA**

MAHAMUDU MOHAMED

**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD
QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF
AGRICULTURE. MOROGORO, TANZANIA.**

2017

ABSTRACT

The factors influencing aflatoxin contamination in maize at harvest and during storage in three villages of Manungu, Mlanga and Kongwa in Kongwa district, Dodoma region, Tanzania, were investigated in the year 2015/2016. The villages were chosen as they were major maize producers and suppliers to the international Kibaigwa grain market located in this area. The number of maize samples analyzed were 91. Twenty three samples were taken during harvest and stored for 90 and 180 days in Purdue Improved Crop Storage bags and polypropylene bags. At harvest (30% (7 out of 23) of maize samples were contaminated with aflatoxins. After storage for 90 days of storage 41% (13 out of 32) of the samples were contaminated with aflatoxins while after 180 days of storage, 67% (12 out of 36 samples) were contaminated with aflatoxins. Aflatoxin contamination in all maize samples was above maximum tolerable limit of 10 $\mu\text{g}/\text{kg}$ by East African Standards, thus posing a health hazard to consumers. Parameter estimates from generalized linear model (GENMOD) indicated that total aflatoxins increase with time and the concentration was 13.12, 14.75 and 19.39 $\mu\text{g}/\text{kg}$ at day 0, 90 and after 180 days of storage, respectively. The effects associated with higher aflatoxin contamination in post-harvest management practices were storage duration, storage type, sorting, treatment of stores and treatment of crops. The storage technique with high risk of aflatoxin development was polypropylene bags without pesticides treatment. Maize stored in polyethylene bags (uncontrolled) for 180 days showed increase in aflatoxin levels with mean value of 19.06 $\mu\text{g}/\text{kg}$. Low aflatoxin was related to the use of insecticides, sorting and use of Purdue Improved Crop Storage bags (PICS) with a mean of 5.4 $\mu\text{g}/\text{kg}$ at 180 days. Proper pesticide application reduces the likelihood for infestation, while the use of improved bags (PICS) had shown minimum/low increased levels of aflatoxin contamination in maize.

DECLARATION

I, Mahamudu Mohamed do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation 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.

Mahamudu Mohamed

(MSc. Candidate)

Date

The above declaration is confirmed;

Professor Jovin K. Mugula

(Supervisor)

Date

COPYRIGHT

No part of this dissertation may be reproduced, stored in any retrieval system, or transmitted in any form or by any means without prior written permission of the author or the Sokoine University of Agriculture on the behalf.

ACKNOWLEDGEMENTS

I would like to thank my main supervisor Prof. Jovin K. Mugula of Sokoine University of Agriculture (SUA), co-supervisors Dr. George Mahuku and Dr. Simon Boniface of the International Institute for Tropical Agriculture (IITA) and Mr. Chacha J. Nyangi of Mbeya University of Science and Technology (MUST) for guidance and support throughout the research. I am also very grateful to the Ghent University and IITA Pathology Laboratory staff for their cooperation during analysing of the maize samples.

Last, but not least, I would like to express my thanks to my classmates Reward Mrema, Jamila Omar, Salama Shekilango, Amina Yassin, Flora Luvanda and Mwanahamisi Msangi for their support during the pursuit of my postgraduate degree studies. In fair honesty, it is impossible to mention everyone who assisted me, but I am immensely indebted for their help and may all be blessed.

DEDICATION

This work is dedicated to my late father Mohamed .A. Sasamalo. May the Almighty God rest his soul in peace; my mother Mwantime Abdi Bakari, my lovely wife Advocate Hanifa Omar Abdalla and my son Saeed .M. Sasamalo (Colonel Jr).

TABLE OF CONTENTS

ABSTRACT.....	ii
DECLARATION	iii
COPYRIGHT	iv
ACKNOWLEDGEMENTS	v
DEDICATION	vi
TABLE OF CONTENTS	vii
LIST OF TABLES	x
LIST OF FIGURES	x
LIST OF APPENDICES	xii
LIST OF ABBREVIATIONS AND ACRONYMS	xiii
CHAPTER ONE.....	1
1.0 INTRODUCTION	1
1.1 Background.....	1
1.2 Problem Statement and Justification of the Study.....	4
1.3 Objectives	5
1.3.1 General objective	5
1.3.2 Specific objectives	5
CHAPTER TWO.....	6
2.0 LITERATURE REVIEW	6
2.1 Overview of Maize Production in Tanzania	6
2.2 Aflatoxins and their Causes	7
2.3 Effects of Aflatoxins to Humans	8

2.4	Chemical Structures of Aflatoxins	10
2.4.1	Aflatoxin B ₁	11
2.4.2	Aflatoxin B ₂	11
2.4.3	Aflatoxin M ₁	12
2.4.4	Aflatoxin M ₂	12
2.4.5	Aflatoxin G ₁ and G ₂	12
2.5	Factors Affecting Aflatoxin Development in Maize	12
2.5.1	Climatic condition	12
2.5.2	Pest infestation	13
2.5.3	Harvest and drying.....	14
2.5.4	Storage conditions	15
2.6	Control of Aflatoxins	15
2.6.1	Pre harvest management practices.....	15
2.6.2	Post-harvest management practices.....	18
	CHAPTER THREE	19
	3.0 MATERIALS AND METHODS.....	19
3.1	Study Area	19
3.2	Collection of Samples	22
3.3	Aflatoxin analysis.....	23
3.3.1	Sample preparation	23
3.3.2	Extraction of aflatoxins	23
3.3.3	Quantification of aflatoxins.....	23
3.3.4	Validation of total aflatoxins using LC-MS/MS.....	24
3.4	Statistical Analysis	24

CHAPTER FOUR	25
4.0 RESULTS AND DISCUSSION	25
4.1 Characteristics of Farmers	25
4.1.1 Storage practices	26
4.1.1.1 Harvest and drying	26
4.1.1.2 Storage structures	29
4.1.1.3 Sorting.....	30
4.1.1.4 Storage pests.....	32
4.1.1.5 Removal of crop residue from stores	34
4.2 Total Aflatoxins Content in Maize	34
4.3 Effect of Storage Practices on Aflatoxin Levels in Maize.....	36
4.3.1 Storage type.....	37
4.3.2 Storage duration	38
4.3.3 Store treatment.....	38
4.3.4 Grain treatment	39
4.3.5 Effect of duration of age of storage facility in use	42
CHAPTER FIVE	43
5.0 CONCLUSION AND RECOMMENDATIONS	43
5.1 Conclusion	43
5.2 Recommendations	44
REFERENCES	45
APPENDICES	69

LIST OF TABLES

Table 1:	Demographic characteristics of farmers.....	26
Table 2:	Occurrence of aflatoxins in maize according to storage practices/ structure	28
Table 3:	Total Aflatoxins content in maize stored in different storage structures for 180 days.....	30
Table 4:	Aflatoxin content in maize during storage for 180 days	35
Table 5:	Aflatoxin content in stored maize in Kongwa district for 180 days	35
Table 6:	Association of storage factors with aflatoxin contamination in maize (Y) across three villages	36
Table 7:	Storage factors significantly associated with aflatoxin contamination in maize (Y) across three villages at the beginning of storage	40
Table 8:	Storage factors that are significantly associated with aflatoxin contamination in maize across three villages at the end of storage	41
Table 9:	Effect of duration of store use on total aflatoxin content in maize	42

LIST OF FIGURES

Figure 1: Aflatoxin and disease pathways: source (Wu, 2010). 9

Figure 2: Chemical structures of aflatoxins 11

Figure 3: Map of Tanzania 20

Figure 4: Map of Kongwa district –Dodoma. 20

Figure 5: 1=24% of farmers sort maize and 2=76% of the farmers do not sort maize... 32

LIST OF APPENDICES

Appendix 1:	Sampling protocol for maize from field, and household villages in Kongwa, Tanzania	69
Appendix 2:	Determination of aflatoxin in maize by Neogen ELISA	75
Appendix 3:	Determination of aflatoxin in maize by means of LC-MS/MS	80

LIST OF ABBREVIATIONS AND ACRONYMS

AFB ₁	Aflatoxin B ₁
AFB ₂	Aflatoxin B ₂
AFG ₁	Aflatoxin G ₁
AFG ₂	Aflatoxin G ₂
EAC	East Africa Community
FB ₁	Fumonisin B ₁
CIAT	International Centre for Tropical Agriculture
CRM	Certified Reference Materials
DAICO	District Agriculture, Irrigation and Cooperative Officer
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FDA	Food and Drugs Authority
FAO	Food and Agriculture Organization
GPS	Global Positioning System
HCC	Hepatocellular Carcinoma
iAGRI	Innovative Agricultural Research Initiative
IITA	International Institute of Tropical Agriculture
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometer
LOD	Limit of Detection
LSMEANS	Least Square Means
MATI	Ministry of Agriculture Training Institute
µg/kg	Microgram per Kilogram (ppm)
mg/kg	Milligram per Kilogram (ppb)
MTL	Maximum Tolerable Limits

MT	Metric Tones
NTD	Neural Tube Defect
RPM	Revolution per minutes
SAS	Statistical Analysis System
SE	Standard Error
SUA	Sokoine University of Agriculture
UK	United Kingdom
URT	United Republic of Tanzania
USA	United States of America
USAID	United States Agency for International Development
USDA-ARS	Southern Regional Research Center of the United States
US\$D	United States Dollar
UV	Ultra Violet

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Mycotoxins are toxic secondary metabolites of fungi that frequently contaminate maize in the field and/or during storage (Smith *et al.*, 2012). Mboya and Bogale (2012) reported that mycotoxins contamination of maize poses a health risk to humans and domesticated animals.

The most important mycotoxin in maize are the aflatoxins, fumonisins, deoxynivalenol, and ochrotoxin (Kimanya *et al.*, 2012). Aflatoxins are naturally occurring mycotoxin produced by some strains of moulds such as *Aspergillus flavus* and *Aspergillus parasiticus* (Pasone *et al.*, 2010 and Sardin *et al.*, 2011).

Aflatoxins cause serious problems in many foods and are most abundant in maize and maize products since maize can be infected while in the field under specific environmental conditions (Krnjaja *et al.*, 2013). Contamination of maize depends on the co-existence of susceptibility of hybrids and environmental conditions favorable for proliferation of mycotoxigenic fungi (Blandino *et al.*, 2009).

Sclerotia are important perennation structures of fungi, especially pathogenic fungi, as they remain dormant during stressful environmental conditions like drought and germinate when more favorable conditions, usually adequate moisture and temperature arise Okoth *et al.* (2016).

Poor harvesting practices, improper storage and less than optimal condition during transportation, marketing and processing can also contribute to fungal growth and increase the risk of mycotoxins production (Mahmoudi *et al.*, 2013). As reported by Hell and Mutegi (2011) and Jaetzold *et al.*, 2006, small-scale farmers store maize under varying and often suboptimal conditions for up to 4 months before home use or sale.

Mycotoxins have negative impact on human health, animal productivity and trade (Wagacha and Muthoni, 2008; WHO, 2006; Wu, 2006). Aflatoxin B₁ is most toxic and is associated with liver cancer and immune suppression (Shephard, 2008). In high exposures aflatoxins cause acute toxicity particularly to the liver with growth retardation and death depending upon the dose and duration of aflatoxin exposure (Bill *et al.*, 2014). These metabolites, aflatoxins M₁ and M₂, are potentially important contaminants in dairy products (Strosnider *et al.*, 2006).

In the field, predisposing conditions leading to fungal growth are high temperatures and humidity, poor soil fertility, drought and insect damage, monsoons and unseasonal rains during harvest (Kamala *et al.*, 2015). In addition, other stresses (example, nitrogen stress) that affect plant growth during pollination can increase the level of aflatoxins produced by the *Aspergillus* (Wagacha and Muthoni, 2008).

Inadequate drying and improper storage also increases the risk of aflatoxins contamination. Countries that are located between 40°N and 40°S latitude such as Tanzania favour suitable growing conditions for the moulds subjecting the populations to risk of exposure (Hussaini *et al.*, 2012).

Aflatoxins cause serious health effects in humans, leading to significant adverse impacts in the form of disease and impacts on a country's agriculture, food security, and commerce (Lamb *et al.*, 2012). IITA estimates that globally, about US\$ 1.2 billion in global commerce is lost annually due to the aflatoxin contamination, with African economies losing US\$ 450 million each year (IITA, 2013). Schmalke III and Munkvold, (2015) reported that over four billion people in developing countries are repeatedly exposed to aflatoxins, contributing to greater than 40% of the disease in these countries.

Maize is the commodity most often contaminated with fumonisins and aflatoxins in Eastern and Southern Africa, as in most developing countries (Hell *et al.*, 2005). Studies in Tanzania have reported high exposure in infants and young children to aflatoxin through maize based diet (Shirima *et al.*, 2014) and through breast milk from mothers whose predominant diet is maize (Magoha *et al.*, 2014).

Reported by Shirima *et al.* (2013) that young children in Tanzania are chronically exposed to aflatoxins through contaminated diet although the level of exposure varies markedly. There is increasing concern about mycotoxin contamination in tropical food systems. Significant attention has been focused on regions where outbreaks of fatal mycotoxicosis tend to occur. For example, the recurrent outbreak of aflatoxins contamination of maize and fatal aflatoxicosis in eastern Kenya have received considerable attention (Daniel *et al.*, 2011; Nyikal *et al.*, 2004), and the recent outbreak in Dodoma on 17 September 2016 in Chemba, Kiteto, Chamwino, Dodoma and Kondoa districts where total reported cases were 65 and 17(26.15%) people died and 48 were in quarantine at Dodoma Regional Hospital-Tanzania. However, little information is available on the occurrence and extent of aflatoxins contamination in most areas of

Africa, including Kongwa district in Dodoma, central Tanzania, where most of the maize is produced.

This study was conducted in Kongwa District of Tanzania whereby aflatoxins in maize was quantified and assessed to the extent in which these toxins are produced in relation to; storage type and management practices focusing on sorting and drying.

1.2 Problem Statement and Justification of the Study

Aflatoxins develop in maize in the field and during storage thus making the grains unsafe and unwholesome for consumption. Several outbreaks of aflatoxins poisoning have been reported in Eastern province of Kenya since 1978 as reported by Moturi (2008) and recently in Dodoma Tanzania. These outbreaks have caused a lot of concern because they have worsened the food security status as maize is a major staple food in several households in the country. Considering that maize is also a staple food for the majority of Tanzanians, it was necessary to assess the factors that influence aflatoxin contamination in stored maize in Tanzania.

In Tanzania, the international maize market is located at Kibaigwa in Kongwa District. Whilst Kongwa, is among the largest producers of maize, there has been no study on factors that influence aflatoxins contamination at harvested and stored maize. The aim of this study was to assess factors influencing aflatoxins contamination in naturally contaminated maize in Kongwa at harvest and during storage as a first and essential step in the selection of intervention and management options to mitigate aflatoxins contamination during growth and storage of maize.

1.3 Objectives

1.3.1 General objective

The general objective of the study was to assess for factors influencing aflatoxins contamination at harvest and during storage of maize in Kongwa district, central Tanzania.

1.3.2 Specific objectives

The specific objectives of the study were to:

- i. determine total aflatoxins in harvested maize;
- ii. determine the total aflatoxins in stored maize; and
- iii. evaluate the effects of harvest and post-harvest management practices on aflatoxins contamination in maize.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Maize Production in Tanzania

Maize is one of the most important food crops grown in all regions of Tanzania (Ismail *et al.*, 2015; Kimanya *et al.*, 2010). Maize is cultivated and used as a staple food by the majority of Tanzanians and used as an ingredient for preparation complementary foods. National maize consumption is estimated to be over 3 million metric tons per year, whereas the daily per capital consumption of maize for people in rural areas is estimated to be 450 g (Smith and Subandoro, 2012). More than half of cultivated land in Tanzania is allocated to cereal crops but, maize is the major and most preferred staple crop among all staple and cash crops being produced (Suleiman and Rosentrater, 2015).

Maize is a suitable substrate for mould contamination and production of mycotoxins harmful to both humans and animals (Kpodo *et al.*, 2000). Mycotoxigenic moulds can invade maize at different production stages especially during pre-harvest and during post-harvest handling (Chulze, 2010). A recent study detected multiple mycotoxins contamination in stored maize in rural Tanzania (Kamala *et al.*, 2015).

Kimanya *et al.* (2008) reported the incidence and extent of mycotoxin contamination of maize grown in four different agro-climatic regions of Tanzania whereby 52% of the samples were contaminated with fumonisin B₁ at levels up to 6125 µg/kg (median, 206 µg/kg) and aflatoxins B₁ was 12% of the samples at levels ranging from 5 to 90 µg/kg (median, 38µg/kg). Nyangi *et al.* (2016) reported that maize products consumed by humans and animals in Babati Northern Tanzania contained aflatoxins at levels below the EAC MTL which was satisfactory, however a small portion of marketed maize was

contaminated with mycotoxins at levels that exceeded EAC standards, indicating the need to improve aflatoxins control.

2.2 Aflatoxins and their Causes

Aflatoxins are secondary fungal metabolites that contaminate agricultural commodities and can cause sickness or death in humans and animals (Guchi, 2015). Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans (De Lucca, 2007).

One mould species may produce many different mycotoxins, and the same mycotoxin may be produced by several species (Robbins *et al.*, 2000). The most important mycotoxins are aflatoxins, ochratoxins, deoxynivalenol (DON), zearalenone, fumonisins, T-2 toxin and T-2 like toxin. However, food-borne mycotoxins likely to be of greatest significance in tropical developing countries are the fumonisins and aflatoxins (Kumar *et al.*, 2008).

Among the factors which cause aflatoxins production are water stress, high-temperature stress and insect damage of the host plant. These are major determining factors in mould infestation and toxin production. Similarly, specific crop growth stages, poor fertility, high crop densities and weed competition have been associated with increased mould growth and toxin production (Dhanasekaran *et al.*, 2011).

Patel *et al.* (2015) reported that under favorable temperature and humidity conditions these fungi grow on certain food stuffs such as groundnuts, maize, rice, figs and other dried foods, spices, crude vegetable oils and tree nuts like almonds, walnuts, pistachios

and Brazil nuts, as a result of fungal contamination before and after harvest. Aflatoxigenic moulds grow exponentially in conventional multi-month storage as a result of a combination of heat and high humidity (Hell *et al.*, 2010).

Aflatoxins cause infertility, abortion, and delayed onset of egg production in birds as well as sudden losses of egg production in actively laying birds. Furthermore, loss of appetite, skin discoloration, or even yellowish pigmentation on skin can be observed in fish (Oladele, 2014).

2.3 Effects of Aflatoxins to Humans

It has been reported that approximately 25-50 % of world's agricultural crops are contaminated with mycotoxins, among which aflatoxins is the most significant (Muthoni *et al.*, 2011). Acute and chronic exposure to mycotoxin may cause various human health effects (Sheriff *et al.*, 2009). Dietary exposure to aflatoxins is one of the major causes of hepatocellular carcinoma, the fifth most common cancer in humans worldwide (Williams and Windham, 2015).

There is sufficient evidence that AFB₁ and mixture of B₁, G₁ and M₁ are proven carcinogens as provided by the International Agency for Research on Cancer who classifies them as Group 1 carcinogens while M₁ and B₂ are designed to group 2B reported by Kaaya *et al.* (2005).

If crops with very high levels of aflatoxins contamination are consumed by humans, poisoning such as aflatoxicosis and even death can occur while chronic exposure to low levels of contamination in crops consumed regularly increases liver cancer and can suppress the immune system particularly for population that test positive for the hepatitis

B virus (TFDA, 2012). Aflatoxins have been implicated in the pathogenesis of protein energy malnutrition (PEM), a condition affecting more than 118 million (32% of) children in the developing world and hence the development of kwashiorkor and marasmus in infants (Scheinfeld and Mokashi, 2010).

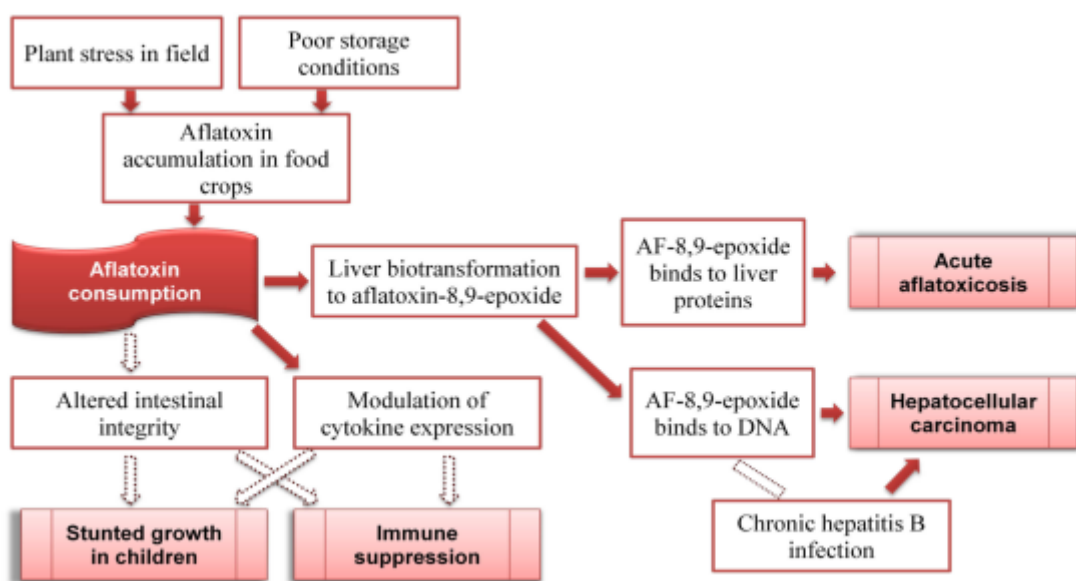


Figure 1: Aflatoxin and disease pathways: source (Wu, 2010).

The darker arrows in Figure 1 denote linkages that are well established in agricultural and toxicological researches, while the white arrows denote linkages that have been relatively well established (Wu, 2010).

Improper management practices and adverse climatic conditions causes stress to the plant in which during harvest and after harvest are predisposing factors for post-harvest aflatoxins contamination (Figure 1). Post-harvest contamination can occur if crop drying is delayed and during storage of the crop if water is allowed to exceed critical values for the mould growth (Waliyar *et al.*, 2014).

Aflatoxin consumption, routine dietary consumption of food that contain aflatoxin have been associated with many diseases. Example, Chronic high levels lead to a gradual deterioration of health through liver damage and immunosuppression also may cause child stunting as reported by Wu (2010). Alvito *et al.* (2015); Lombard (2014) reported that infants and children below 12 years are more vulnerable to the effects of mycotoxins because of their less developed immune systems and high intake of foods and water per kg body weight. Mycotoxins come in the organism of animal or human by contaminated food infested with spores, conidiospores and/or with fragments of mycelium, alimentary ingestion of these fungal toxins in organism of animal or human cause intoxication called mycotoxicosis (Duarte-Vogel and Villamil-Jimenez, 2006).

Exposure to foods contaminated with high levels of aflatoxin can cause immediate death to humans and animals. In 2004, a year in which 317 hospitalizations and 125 deaths due to acute aflatoxin poisoning were recorded in Kenya as reported by Lewis *et al.* (2005).

2.4 Chemical Structures of Aflatoxins

The chemical structures of some aflatoxins are shown in Figure 2:

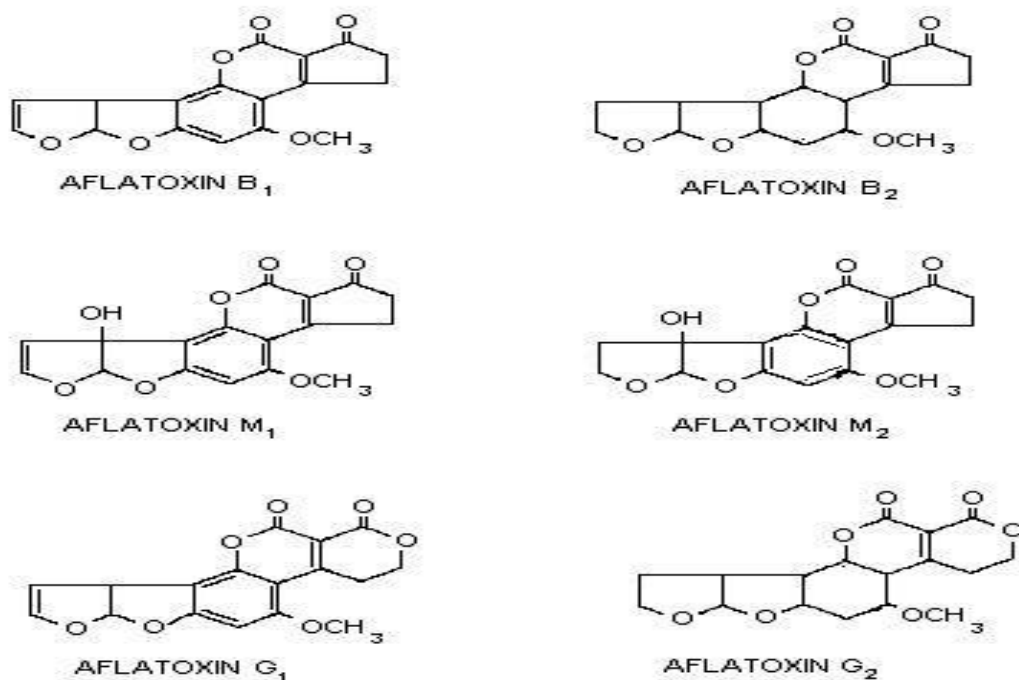


Figure 2: Chemical structures of aflatoxins

2.4.1 Aflatoxin B₁

Aflatoxin B₁ (AFB₁) is the most toxic congener, and has potent hepatotoxicity, carcinogenicity, cytotoxicity, genotoxicity and immunotoxicity (Golli-Bennour *et al.*, 2010 and Meissonier *et al.*, 2006). Aflatoxin B₁ has a molecular weight of 312 and a formula C₁₇H₁₂O₂. Against ultraviolet light shows, relatively strong blue fluorescence of their. It is a colorless crystalline.

2.4.2 Aflatoxin B₂

Aflatoxin B₂ has a formula C₁₇H₁₄O₆ and molecular weight 330. Its crystals have melting points between 286 and 289 °C. The compound exhibits blue fluorescence.

2.4.3 Aflatoxin M₁

Aflatoxin M₁ (AFM₁) or milk toxin is a hydroxylated metabolite of aflatoxin B₁ and is secreted in milk of dairy cattle after consumption of feed contaminated with aflatoxin B₁ (Darshti *et al.*, 2009; Iha *et al.*, 2013). AFM₁ is a relatively small molecule (328.3 g mol⁻¹) which exhibits slight affinity towards water (10–30 µg mL⁻¹).

2.4.4 Aflatoxin M₂

Aflatoxin M₂ is a natural oxidative metabolic product of the mycotoxin aflatoxin B₂. It has molecular formula of C₁₇H₁₄O₇ and molecular weight 330.3. Like other aflatoxins, aflatoxin M₂ is acutely toxic, immunosuppressive, mutagenic, teratogenic, and carcinogenic. It has Off-White to Pale Yellow Solid (Peraica *et al.*, 1999).

2.4.5 Aflatoxin G₁ and G₂

Their molecular weight and molecular formula for aflatoxin G₁ and G₂ are 328.06; 330.07 and C₁₇H₁₂O₇; C₁₇H₁₄O₇ respectively. Both show yellow-green fluorescence of the relevant structures under UV-light (Barbas *et al.*, 2015).

2.5 Factors Affecting Aflatoxin Development in Maize

2.5.1 Climatic condition

Climate change has been reported as a driver for emerging food and feed safety issues worldwide and its expected impact on the presence of mycotoxins in food and feed is of great concern (Battilani *et al.*, 2016).

Fungal development and aflatoxins contamination in foods occur as a consequence of interaction among the mould, the host and the environment (Milani, 2013). The environmental factors that favour *A. flavus* development and aflatoxins production

include high soil and/or air temperature, high relative humidity and high rates of evapotranspiration, water availability, drought stress, crowding of plants and condition aiding dispersal of fungi during silking (Hell *et al.*, 2000). Rainfall is more important than altitude in predicting mycotoxins (Nyangi *et al.*, 2016).

Aflatoxins contamination is more common in the tropics and Sub-Sahara-tropics, such as Tanzania, and these conditions relate well to temperature and rainfall that are strongly suitable for growth of *A. flavus* reported by Pratiwi *et al.* (2015). High temperature increases the production of *A. flavus* conidia, their dispersal and kernel infection rate, thereby contributing to high levels of aflatoxins accumulation under these conditions (Reddy *et al.*, 2014).

The aflatoxins contamination pattern is due to the fact that lower altitude areas are usually warmer with high temperatures and humidity compared to higher altitude areas which are usually cooler with low temperatures and humidity (Nyangi *et al.*, 2016). The prevailing conducive weather in sub-Sahara Africa characterized by high temperatures and high humidity coupled with dryness; promote fungal growth and aflatoxins production (Abbas *et al.*, 2009). In addition, studies have reported significant correlation in aflatoxins levels in maize after long storage in agro-ecological zones with wet and humidity climates in dry regions (Hell *et al.*, 2000).

2.5.2 Pest infestation

In Tanzania, the major constraints to maize production include insect pests, diseases, weeds, rodents, fungi, and pathogens (Suleiman and Rosentrater, 2015), also reported by Akowuah *et al.* (2015).

Insects are primary biotic stress factor that influence fungal colonization and mycotoxin contamination in maize. Further, kernel feeding insects are more important stress than silk or cob-feeding insects under warm climatic conditions (Scully *et al.*, 2009). Also, reported by Widstrom *et al.* (2003), several studies have shown a positive association between ear-feeding insects and mycotoxin contamination in kernels.

Damaged grains are more prone to fungal invasion and, therefore, to mycotoxin contamination as well (Ostry *et al.*, 2014). Kebede *et al.* (2012) reported that inadequate plant nutrition, insects feeding on developing kernels, weed competition, excessive plant density, plant disease and other biotic and abiotic stress facilitate the infection and production of aflatoxins by the fungus.

According to Mihale *et al.* (2009), insects are responsible for 15-100 % and 10-60 % of the pre- and post-harvest losses of grains in developing countries, respectively. Control of weeds in maize is very essential for obtaining good harvest. Weed control practices in maize resulted in 77 to 96.7% higher grain yield than the weed control (Amare *et al.*, 2015).

2.5.3 Harvest and drying

Timely harvest and proper drying of maize are very important factors. Extended field drying of maize could result in serious grain losses during storage. Also, observed, aflatoxin levels increased by about 4 times by a third week and more than 7 times when maize harvest was delayed for 4 weeks (Kaaya *et al.*, 2006). Wu *et al.* (2011) reported that occurrence of aflatoxin in maize is influenced by favorable conditions such as high moisture content and temperature.

2.5.4 Storage conditions

Infection of stored products by toxigenic fungi and subsequent contamination with aflatoxins are generally influenced by many factors including fungal populations, environmental conditions (general climate, temperature, and humidity O₂ and CO₂), Insect infestation and pre- and post- harvest handling, but in most cases there complex interactions among the different factors (Gnonlonfin *et al.* , 2013).

Jian and Jayas (2012) reported that some fungi attract insects and promote their growth, while others prevent through secretion of toxic metabolites. High moisture levels during storage have shown to increase grain vulnerability to aflatoxin contamination reported by Morenoa *et al.* (2009). Hence, crops must be stored under optimum condition for longer storage.

2.6 Control of Aflatoxins

Control of aflatoxin in Tanzania is a matter of importance not only for health implications, but also for improvement of the economy of people. Thus, a number of strategies for reduction and control of aflatoxin have been developed by researchers. The control of aflatoxin involves pre- and post-harvest management practices.

2.6.1 Pre harvest management practices

It is well established that most of the mycotoxin contamination of maize starts in the field and continue during storage (Akowual *et al.*, 2015). Thus, prevention at field stage is crucial to prevent the development of mycotoxins during drying and storage (Strosnider *et al.*, 2006). At farm level, it has been found that irrigated maize has fewer problems with *Aspergillus* infection due to better growing conditions leading to less drought and heat stress (Summer *et al.*, 2009).

Hell *et al.* (2010) reported that pre-harvest measures that are efficient in reducing aflatoxins contamination in maize are the same as those that will enhance yields. These pre-harvest practices include timely planting, ensuring optimum plant density, proper plant nutrition, avoiding drought stress, controlling other plant pathogens, weeds, insect pests and proper harvesting (Bruns, 2003).

Harvesting during heavy rains should be avoided as this could have serious effects on quality of maize including rotting (Hosney, 2015). It is also important that during handling of grains, physical damage should be minimized and high moisture maize should not be held longer than six hours in transportation wagons or trucks (Summer *et al.*, 2009). Several strategies that have been investigated to manage, prevent, and reduce mycotoxins contamination in crops include biological, chemical and cultural control practices.

(i) Biological control

Biological control is potential means for aflatoxin control of fungal growth in the field by use of organisms to reduce the incidence of pests, diseases, or toxins (Wu and Khlangwiset, 2010). Numerous organism have been tested for biological control of aflatoxin contamination including bacteria, yeasts and non- toxigenic (atoxigenic) strains of the causal organisms (Yin *et al.*, 2008) of which only atoxigenic strains have reached commercial stage (Dorner, 2009).

Modifying the structures of fungal communities to favour the growth of atoxigenic strains can result in drastic reduction of aflatoxin because the chief causal agent of contamination has been reduced (Mehl *et al.*, 2012).

The atoxigenic strain is applied once per growing season when the overall *A. flavus* inoculum level in the field are low, thereby providing the applied strain the time and preferential exposure to be established in the crop, as well as subsequent advantage when competing for crop resources with the toxigenic strains (Cleveland *et al.*, 2003; Cotty and Melon, 2006).

(ii) Chemical control

Aflatoxin G₁ and G₂ are more susceptible to chemical hydrolysis than aflatoxin B₁ and B₂ because of the linkage in the G group compared to the B group which possess a single ether linkage (Ogunsanwo *et al.*, 2004).

Thus, insect damage and fungal infection must be controlled in the vicinity of the crop by proper application of insecticides and fungicides (Kabak *et al.*, 2006). For instance, application of itraconazole and amphotericin B fungicides to control *Aspergillus species* (Wagacha and Muthomi, 2008). Also, application of tridemorph on T-2 toxin and diacetoxyscirpenol (DAS) to inhibit growth and development of *F. sporotrichioides* in vitro (Pirgozliev *et al.*, 2003).

(iii) Cultural control

Cultural Control are practices designed to reduce mycotoxin contamination of crops that have their roots in plant disease epidemiology (Munkvold, 2003). Cultural control strategies include crop rotation, tillage practices, appropriate application of fertilizers, weed control, plant density, irrigation, insect control, planting and harvesting dates, genotypes of seed planted, competitive exclusion and good agricultural practices (Wagacha and Muthomi, 2008; Pirgozliev *et al.*, 2003; Strosnider *et al.*, 2006).

The main principle of cultural control is to alter the conditions under which the crop is grown so that infection by the offending fungus is avoided and discourage disease development (Battilani *et al.*, 2008).

2.6.2 Post-harvest management practices

Pre-harvest contamination is very much related to post-harvest accumulation as higher aflatoxin loads at harvest provide inoculum sources for subsequent contamination during storage (Craufurd *et al.*, 2006).

Sorting to remove physically damaged and infected grains (based on their coloration, odd shapes, shriveled and reduced size) from the intact commodity can reduce aflatoxin levels by 40-80% (Park, 2002; Afolabi *et al.*, 2006). Channaiah *et al.* (2014) reported that the best management for successful storage of maize includes sanitation, loading aeration and monitoring.

ICRISAT reported that drying methods (avoiding high moisture, slow drying and air circulation) were common practices that could help to reduce or stop aflatoxin contamination (Diaz Rios and Jaffee, 2008; ICRISAT, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in the three villages of Manungu, Mlanga and Kongwa in Kongwa district, Tanzania. The number of households growing maize in Dodoma region was 260 043, representing 94 percent of agricultural households. Most households grew maize in Kondoa (81 069 hh) followed by Kongwa (45 098 hh), Mpwapwa (6852 hh), Chamwino (39 478 hh), Dodoma Urban (31 244 hh) and Bahi (21 441 hh). On average, the area under maize per household was 1.3ha (Census of agriculture 2007/2008).

The district is located between latitude 5°30' to 6°0' South and longitude 36° 15' to 36° East. It borders Kilosa district in the East, Chamwino district in the West, Kiteto district in the North and Mpwapwa district in the South. The elevation of Kongwa district ranges from 900 to 1000 metres above sea level. Generally, the district lies on the leeward side of Ukaguru Mountains.

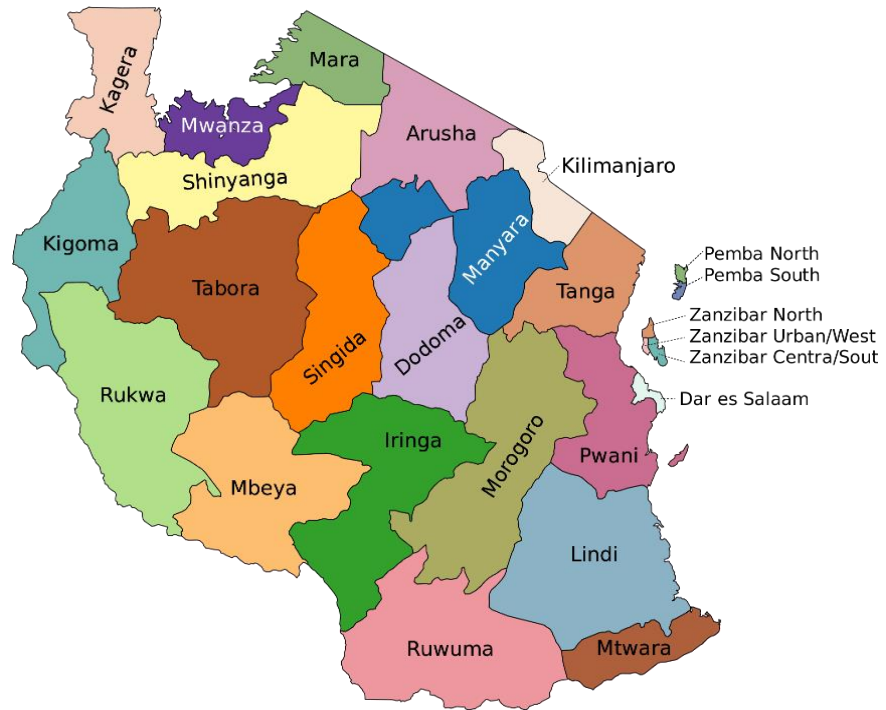


Figure 3: Map of Tanzania



Figure 4: Map of Kongwa district –Dodoma.

The Headquarter for Kongwa district bears the same name with the district and is located 89 kilometres East of Dodoma Municipality. Kongwa district covers 4041 square kilometers with a population of 309 973 people where 149 221 are male 160 752 are female (URT, 2012). Administratively, Kongwa district is divided into three divisions namely Zoissa, Kongwa and Mlali with 14 wards and 67 villages. The main activity of the people is agriculture. Crop production is the major activity, while livestock keeping is the second important occupation. The crops grown include maize, sorghum, finger millet, and legumes. Livestock kept include cattle, goats, sheep and pigs.

The district has two agro-ecological zones, based on rainfall and agronomic factors. These are maize belt and sorghum belt, respectively. The maize belt occupies the whole division of Mlali and major part of Zoissa division. The soil is relatively fertile based on total amount of annual precipitations received, the district can be categorized into two zones visualized as zone one with rainfall between 600 – 800 mm per annum and zone two that receives between 400 and 600 mm of rainfall annually. The rainfall pattern in the zones is bi-modal with short rains commencing in November/December to January and long rains falling from mid-February to May. The annual temperature varies from mean minimum of 18°C to a maximum of 34°C; being relatively suitable for maize growing. The area is the powerhouse of Kongwa cereals production, making it among big cereals producers in the country. The International Kibaigwa grain market is located in this area.

The sorghum belt occupies Kongwa division and Hogora ward. The area is relatively dry with rainfall between 400 mm to 600 mm per annum; as such only drought resistant crops have big chance of being grown. Crops grown are maize, sorghum, millet, groundnut and pulses (Okori, 2014).

3.2 Collection of Samples

Ten farmers from three villages namely Mlanga, Manungu and Kongwa were selected to provide samples that are representative of those consumed on farm as food and feed from harvest to store, for aflatoxin analysis. Collection sites were selected based on information provided by village leaders, farmer organizations, District Agriculture and Livestock Development Officer (DALDO) and local extension officers.

Ninety one samples were collected with distribution as shown in brackets, from Mlanga village (32), Kongwa (33) and Manungu (26). Detailed procedures for sample collection are indicated in the sampling protocol (Appendix 1). Samples were collected in polythene bags and taken for analysis to the International Institute of Tropical Agriculture (IITA) laboratory in Dar es Salaam. One kilogram sample was collected in each household on day 1 (after harvesting and when maize grains were properly dried and ready for storage), after 90 and 180 days in traditional storage and improved storage. Alongside maize sampling, a questionnaire was administered to farmer to obtain information as shown in Appendix 2. Briefly, the information solicited included; GPS coordinates and basic demographic details of supplier, processing and storage methods such as use of improved bags namely, Purdue improved crop storage (PICS) bags and traditional storage facility namely, *vihenge* (cribs) or polypropylene (POP) bags, and knowledge of aflatoxins. The information was catalogued in a database to correlate analysed aflatoxin levels with production, and storage practices.

3.3 Aflatoxin analysis

3.3.1 Sample preparation

Clearly labelled samples were dried to maximum of 13% moisture to avoid mould growth and aflatoxin production. Due to the heterogeneous contamination of aflatoxin in grains, sampling is the largest source of variation associated with the analysis of these naturally occurring contaminants (Whitaker, 2004). To overcome this problem the whole 1kg sample was milled using the Bunn and Waring grinder®(Mann: Bunn-o-Matic Corporation Springfield, Illinois, U.S.A) and sub sampled in order to produce a blended sub-sample that was deemed to be representative of the whole sample and cross referenced with codes linking them to accompanying questionnaires.

3.3.2 Extraction of aflatoxins

A 50 gm of ground sample was mixed with 250 mL of 65% ethanol (v/v) and shaken vigorously for 3 min using a laboratory shaker (IKA ®. Werker, German). The sample was allowed to settle, and then filtered through Whatman No.1 filter paper (Whatman International Ltd, Maidstone, UK).

3.3.3 Quantification of aflatoxins

The extracts were assayed for total aflatoxins using enzyme-linked immunosorbent assay (ELISA) as indicated by the manufacturer's protocol using Reveal Accuscan®III Reader (Neogen, USA). The lower limit of detection (LOD) of the ELISA was 2µg/kg, with a qualitative range of 2-150 µg total aflatoxins/kg. The concentration below the LOD was reported as not detected (n.d.). The analytical quality of the ELISA methods was assured by the use of certified reference material (CRM), a naturally contaminated maize sample with certified total aflatoxins of 18.1±3.6 µg/kg supplied by Neogen USA.

3.3.4 Validation of total aflatoxins using LC-MS/MS

Validation tests were carried out using liquid chromatography tandem mass spectrometry (LC-MS/MS) at the Department of Bioanalyses Laboratory, Ghent University in Belgium on 30 randomly selected samples previously analysed using Reveal AccuScan[®] III reader (Neogen, USA) at the Plant Pathology Laboratory, IITA in Dar es Salaam, Tanzania. Results from the study showed that sensitivity and specificity of LC-MS/MS system is more than Elisa method. LC-MS/MS system determined the concentration of aflatoxin with more sensitivity, determining the small amounts of aflatoxin in maize. There was no significant difference between two methods during this study (Appendix 2 and 3).

3.4 Statistical Analysis

The data were analysed using Statistical Analysis System (SAS[®] Version 9.4 (SAS- Institute Incorporation, USA). A generalized linear model (GENMOD) was run to identify the factors that significantly influenced contamination of maize with aflatoxins. The differences in mean total aflatoxins amongst the climatic zones and storage practices were determined using the least square means (LSMEANS). Aflatoxin levels were transformed using the natural log to normalise the data before analysis.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Characteristics of Farmers

Overall, 89 % of the farmers interviewed were males. In terms of awareness, 11% of the farmers were aware of aflatoxins and thought that it was a “poison found in spoiled maize”. These insights revealed limited understanding of what aflatoxins were and how they are formed. Aflatoxins and mycotoxins in general have not been widely prioritized from a public health perspective in low-income countries. This is because knowledge of mycotoxins and the full range and scale of their adverse health effects is incomplete and the known risks are poorly communicated to governments in regions where the contamination is greatest (Wild and Gong, 2010).

Creation of awareness and sensitization on aflatoxins to farmers and all the stakeholders was therefore an important part of intervention strategies to prevent and control mycotoxicosis in Tanzania. This might include the development and distribution of training manuals and brochures in major languages used in their areas, conducting training, workshops, and use of media for dissemination of information. A more effective method of controlling aflatoxins requires a combination of technologies and improvement on existing cultural practices by subsistence farmers (Atehnkeng *et al.*, 2015). It was not easy to determine if anyone in the whole population had illnesses associated with aflatoxin contamination in their foods.

In terms of formal education, 96% of the farmers had primary school education, 2% had secondary education and 2% had tertiary education (diploma and technical education). Dosman *et al.* (2001) found that people with higher levels of education were likely to be better informed, and may be aware of some type of risks of food additive in food than people with less education. Results for demographic data are represented in Table 1.

Table 1: Demographic characteristics of farmers

Characteristic	Number of farmers (n = 91)	Occurrence of aflatoxins (%)
Sex		
Male	81	89
Mycotoxin awareness		
Yes	10	11
Health problem related to eating food		
Yes		-
Education		
Primary	87	96
Secondary	2	2
Tertiary	2	2
None		

4.1.1 Storage practices

4.1.1.1 Harvest and drying

Harvesting of maize is done during early August. This was observed in all three villages. Immediate after harvest, farmers use different means of transport to send maize to their stores. Some use donkey, bicycles, tricycles and others use tractors. According to Kaaya *et al.* (2005) timing of harvest greatly affected the extent of aflatoxin contamination; extended field drying of maize increases insect infestation and fungal contamination and delayed harvest increased mould incidence, insect damage and aflatoxin levels in Uganda. In all three villages maize was dried to the safe storage moisture content of 13-15% mainly on the bare ground by 60 % of the farmers, in the field by 6% of the farmers and on platforms by 34% of the farmers. Sometimes the maize was stored before it was well dried.

Previous studies from neighboring countries of Tanzania like Kenya and Uganda demonstrate that *A. flavus* and *A. parasiticus* can invade maize seed in the field before harvest, during post-harvest, drying and curing as well as during storage and transportation. Since, spores of both the species can survive for a long period of time in

air and can get disseminated over a long period of distance from one place to another (Bhat *et al.*, 1997; Gao *et al.*, 2007).

Farmers used traditional methods to test moisture content in grains such as biting dry grains or shaking grains in a can and listening to the sound produced to determine whether the crops were dry or not. Maize was further dried before packing into bags for sale especially to markets which had grading systems in place to check moisture content. In those circumstances, maize was dried on canvass spread on the ground in order to prevent contact with the soil. In many instances, such maize was also dried along the road sides or in open fields where dust was easily blown into the drying maize on canvass. Hodges and Maritine (2012) reported that harvesting, drying and threshing losses for different cereal grains in some regions of Africa.

Results from this study indicated that the likelihood of aflatoxin contamination of maize was more than three times higher in maize dried on bare ground than that of maize not dried on bare ground. This observation is consistent with other reports on the association of drying maize on the bare ground with aflatoxin contamination (Atukwase *et al.*, 2009; Kaaya *et al.*, 2006).

Post-harvest aflatoxin contamination can occur when grain is improperly managed through faulty drying and storage processes under humidity and temperature levels favorable for mould proliferation (Oliveira *et al.*, 2009). It is also important that during handling of grains, physical damage should be minimized and high moisture maize should not be held longer than six hours in transportation wagons or trucks (Summer *et al.*, 2009). Simple devices should be developed so that Tanzania farmers can determine if their products have reached a safe moisture level or use other cost-effective and safe means of dehydration.

Table 2: Occurrence of aflatoxins in maize according to storage practices/structure

Storage practices	Number of samples n = 91 (%)	Mean aflatoxin ($\mu\text{g}/\text{kg}$)	Range of aflatoxin ($\mu\text{g}/\text{kg}$)
Storage structures			
Polypropylene bags	56 (62)	10.42	0-70.5
Cribs/granaries	1 (1)	3.2	3.2-3.2
Improved bags	34 (37)	3.397	0-70.4
Storage length/time			
Day 90	32 (35)	6.34	0-43.8
Day 180	36 (40)	13.09	0-70.5
Day 0	23 (25)	4.17	0-24.5
Storage pests			
Yes	91 (100)	8.466	0.70.5
No	-		
Remove previous crop residue from stores			
Yes	91 (100)	8.466	0-70.5
No	0 (0)	-	-
Storage with other crops			
Yes	49 (54)	7.027	0-52.0
No	42 (46)	9.646	0-70.5
Stores treatment			
			9.40-70.50
Traditional pesticides	3 (3)	32.9	0.00-70.40
Chemical spray	66 (73)	8.02	0.00-28.30
No stores treatment	22 (24)	6.48	
Grain treatment			
Traditional pesticides	3 (3)	28.93	18.5-43.8
Chemical spray	58 (64)	8.06	0.00 -70.40
No stores treatment	30 (33)	7.21	0.00 - 70.40
Drying method			
On ground	55 (60)	9.457	0-70.5
Dry on field	5 (6)	3.655	0-11.5
On platform	31 (34)	7.552	0-43.8
Season storage in use (years)			
1 to 5 years	47 (52)	8.885	0-70.5
5 to 10 years	21 (23)	3.433	0-21.4
Above 10 years	23 (25)	12.2	0-53.2
Sorting			
Yes	22 (24)	6.336	0-43.8
No	69 (76)	9.145	0-70.5

4.1.1.2 Storage structures

Total aflatoxins in the three villages at 180 days:

Traditional storage structures used in all (three) villages were almost similar, and the commonly used being the locally made granaries or cribs known as “*vihenge*” in Swahili. These were made of bamboo and wood sticks or mud walled and placed on raised platform, and grass-thatched or with metal roofing sheet. This storage method was used by 1% of the farmers and the sample collected from these stores was found positively contaminated with aflatoxins, since only one sample collected from this storage facilities, the results obtained could not be discussed.

It is reported in Guatemala, due to a lack of storage structures along with the region’s high humidity, storage losses were estimated between 40% and 45 % (IICA, 2013). Thirty four samples were stored in improved bags (PICS) out of which 13(38%) samples were positively contaminated with aflatoxins, Triple-layer hermetic (Purdue Improved Crop Storage) bags have been disseminated and adopted by millions of farmers due to their exceptional ability to control insect pests in cowpea, maize, and peanut (Baoua *et al.*, 2014; Anankware *et al.*, 2013; Mutungi *et al.*, 2014).

Similar observation was also reported by Williams *et al.* (2014) that Purdue bags can prevent spoilage by moulds aflatoxin accumulation. Fifty six samples stored in polypropylene 30(54%) samples were positively contaminated with aflatoxins. The polypropylene bags are neither moisture nor insect resistant, thus causing the maize grains to be very susceptible to moisture and insect infestation. Mboya *et al.* (2011) studied the quality of maize stored using polypropylene in Katumba ward, Tanzania and concluded that the method was inadequate for protecting maize against fungal infection. The result of total aflatoxins content in maize stored in different storage structures in the three villages for 180 days are given in Table 3.

Table 3: Total Aflatoxins content in maize stored in different storage structures for 180 days

Maize storage structure	Number of samples	Positive Samples (%)	Aflatoxin ($\mu\text{g}/\text{kg}$)	
			Range	Means \pm SE
PICS	34	13(38)	2.30 - 70.40	13.40 ^a \pm 5.19
POP bags	56	30 (54)	2.70 - 70.50	19.06 ^{ac} \pm 2.95
Granaries	1	1 (100)	3.20 - 3.20	3.20 ^{bc}

Values are means of positive total aflatoxin levels of maize samples stored in different storage structures.

Means with the different letters (by column) are significantly different ($P < 0.05$).

Positive samples are all analysed samples with value $>$ limit of detection (LOD)

POP represents polypropylene bags commonly used as a storage facility.

4.1.1.3 Sorting

Sorting processes seek to eliminate maize with substandard quality. Normally sorting can be achieved based on differentiation of physical properties such as colour, size, shape, and density as well as visible identification of fungal growth in affected crops.

Sorting was one of the post-harvest handling practices used by farmers in all three villages (Table 2). This was done manually by removing physically damaged and discolored and intact grains colonized by moulds. In the whole population of 30 farmers, 24% sorted their maize before shelling and after they were properly dried. Aflatoxin contamination is usually heterogeneous so that separating damaged kernels can effectively reduce contamination (Kabak *et al.*, 2006). The observation was similar to those reported in other studies on the use of sorting to reduce mycotoxin contamination of maize (Afolabi *et al.*, 2006; Kimanya *et al.*, 2009; Matumba *et al.*, 2015; Nyangi *et al.*, 2015). The sorted bad portion was mainly used as animal feeds and few farmers were using the portion for food after they de hull and mill it. The portion was mixed with sorghum to obtain flour. Animals fed contaminated food can pass aflatoxin

transformation products into eggs, milk products, and meat. Mazzete *et al.* (2009) reported that feeding of contaminated feed can lead to aflatoxin in animal's milk.

Effect of sorting of bad or molded maize in the stored lot indicated that the bad portion had the highest levels of aflatoxin of mean 32.9 μ /kg compared with good portion which had a mean aflatoxin levels of 5.4 μ /kg . Seventy six percent of the farmers did not sort their crops and sold or consumed their crops with bad portions.

Consuming damaged and discolored maize samples, increase the chances of aflatoxins as well as contaminating materials in food and feed, hence expose the population to the risks of aflatoxins in food. The diseases caused by aflatoxin consumption are loosely called aflatoxicosis. Acute aflatoxicosis results in death; chronic aflatoxicosis results in cancer, immune suppression, and other "slow" pathological conditions to human (Herrman, 2006) and in livestock, Consumption of very high levels of aflatoxins causes acute toxicity and death, while chronic consumption of lower levels can cause liver damage, gastrointestinal dysfunction, and decrease in appetite, reproductive function, growth, average daily gain, body weight and production (Khlungwiset, 2011).

Sorting of maize for farmers

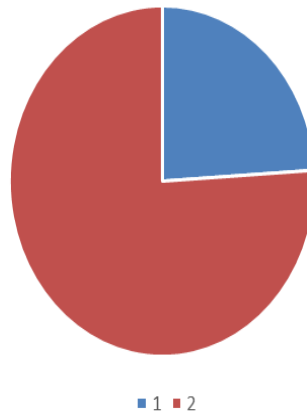


Figure 5: 1=24% of farmers sort maize and 2=76% of the farmers do not sort maize

4.1.1.4 Storage pests

Maize is attacked by many insect pests during all stages of growth from seedling to storage (Shiferaw *et al.*, 2011). Insects and mites may damage stored grain, but they also interact with fungal colonization in many different ways. Thus, it is important that insects are controlled both pre and post-harvest (Hell, 2000). Prevention of pests is important as losses during storage reduce food availability, quality, and the stability of farmers' food supply and income (Boxall, 2002).

A high incidence of insect damage was observed in stored maize in all three villages and only few stores were free from insects. Cardwell *et al.* (2000) and Udoh *et al.* (2000) studied the effect of insects' activities and qualified them as favorable for aflatoxin contamination when they increased the level of infection of *A. flavus*. In all villages the common pest infesting maize was identified as *Sitophilus zeamais*.

It has also been reported that maize weevils and the larger grain borer are the main and most serious pests of stored maize (Holst *et al.*, 2000). Insect infestation in the stored maize grains not only reduced their quality, but also associated with higher levels of aflatoxin contamination. This presented a health hazard since maize is the main staple cereal in Tanzania. Insects carry spores of mycotoxicogenic moulds from plants to the interior of the stack or kernels and may create infection wounds through their feeding habits (Munkvold, 2003). Insects' damage have previously been associated with mycotoxin contamination (Hell *et al.*, 2000; Wu, 2007).

As a consequence, proper management of insect pests through appropriate control strategies will reduce mycotoxin contamination. In this study no aflatoxins or lower total aflatoxin levels were found in stored maize that was free of insect damage such as those stored in controlled improved bags (Purdue Improved Crop Storage Bags-PICS), this bag use two liners of high-density polyethylene (HDPE) and an outer layer composed of woven polypropylene. Together, they create low-oxygen environments that reduce insect development (Murdock *et al.*, 2012). As much as 98% of all insect pests can be eliminated within just 1 month of storage, reducing damage and weight loss caused by feeding (Baoua *et al.*, 2012).

According to Mihale *et al.* (2009) insects are responsible for 15-100% and 10-60% of the pre- and post-harvest losses of grains in developing countries. Also, Philip and Throne, (2010) reported that stored grains contaminations with insect pests and fungi are a serious problem resulting in more than 20% losses in overall production by decreasing seed germination and downgrading of grains. One of the possible strategies to reduce aflatoxin contamination in stored maize would be to remove insect damaged maize at harvest

(Hell *et al.*, 2000). Some farmers use smoke to discourage presence of insects from their stores.

Another major pest for the stored maize observed were rodents. Rodents destroy crops and packaging materials hence giving favorable environment for insects to attack crops for example, hermetic storage plastic bags technologies which have been proven as an effective storage alternative for small-scale farmers to control insects (De Groote *et al.*, 2013; Moussa *et al.*, 2014), can be damaged by rodents (Ndegwa *et al.*, 2016) if care is not taken for their control, making these bags ineffective.

Rodents are well known vectors for diseases such as typhoid, paratyphoid, trichinosis, scabies, plague, and hemorrhagic fevers like ebola which are of public health concern (Cao *et al.*, 2002).

4.1.1.5 Removal of crop residue from stores

In all three villages, 99% of the farmers removed previous season's crops before introducing new crops in their stores. Mixing old and new crops increases the risk of contamination. Hell *et al.* (2008) reported that cleaning of stores before loading in the new harvests was correlated with reduction in aflatoxin levels.

4.2 Total Aflatoxins Content in Maize from day zero to 180 days

The results from storage duration for maize indicated that the mean aflatoxin levels increased from day 0 to day 180 (at harvest total mean aflatoxin was 13.12 $\mu\text{g}/\text{kg}$, at day 90 (14.75 $\mu\text{g}/\text{kg}$) and day 180(19.39 $\mu\text{g}/\text{kg}$), reported by Wild and Hall (2000) Aflatoxin contamination occurs more during post-harvest than during pre-harvest conditions. The observed increase was statistically significant in day 180 from the rest of storage period ($p < 0.05$). Also, Cotty *et al.* (2008) and Jaime *et al.* (2013) reported that Aflatoxins

increase in poorly stored crops after harvest. The results of total aflatoxins content in maize from day 0 to 180 days are shown in Table 4.

Table 4: Aflatoxin content in maize during storage for 180 days

Maize storage days	Samples	Contaminated samples (%)	Aflatoxin ($\mu\text{g}/\text{kg}$)	
			Means \pm SE	Range
Day 0	23	30	3.20-24.50	13.12 ^{ad} \pm 3.56
Day 90	32	41	2.50 – 43.80	14.75 ^a \pm 3.08
Day 180	36	67	2.30 – 70.50	19.39 ^{bc} \pm 4.25

Values are means of positive total aflatoxin levels of maize samples

Means with different letters (by column) are significantly different ($P < 0.05$).

Results obtained from farmers storage structures during the storage period of 180 days, aflatoxins contaminated samples were 18(56%), 12(36%) and 14(53%) of Mlanga, Kongwa and Manungu of maize samples, respectively were contaminated with aflatoxins. The range of aflatoxins concentrations in maize is shown in Table 4. The highest mean aflatoxin value was 70.50 $\mu\text{g}/\text{kg}$ was observed in maize from Mlanga village (Table 5) the maximum set limit in Tanzania for total Aflatoxin (10 $\mu\text{g}/\text{kg}$), (TBS 2004). According to Nyangi *et al.* (2016) maize processing reduce the levels of aflatoxin contamination in flour.

Table 5: Aflatoxin content in stored maize in Kongwa district for 180 days

Village	Number of samples	Contaminated samples (%)	Total aflatoxins ($\mu\text{g}/\text{kg}$)	
			Range	Means \pm SE
Mlanga	32	56	3.00 - 70.50	20.21 ^a \pm 5.37
Kongwa	33	36	2.30 – 52.00	15.23 ^{ac} \pm 4.20
Manungu	26	53	2.70 – 24.50	14.46 ^{bc} \pm 2.10

Values are means of positive total aflatoxin of maize samples from each Village.

Means with different letters (in column) are significantly different ($P < 0.05$).

4.3 Effect of Storage Practices on Aflatoxin Levels in Maize

The occurrence of aflatoxin in maize during storage was correlated with three practices/factors. These are storage duration and storage with other crops, sorting and storage of maize in improved bags and the use of traditional protectant as pesticides. These practices were negatively associated with aflatoxin contamination. Parameter estimates from regression model indicated that sorting was the major factor reducing the contamination of maize to aflatoxin. Also, Wicklow and Pearson (2014) reported that removal of the contaminated kernels is a reasonable approach for reducing aflatoxin contamination.

Table 6: Association of storage factors with aflatoxin contamination in maize (Y) across three villages

Parameter	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept	0.4452	0.1861	5.7222	0.0168*
Polypropylene bags	0.6142	0.1507	16.6047	<.0001*
Cribs/granaries	1.1895	0.4450	7.1463	0.0075*
Storage at day 180	0.6289	0.1273	24.3964	<.0001*
Chemical pesticides	-0.4363	0.1336	10.6713	0.0011*
Traditional pesticides	0.7222	0.2968	5.9208	0.0150*
6 - 10 year storage facilities	-0.3932	0.1297	9.1937	0.0024*

$$\text{Aflatoxin Y} = 0.4452 + 0.6142X_1 + 1.1895X_2 + 0.6289X_3 - 0.4363 X_4 + 0.7222 X_5 - 0.3932 X_6;$$

* = Statistically significant at P < 0.05

From the results (Table 6), the use of polypropylene bags without any chemical treatment increased aflatoxin contamination by estimates of 0.6142, cribs or granaries increased by estimates of 1.1895, use of chemical pesticides/insecticides reduced aflatoxin contamination by estimates (-0.4363) and use of traditional pesticides increased contamination by estimates (0.7222).

4.3.1 Storage type

Type of storage used had direct influence on the contamination of grain by aflatoxin. Maize stored in traditional storage structures both POP and cribs had higher levels of aflatoxin contamination compared to the maize stored in improved bags (PICS) if other variables are constant. Mwihia *et al.* (2008) reported that nylon sacks and polyethylene sacks maintained moisture and are impervious to free air circulation within the grain store and may promote aflatoxin contamination. On the other hand, high moisture content in polypropylene and polyethylene bags could result to lack of aeration in the bags.

PICS bag also known as a triple layer bags consist of three plastic liners. Two 80 micron high density polyethylene plastic bags, one surrounded by second, both are enclosed by a third bag made of woven polypropylene bag for reinforcement (Murdock and Baoua *et al.*, 2014). PICS are based on the principle of the bio-generated modified atmosphere, where the low oxygen environment inhibits the growth and development of insect pests (Sanon *et al.*, 2011). It takes advantage of an airtight seal where oxygen concentration dramatically decreases while carbon dioxide levels proportionally increases within a few days after sealing through respiration of insects, fungus, and grains/seed (Quezada *et al.*, 2006). Many studies have proven PICS to be effective storage systems for a variety of crops, including cowpeas, maize, peanuts, sorghum, wheat and common beans against insect infestation, fungal growth and aflatoxin accumulation (Zorya *et al.*, 2011; William *et al.*, 2014). However the effectiveness of hermetic technology depends on several factors such as airtightness of the seal, the commodity stored, agro-climatic conditions type and prevalence of insect pests and mechanical strength of the barrier material (Njoroge *et al.*, 2014). Thirty four percent of the farmers used improved bags. Thirty eight percent of the samples were contaminated though the increase was minimal (Table 2).

4.3.2 Storage duration

Several studies have reported increases in aflatoxins in stored maize, especially in the tropics (Kankolongo *et al.*, 2009; Hell *et al.*, 2000; Tedihou *et al.*, 2012). Storage helps to even out fluctuations in market supply, both from one season to the next and from one year to the next, by taking produce off the market in surplus seasons, and releasing it back into the market in lean seasons (Proctor, 1994). Storage durations of the maize crops in the three villages were taken during, day 0, day 90 and day 180. In all three villages 23% of the samples were taken during day 0, 35% of samples taken during day 90 and 40 % of the samples taken during day 180 (Hell *et al.*, .2000).

The influence of storage time on aflatoxin content was only noticed for period of 90 – 180 days, which generally resulted in higher aflatoxin content in stored maize samples (Table 6). It seemed that farmers, who stored maize for a short period, did not take as many precautions nor care as much as those that stored maize for long period. Results from analysis indicated that in traditional/no treatment maize as the number of days increased from zero to day 180, there was an increase of total aflatoxin in maize, while those stored in improved bags there was no/or minimal increase in aflatoxin levels. William *et al.* (2014) reported that spread of *A. flavus* and aflatoxin accumulation in moist maize ($\geq 18\%$ MC) can be controlled by placing the grain in a PICS bags.

4.3.3 Store treatment

Store treatment with chemical pesticides was related to lower aflatoxin level compared to traditional application of insecticides and to farmers who didn't apply any insecticides. Seventy six percent of the farmers in the study area had treated their stores against insect infestation before introducing their crops for storage and 24 % used no treatment in their stores during storage. Chemical pesticides were applied by 73% of the farmers and

traditional pesticides were used by 3% of the farmers. Chemical pesticides were sprayed in the store especially on walls, floor and ceiling before introducing crops to be stored. The common pesticides used were acetic acid (pilmophos methyl) and Bami force (permethrin and Malathion). An alternative treatment involved the use of natural protectant to smear the granaries/ cribs prior to introduction of crops and this comprised of the mixture of dried plant leaves with burnt cow dung or goat dung (Table 7).

4.3.4 Grain treatment

Farmers in all three villages have different perceptive relating on treatment of their maize. Some farmer's treat their maize using chemical pesticides/insecticides which they expect to sell and leave some maize untreated for house hold consumption. This result to untreated maize spreading pests to treated maize. Others use traditional method to treat their crops like smoking and other farmers do not treat their crops completely. About 4 to 12% of farmers in the various ecological zones in Nigeria used smoke to preserve their grains, and this practice was found to be correlated with lower aflatoxin levels in farmers' stores (Udoh *et al.*, 2000).

Grain treatment with traditional pesticides was related to higher aflatoxin levels compared to farmers who did not apply any insecticides because but these products have not been sufficiently tested for their efficiency in controlling aflatoxin in stored crops. The effect of the application of synthetic insecticides during maize storage on reduction of aflatoxin or fumonisins contamination was reported in several studies (Hell *et al.*, 2000; Atukwase *et al.*, 2012).

Some of the farmers used Chemical pesticide which is 3% Actellic super a cocktail of 1.6% Pirimiphos-methyl and 0.3% Permethrin. Chemical pesticides have been promoted

as effective against the LGB in combination with practices like immediate shelling and treating (Farrell and Schulte, 2002). Sixty four percent of the farmers used Chemical spray which is specific formulation for stored grains such as super shumba (pirimiphos methyl), Bami force (permethrin and Malathion).

Appropriate use of pesticides during production process could help in reducing the fungal infection or insect infestation and subsequently mycotoxin contamination. Fungicides such as intraconazole and amphotelicin B have shown to effectively control the aflatoxin producing *Aspergillus species* (Ni and street, 2005). In most cases chemical pesticides were applied once during the storage period and in few cases it was applied twice depending on the length of the storage and the extent of insect infestation. Thirty three percent of the farmers did not use any chemical to store their crops. High levels of insects' infestation were observed in their crops and during test most of the samples were positive in aflatoxin.

Table 7: Storage factors significantly associated with aflatoxin contamination in maize (Y) across three villages at the beginning of storage

Parameter	Estimate	Standard Error	t Value	Pr > t
Intercept	-0.247	0.938	0.26	0.7965
X₁	2.252	0.807	2.79	0.0154*

For Aflatoxin $Y = -0.247 + 2.252X_1$; where X_1 represents grain treatment with traditional Pesticides/insecticides.

$R^2 = 0.60$; $F = 2.15$

* = Statistically significant at $P < 0.05$

From the beginning of storage maize grains stored with pesticides/ insecticides reduced the levels of aflatoxin contamination. This is because preventing presence of insects or fungi from the grains reduce the chances of aflatoxin formation in maize. Many of the

insects act as vectors for fungal pathogens of maize and other plants (Abbas *et al.*, 2009). Among the chemical compounds tested in feeds, propionic acid, sodium propionate, benzoic acid and ammonia were the best anti-fungal compounds, followed by urea and citric acid (Gowda *et al.*, 2004).

Table 8: Storage factors that are significantly associated with aflatoxin contamination in maize across three villages at the end of storage

Parameter	Estimate	Standard Error	t Value	Pr > t
Intercept	-0.0198	0.327	0.06	0.9522
X₁	0.863	0.402	2.15	0.0404*
X₂	0.798	0.134	5.94	<.0001*

For Aflatoxin $Y = -0.0198 + 0.863X_1 + 0.798X_2$; where Y.....X₁ represents store treatment with traditional pesticides/insecticides and X₂ represents grain treatment with chemical pesticides/insecticides

$R^2 = 0.60$; $F = 7.4$

* = Statistically significant at $P < 0.05$

From Table 8 use of chemical pesticides/ insecticides reduced the aflatoxin contamination of the stored maize compared to the use of traditional pesticides. This is because chemical pesticides/insecticides undergone several researches to prove its ability as compared to traditional insecticides/pesticides. Some of the biorational insecticides like spinosad, thiamethoxam have been proved as potential grain protectants against stored insect pests in different parts of the world (Vayias *et al.*, 2010; Arthur, Yue and Wilde, 2004). Hence, the use of pesticides/insecticides reduced the levels of aflatoxin contamination of maize by killing/preventing the spread of insects in grains.

4.3.5 Effect of duration of age of storage facility in use

In maize stored in storage structures that were in use between 1 to 5 years to more than 10 years, the total aflatoxins levels increased with time. However, from 5 to 10 years, the levels of aflatoxin contamination lowered as most of the farmers carried out maintenance to their warehouses. Fifty three percent of the farmers who had stored their crops in structures that are 1 to 5 years old had total aflatoxin means of (16.18 $\mu\text{g/kg}$), 23 % (13.18 $\mu\text{g/kg}$) stored in structure that are 6 to 10 years old and 25 % (19.91 $\mu\text{g/kg}$) stored their maize crops in structures that were more than 10 years old (Table 9).

Table 9: Effect of duration of store use on total aflatoxin content in maize

Duration of store in use	Number of samples	Contaminated samples (%)	Aflatoxin ($\mu\text{g/kg}$)	
			Range	Means \pm SE
1 to 5 years	47	25 (53)	2.30 - 70.50	16.18 ^a \pm 3.88
5to 10 years	21	5 (24)	3.20 - 21.40	13.18 ^a \pm 3.26
More than 10 years	23	14 (61)	3.30 – 53.20	19.91 ^a ^e \pm 3.93

Values are means of positive total aflatoxin levels of maize samples stored in different storage structures.

Means with the different letters (by column) are significantly different ($P < 0.05$).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The conclusion drawn from the research findings was that, storage duration and storage types play important role and influence on the aflatoxin contamination in maize. Thus, the maize stored in polyethylene bags (uncontrolled) for 180 days showed increase on aflatoxin levels with time. At the beginning of storage (day 0), the mean aflatoxin level was low and few maize samples were free from contamination; after 90 days of storage the mean aflatoxin level was increased ; and after 180 days of storage, the mean aflatoxin level was higher than 90th days stored maize samples.

Maize stored while treated by insecticides showed minimum increase in total aflatoxin levels and those stored in control improved bags had low/or no aflatoxin contamination. The mean aflatoxin level for control improved bags also increased with storage time but to lower levels than that of maize in the polythene bags. Maize stored in PICS and chemical treatment maize had low levels of aflatoxin compared to maize treated by traditional method which had higher levels of aflatoxin. It was observed that there was an association and interaction between insect and fungi in stored maize.

Prevention strategies such as proper farm management, introduction of aflasafe can only be effective for aflatoxins that are formed from the farm, Aflasafe is based on the use of atoxigenic strains of *Aspergillus spp.* that work through competitive exclusion of toxigenic strains from the substrate (Abbas *et al.*, 2006). Pre-harvest natural contamination can only be minimized post-harvest by application processing techniques that will minimize subsequently entry into the grains. Pesticide use decreased pest

infestation. Hence, prevention strategies for aflatoxin must be started from the farm, proper drying of grains (to moisture less than 15%) and cleaning of stores to make them free from insect and mould contamination is recommended. Also, use of recommended chemicals during storage and at the required dose for effective protection of the maize and the ultimate consumer of the grain showed positive results in lowered total aflatoxin levels in grains.

5.2 Recommendations

- i. There is need for more research and technology for the prevention and control of aflatoxins, to adapt and promote the application of existing technologies and to generate new technologies that enhance the control of aflatoxins in Tanzania.
- ii. Increased awareness of the impacts of aflatoxins and of potential technologies and approach to address aflatoxin.
- iii. Hazards analysis critical control points (HACCP) should be employed in the agricultural production chain (such as from farm to fork) to minimize the aflatoxin contamination foods.
- iv. Investigation should focus on different pre-and post-harvest crop management systems on aflatoxin contamination in different agro ecologies in Tanzania and the effect of different traditional food processing methods on aflatoxin production so that technologies that result in a significant reduction in aflatoxin levels could be promoted.
- v. There is need to conduct food baskets survey for aflatoxin contamination using uniform sampling protocols and modern analytical methods. This will help to obtain sound and reliable data on aflatoxin incidence in different food crops. This could then be used to design and define control strategies.

REFERENCES

- Abbas, H. K. and Shier, T. (2009). Mycotoxin contamination of agricultural products in the southern United States and approaches to reducing it from pre-harvest to final food products. Mycotoxin Prevention and Control in Agriculture. *American Chemical Society Symposium Series 1031*: 37 – 58.
- Abbas, H. K., Wilkinson, J. R., Zablutowicz, R. M., Accinelli, C., Abel, C. A., Bruns, H. A. and Weaver, M. A. (2009). Ecology of *Aspergillus flavus*, regulation of aflatoxin production and management strategies to reduce aflatoxin contamination of corn. *Toxin Revision 2*(3): 142 – 152.
- Abbas, H. K., Zablutowicz, R. M., Bruns, H. A. and Abel, C. A. (2006). Bio-control of aflatoxin in corn by inoculation with non-aflatoxigenic *Aspergillus flavus* isolates. *Biocontrol Science and Technology 16*: 437 – 449.
- Afolabi, C. G., Bandyopadhyay, R., Leslie, J. F. and Ekpo, E. J. A. (2006). Effect of sorting on incidence and occurrence of fumonisin and fumonisin-producing *Fusarium* species on maize (*Zea mays L.*) in Nigeria. *Journal of Food Protection 69*: 2019 – 2023.
- Akokuah, J. P., Mensah, L. D., Chan, C. and Roskilly, A. (2015). Effect of practices of maize farmers and traders in Ghana on contamination of maize by aflatoxins: Case study of Ejura-Sekyeredumase Municipality. *African Journal of Microbiology Research 9*(25): 1658 – 1666.
- Alvito, P., Martins, C., Assunção, R., Pires, M. J. and Calhau, M. A. (2015). Syndromes associated with children exposure to mycotoxins and health risk assessment to multiple mycotoxins in infant foods. In: *Proceedings of the ASPOMM Meeting Local Mycology Meeting*. Lisbon, Portugal. 10 October, 2015. pp. 1 – 17.

- Amare, T., Mohammed, A., Negeri, M. and Sileshi, F. (2015). Effect of weed control methods on weed density and maize (*Zeamays* L.) yields in west Shewa Orimia Ethiopia. *African Journal of Plant Science* 9(1): 8 – 12.
- Anankware, J. P., Obeng-Ofori, D., Afreh-Nuamah, K., Fatunbi, A. O. and Ansah, F. A. (2013). Use of the triple-layer hermetic bag against the maize weevil, *Sitophilus zea mais* (Mots) in three varieties of maize. *Journal of Biology, Agriculture and Health* 3: 67 – 73.
- Arthur, F. H., Yue, B. and Wilde, G. E. (2004). Susceptibility of stored-product beetles on wheat and maize treated with thiamethoxam: effects of concentration, exposure interval, and temperature. *Journal of Stored Products Research* 40: 527 – 546.
- Atehnkeng, J., Donner, M., Ojiambo, P. S., Ikotun, B., Augusto, J., Cotty, O, J. and Bandyopadhy, R. (2015). Environmental distribution and genetic diversity of vegetative compatibility groups determine biocontrol strategies to mitigate aflatoxin contamination of maize by *Aspergillus flavus* .*Microbial Biotechnology* 9: 75 – 88.
- Bandyopadhyay, R., Kumar, M. and Leslie, J. F. (2007). Relative severity of aflatoxin contamination of cereal crops in West Africa. *Food Additives and Contaminants* 24(10): 1109 – 1114.
- Baoua, I. B., Amadou, L., Ousmane, B., Baributsa, D. and Murdock, L. L. (2014). PICS bags for post-harvest storage of maize grain in West Africa. *Journal Stored Products Research* 58: 20 – 28.
- Baoua, I. B., Margam, V., Amadou, L. and Murdock, L. L. (2012). Performance of triple bagging hermetic technology for postharvest storage of cowpea grain in Niger. *Journey Stored Products Research* 51: 81–85.
- Barbas, C., Dams, A. and Majors, R. E. (2015). Separation of Aflatoxins by HPLC. *Environmental, Food Safety* 67: 1 – 2.

- Battilani, P., Pietri, A., Barbano, C., Scandolara, A., Bertuzzi, T. and Marocco, A. (2008). Logistic regression modeling of cropping systems to predict fumonisin contamination in maize. *Journal of Agricultural and Food Chemistry* 56(21): 10433 – 10438.
- Battilani, P., Toscano, P., Van der-Klerx, H. J., Moretti, A., Camordo, L. M., Brera, C., Rortais, A., Goumpens, T. and Robinson, T. (2016). Aflatoxin B₁ contamination in maize in Europe increases due to climate changes. *Scientific Reports* 6: 1038 – 24328.
- Bhat, R. V., Sherry, P. H., Amruth, R. P. and Sudershan, R. V. (1997). A food borne disease outbreak due to the consumption of mouldy sorghum and maize containing fumonisin mycotoxins. *Journal Toxicology* 35: 249 – 255.
- Binder, E. M., Tan, L. M., Chin, L. J., Handl, J. and Richard, J. (2007). Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal Feed Science and Technology* 137(4): 265 – 282.
- Blandino, M., Reyneri, A. and Vanara, F. (2009). Effect of sowing time on toxigenic fungal infection and mycotoxin contamination of maize kernels. *Journal of Phytopathology* 157: 7 – 14.
- Candida, P., Shirima, M. E., Kimanya, J. L., Kinabo, M. N., Routledge, I., Chou, S., Christopher, P. W. and Yun, Y. G. (2013). *A Prospective Study of Growth and Biomarkers of Exposure to Aflatoxin and Fumonisin During Early Childhood In Tanzania*: Queen's University Belfast, United Kingdom. 28pp.
- Cao, D., Pimentel, D. and Hart, K. (2002). Post-harvest food losses (vertebrates). In: *Encyclopedia of Pest Management*. (Edited by Pimentel, D.), Marcel Dekker, New York. pp. 648 – 649.

- Cardwell, K. F., Kling, J. G., Maziza-Dixon, B. and Bosque-Pérez, N. A. (2000). Interaction between *Fusarium verticillioides*, *Aspergillus flavus*, and insect infestation in four maize genotypes in lowland Africa. *Phytopathology* 90: 276 – 284.
- Chala, A., Taye, W., Ayalew, A., Krska, R., Sulyok, M. and Logrieco, A. (2014). Multimycotoxin analysis of sorghum (*Sorghum bicolor* L. Moench) and finger millet (*Eleusine coracana* L. Garten) from Ethiopia. *Food Control* 45: 29 – 35.
- Channaiah, L. H. and Maiyer, D. E. (2014). Best stored maize management practices for the prevention of mycotoxin contamination. In: *Mycotoxin Reduction in Grain Chains*. (Edited by Leslie, J. F. and Logrieco, A. F.), John Wiley and Sons Limited, Chichester, UK. 15pp.
- Chulze, S. (2010). Strategies to reduce mycotoxin levels in maize during Storage. *Food Additive Contamination* 27: 651 – 657.
- Cleveland, T. E., Dowd, P. F., Desjardins, A. E., Bhatnagar, D. and Cotty, P. J. (2003). United States Department of Agriculture-Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Management Science* 59(7): 629 – 642.
- Cotty, P. J. (1994). Comparison of four media for the isolation of *Aspergillus flavus* group fungi. *Mycopathologia* 125: 157 – 162.
- Cotty, P. J. and Mellon, J. E. (2006). Ecology of aflatoxin producing fungi and biocontrol of aflatoxin contamination. *Mycotoxin Research* 22(2): 110 – 117.
- Cotty, P. J., Probst, C. and Jaime-Garcia, R. (2008). Etiology and management of aflatoxin contamination. In: *Mycotoxins Detection Methods, Management, Public Health and Agricultural Trade*. Edited by Leslie, J. F., Bandyopadhyay, R. and Visconti, A.), Commonwealth for Agriculture Bureau International, Oxfordshire, UK. pp. 287 – 299.

- Craufurd, P. Q., Prasad, P. V. V., Waliyar, F. and Taheri, A. (2006). Drought, pod yield, pre-harvest *Aspergillus* contamination on peanut in Niger. *Field Crops Research* 98: 20 – 29.
- Daniel, J. H., Lewis, L. W., Redwood, Y. A., Kieszak, S., Breiman, R. F., Flanders, W. D., Bell, C., Mwihi, J., Ogana, G., Likiman, S., Sraetemans, M. and McGreehin, M. A. (2011). Comprehensive assessment of maize aflatoxin levels in Eastern Kenya. *Environmental Health Perspective* 119: 1794 – 1799.
- Dashti, B., Al-Hamli, S., Alomirah, H., Al-Zenki, S., Bu Abbas, A. and Sawaya, W. (2009). Levels of aflatoxin M₁ in milk, cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed. *Food Control* 20: 686 – 690.
- De Groote, H., Kimenju, S. C., Likhayo, P., Kanampiu, F., Tefera, T. and Hellin, J. (2013). Effectiveness of hermetic systems in controlling maize storage pests in Kenya. *Journal of Stored Products Research* 53: 27 – 36.
- De Lucca, A. J. (2007). Harmful fungi in both Agriculture and Medicine. *Review Iberoam Micol* 24: 3 – 13.
- Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N. and Panneerselvam, A. (2011). *Aflatoxins and Aflatoxicosis in Human and Animals. Aflatoxins Biochemistry and Molecular Biology*. INTECH, India. 36pp.
- Diaz Rios, L. and Jaffee, S. (2008). *Standards, Competitiveness, and Africa's Groundnut Exports to Europe: Barrier, Catalyst, or Distraction?* Agriculture and Rural Development Department. Discussion Paper No. 39. The International Bank for Reconstruction and Development, Washington DC. 97pp.
- Dorner, J. W. (2009). Development of biocontrol technology to manage aflatoxin contamination in peanuts. *Peanut Science* 36: 60 – 67.

- Dosman, D. M., Adamowicz, W. L. and Hrudey, S. E. (2001). Socioeconomic determinants of health- and food safety-related risk perceptions. *Risk Anal. International Journal* 21: 307 – 317.
- Duarte-Vogel, S. and Villamil-Jimenez, L. C. (2006). Mycotoxins in public health. *Review Salud Public* 1: 129 – 135.
- Galvano, F., Ritieni, A., Piva, G. and Pietri, A. (2005). Mycotoxins in the human food chain. *The Mycotoxin Blue Book*. (Edited by Diaz, D. E.), Nottingham University Press, Nottingham, United Kingdom. pp. 187–224.
- Gao, J., Liu, Z. and Yu, J. (2007). Identification of *Aspergillus* section *flavi* in maize in northeastern China. *Mycopathologia* 11: 91 – 95.
- Gnonlonfin, G. J. B., Hell, K., Adjovi, Y., Fandohan, P., Koudande, D. O., Mensah, G. A., Sanni, A. and Brimer, L. (2013). A review on aflatoxin contamination and its implications in the developing world: a sub-Saharan African perspective. *Critical Revision Food Science* 53: 349 – 365.
- Golli-Bennour, E. E., Kouidhi, B., Bouslimi, A., Abid Essefi, S., Hassen, W. and Bacha, H. (2010). Cytotoxicity and genotoxicity induced by aflatoxin B1, ochratoxin A, and their combination in cultured Vero cells. *Journal Biochemical Molecular Toxicology* 24: 42 – 50.
- Gowda, N. K. S., Ramana, J. V., Prasad, C. S. and Khub, S. (2004). Micronutrient content of certain tropical conventional and unconventional feed resources of Southern India. *Tropical Animal Health Produce* 36(1): 77 – 94.
- Guchi, E. (2015). Implication of aflatoxin contamination in agricultural products. *American Journal of Food and Nutrition* 3(1): 12 – 20.
- Hawkins, L. K., Windham, G. L. and William, W. P. (2005). Effect of different post harvesting drying temperature on *Aspergillus flavus* survival and aflatoxin content in five maize hybrid. *Journal of Food Production* 68(7): 1521 – 1524.

- Hell, K. (1997). Factors contributing to the distribution and incidence of aflatoxin producing fungi in stored maize in Benin. Thesis for Award of PhD Degree at University of Hannover, Germany, 182pp.
- Hell, K. and Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Sahara Africa. *African Journal Microbiology Research* 5: 459 – 466.
- Hell, K., Cardwell, K. F., Setamou, M. and Schulthess, F. (2000). Influence of insect infestation on Aflatoxin contamination of stored maize in four agro-ecological regions in Benin. *African Entomology* 8: 169 – 177.
- Hell, K., Cardwell, K. F., Setamou, M. and Schulthess, F. (2000a). Influence Of insect infestation on aflatoxin contamination of stored maize in four agro-ecological regions in Benin. *African Entomology* 8: 169 – 177.
- Hell, K., Fandoham, P., Bandyopadhyay, R., Kiewnicks, S., Sikora, R. and Cotty, P. J. (2005). Pre and Post-harvest management of Aflatoxin in maize. *An Africa Perspective Mycotoxins Detection Methods, Managements, Public Health and Agriculture Trade*. (Edited by Leslie, J. F., Bandyopadhyay, R. and Visconti, A.). Cromwell Press Town Bridge, UK. pp. 219 – 229.
- Hell, K., Fandohan, P., Bandyopadhyay, R., Cardwell, K., Kiewnick, S., Sikora, R. and Cotty, P. (2008). Pre-and post-harvest management of aflatoxin in maize. In: *Mycotoxins Detection Methods, Management, Public Health and Agricultural Trade*. (Edited by Leslie, J. F., Bandyopadhyay, R. and Visconti, A.), Commonwealth for Agriculture Bureau International, Wallingford, UK. pp. 210 – 219.
- Hell, K., Mutegi, C. and Fandohan, P. (2010). Aflatoxin control and prevention strategies in maize for Sub-sahara Africa. *Green Journal Agriculture Science* 2(280): 534 – 540.

- Hell, K., Ognakossan, K. E., Tonou, A. K., Lamboni, Y., Adabe, K. E. and Coulibaly, O. (2010). Maize stored pests control by PICS-Bags: Technological and Economic Evaluation. [<http://ag.purdue.edu/ipia/pics/document/presentation-%20hell%20-wcc>] site visited on 12/9/2017.
- Hell, K., Setamou, M., Cardwell, K. F. and Poehling, H. M. (2000b). The Influence of storage practices on aflatoxin contamination in maize in four agro ecological zones in Benin, West Africa. *Journal of Stored Products Research* 36: 365 – 382.
- Herrman, T. (2006). *Mycotoxins in Feed Grains and Ingredients*. Grain Science and Industry. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. USA. 8pp.
- Hodges, R. J. and Maritime, C. (2012). Post-harvest weight losses of cereal grains in Sub-Saharan Africa. [[http://www.aphlis.net/downloads/Postharvest_quality_losses %20review_Sept_2012_revised.pdf](http://www.aphlis.net/downloads/Postharvest_quality_losses_%20review_Sept_2012_revised.pdf)] site visited on 6/8/2016.
- Holst, N., Meikle, W. G. and Markham, R. H. (2000). Grain injury model for *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) in rural maize store in West Africa. *Journal of Economic Entomology* 93(4): 1338 – 1346.
- Hosney, R. (2015). The Organic Farmer. Principles of Cereal Science and Technology. *Journal of Science of Agriculture* 86(11): 21 – 24.
- Hussaini, A. M., Michael, F. D., Patrick, B. N., Timothy, A. G. and Godwin, H. O. (2012). Aflatoxin contamination in foods and feeds: A special focus on Africa. *Trends in Vital Food and Control Engineering* 2012: 56 – 90.
- ICRISAT (2006). *Nurturing the Seeds of Success in the Semi-Arid Tropics*. International Crop Research Institute for the Semi-Arid Tropics, Patancheru, India. 60pp.

- Iha, M. H., Barbosa, C. B., Okada, I. A. and Trucksess, M. W. (2013). Aflatoxin M₁ in Milk and Distribution and Stability of Aflatoxin M₁ during Production and Storage of Yoghurt and Cheese. *Food Control* 29: 1 – 6.
- Inter-American Institute for Cooperation on Agriculture (2013). *Post-Harvest Losses in Latin America and the Caribbean: Challenges and Opportunities for Collaboration*. Inter-American Institute for Cooperation on Agriculture, Washington DC, USA. 15pp.
- International Institute of Tropical Agriculture (2010). Trade losses due to aflatoxins. [http://www.aflatoxinpartnership.org/uploads/Trade%20Losses%20Due%20to%20Aflatoxins_Aubee.pdf] site visited on 10/7/2016.
- Ismail, I. J., Srinivas, M. and Tundui, H. (2015). Transaction costs and market participation decisions of maize small holder farmers in Dodoma region, Tanzania. *Global Journal of Biology Agriculture and Health Sciences* 4(2): 12 – 20.
- Jaetzold, R., Schmidt, H., Hornetz, B. and Shisanya, C. A. (2006). *Farm Management Handbook of Kenya. Natural Conditions and Farm Information*. (2nd Edition), Ministry of Agriculture, Nairobi, Kenya. 573pp.
- Jaime, R., McKamey, J. and Cotty, P. J. (2013). Module storage time, leaf grade and seed moisture influence fiber quality and aflatoxin contamination of cotton in South Texas. *Journal of Cotton Science* 17: 60 – 68.
- Jian, F. and Jayas, D. S. (2012). Temperature monitoring. In: *Stored Product Protection*. (Edited by Hagstrum, D. W., Phillips, T. W. and Cuperus, G.), Kansas State University, USA. pp. 217 – 282.

- Kaaya, A. N. and Kyamuhangire, W. (2006). The effect of storage time and agro-ecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. International Past successes and future challenges to the role played by maize in global food security. *Food Security* 3(3): 307 – 327.
- Kaaya, A. N., Kyamuhangire, W. and Kyamanywa, S. (2006). Factors affecting aflatoxin contamination of harvested maize in the three agro-ecological zones of Uganda. *Journal of Applied Science* 6(11): 2401–2407.
- Kaaya, A. N., Warren, H. L., Kyamanywa, S. and Kyamuhan, W. (2005). The effect of delayed harvest on moisture content, insect damage, moulds and aflatoxin contamination of maize in Mayuge district of Uganda. *Journal of the Science of Food and Agriculture* 85: 2595 – 2599.
- Kaaya, N. A. and Warren, H. L. (2005). A review of past and present research on Aflatoxin in Uganda. *African Journal of food Agriculture and Nutritional Development* 5:1 – 17.
- Kabak, B., Dobson, A. D. and Var, I. I. L. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: a review. *Critical Reviews in Food Science and Nutrition* 46(8): 593 – 619.
- Kaliba, A. R. M., Verkuijl, H., Mwangi, W., Byamungu, D. A., Anandajayasekeram, P., and Moshi, A. J. (1998). Adoption of maize production technologies in Western Tanzania. *Southern Africa Center for Cooperation in Agricultural Research* 3(3): 307 – 327.
- Kamala, A., Kimanya, M. E., Audenaert, K., Madeje, R. R., Meulenaar, B., Tiisekwa, B., Degraeve, S., Haesaert, G. and Ortiz, J. (2015). Impact of local pre-harvest management practices in maize on the occurrence of *Fusarium species* and associated mycotoxins in two agro-ecosystems in Tanzania. *Journal in Food Control* 59: 225 – 233.

- Kamala, A., Ortiz, J., Kimanya, M. E., Haesaert, G., Donoso, S., Tiisekwa, B. and De Meulenaer, B. (2015). Multiple mycotoxin co-occurrence in maize grown in three agro-ecological zones of Tanzania. *Food Control* 54: 208 – 215.
- Kankolongu, M. A., Hell, K. and Nawa, I. N. (2009). Assessment for fungal, mycotoxin and insect spoilage in maize stored for human consumption in Zambia. *Journal Science Food Agricultural* 89: 1366 – 1375.
- Kebede, H., Abbas, K. H., Fisher, K. D. and Bellaloui, N. (2012). Relationship between aflatoxin contamination and physiological response of corn plants under drought and heat stress. *Journal Toxins* 4: 1385 – 1403.
- Khlangwiset, P. (2011). Reducing the risks of aflatoxin through public health interventions. Thesis for Award of PhD Degree at University of Pittsburgh, 212pp.
- Kimanya, M. E., De Meulenaer, B., Roberfroid, D., Lachat, C. and Kolsteren, P. (2010). Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania. *Molecular Nutrition Food Research* 54: 1659 – 1667.
- Kimanya, M. E., Meulenaer, B., Caupa, J., Baert, J. and Kolsteren, P. (2012). Strategies to reduce exposure of fumonisins from complementary foods in rural Tanzania. *Maternal and Child Nutrition* 8(4): 503 – 5110.
- Kimanya, M., Tiisekwa, B., Devlieghere, F., Ndomondo-Singoma, M., Van Camp, J. and Kolsteren, P. (2008). Co-occurrence of fumonisins with aflatoxin in home stored maize for human consumption in rural village of Tanzania. *Food Additives and Contaminants* 25: 1353 – 1364.
- Kpodo, K., Thrane, U. and Hald, B. (2000). Fusaria and fumonisins in maize from Ghana and their co-occurrence with aflatoxins. *International Journal of Food Microbiology* 61: 147 – 157.

- Krnjaja, V., Levic, J., Stankovic, S., Petrovic, T., Tomic, Z., Mandic, V. and Bijelic, Z. (2013). Moulds and Mycotoxins in stored maize. Institute for animal husbandry. *Journal in biotechnology in Animal Husbandry* 29(3): 527 – 536.
- Kumar, V., Basu, M. S. and Rejendran, T. P. (2008). Mycotoxin research and mycoflora in some commercially important agricultural commodities. *Crop protection* 27: 891 – 905.
- Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Lubber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M. and Misore, A. (2005). Aflatoxin Contamination of Commercial Maize Products during an Outbreak of Acute Aflatoxicosis in Eastern and Central Kenya. *Environmental Health Perspectives* 113: 1763 – 1767.
- Lombard, M. J. (2014). Mycotoxin exposure and infant and young child growth in Africa: What do we know? *Annals Nutrition and Metabolism* 64: 42 – 52.
- Magoha, H., Kimanya, M., De Meulenaer, B., Roberfroid, D., Lachat, C. and Kolsteren, P. (2014c). *Risk of Dietary Exposure to Aflatoxins and Fumonisin in Infants Less Than 6 Months of Age in Rombo, Northern Tanzania*. John Wiley and Sons Ltd., New York. 12pp
- Mahamoudi, R., Norian, R., Katirae, F. and Pajoni-Alamoti, M. R. (2013). Total aflatoxin of maize produced in different regions of Qazvin-Iran. *International Food Research Journal* 20(5): 2901 – 2904.
- Mazzette, A., Decandia, M., Acciaro, M., Fenu, Francesconi, A. D. and Battacone, A. H. (2009). Excretion of Aflatoxin M₁ in milk of goats fed diet contaminated by Aflatoxin B₁. *Italian Journal of Animal Science*: 8(2): 631 – 633.
- Mboya, R. and Bogale, A. (2012). Mycotoxin Contamination of Food and its possible implications on sustainable development in Rungwe district, Tanzania. *International Journal of Sustainable Development* 5(7): 37 – 46.

- Mboya, R., Tongoona, P., Yoo, K. S., Derera, J., Mudhara, M. and Langyintuo, A. (2011). The quality of maize stored using roof and sack storage methods in Katumba Ward, Rungwe District, Tanzania: Implications on household food security. *Journal of Stored Products and Postharvest Research* 2: 189 – 199.
- Mehl, H. L. and Cotty, P. J. (2012). Variation in competitive ability among isolates of *Aspergillus flavus* from different vegetative compatibility groups during maize infection. *Journal of Phytopathology* 100: 150 – 159.
- Meissonier, G., Marin, D., Galtier, P., Bertin, G., Taranu, I., Oswald, I. and Mengheri, E. (2006). Modulation of the immune response by a group of fungal food contaminant, the aflatoxins. In: *Nutrition and Immunity*. (Edited by Mengheri, E., Roselli, M., Britti, M. S. and Finamore, A.), Research Signpost Kerala, India, pp. 147–166.
- Menale, K., Bekele, S., Asfaw, S., Tsedeke, A., Muricho, G., Ferede, S., Eshete, M. and Assefa, K. (2009). Current Situation and future outlook of the chickpea sub-sector in Ethiopia. [http://www.icrisat.org/tropicallegumesII/pdfs/Current_Situation.pdf] site visited on 20/9/2016.
- Mihale, M. J., Selemani, H. O., Kamatenesi, M. M., Kidukuli, A. W. and Ogendero, J. O. (2009). Use of indigenous knowledge in the management of field and storage pest around Lake Victoria basins in Tanzania. *African Journal of Environmental Science and Technology* 3(9):251 – 259.
- Milani, J. M. (2013). Ecological conditions affecting mycotoxin production in cereals: A review of *Veterinarian Medicine* 58(8): 405 – 411.
- Mollea, C., Marmo, L. and Bosco, F. (2013). Valorization of cheese whey, a byproduct from the dairy industry. [<http://www.intechopen.com/books/food-industry/valorization-of-cheese-whey-a-by-product-from-the-dairy-industry>] site visited 22/8/2016.

- Moturi, W. K. N. (2008). Factors likely to enhance mycotoxin introduction into the human diet through maize in Kenya. *African Journal of Food Agriculture Nutrition and Development* 8(3): 1 – 13.
- Moussa, B., Abdoulaye, T., Coulibaly, O., Baributsa, D. and Lowenberg-DeBoer, J. (2014). Adoption of on-farm hermetic storage for cowpea in West and Central Africa in 2012. *Journal of Stored Products Research* 58: 77 – 86.
- Munkvold, G. P. (2003). Cultural and genetic approaches to managing mycotoxins in maize. *Annual Review of Phytopathology* 41(1): 99 – 116.
- Murdock, L. L. and Baoua, I. B. (2014). On Purdue Improved Cowpea Storage (PICS) technology: background, mode of action, future prospects. *Journal of Stored Products Research* 58: 3 – 11.
- Murdock, L. L., Margam, V., Baoua, I., Balfe, S. and Shade, R. E. (2012). Death by desiccation: Effects of hermetic storage on cowpea bruchids. *Journal of Stored Products Research* 49: 166 – 170.
- Muthoni, J. M., Njenga, L. M., Gathumbi, J. K. and Chemimingwa, G. N. (2009). The occurrence of aflatoxins in maize and distribution of mycotoxin-producing fungi in Eastern Kenya. *Plant Pathology Journal* 8(3):113 – 119.
- Muthoni, J. W., Mereithi, B. K., Cheminingiwa, G. N., Gathumbi, J. K. and Mutit, E. W. (2011). *Aspergillus* species and aflatoxin b₁ in soil, maize grain and flour samples from semi- arid and humidity regions of Kenya. *International Journal of Agriscience* 2(1): 22 – 34.
- Muthoni, J. W., Otien, P. E., Chemining, Wag, G. N., Nderitu, J. H. and Wagacha, J.M. (2008). Effect of Chemical sprays on insect pests and yield quality of food grain legumes. *Journal Entomology* 5(3): 156 – 163.

- Mutungi, C. M., Affognon, H., Njoroge, A. W., Baributsa, D. and Murdock, L. L. (2014). Storage of mung bean (*Vigna radiate* [L] Wilczek) and pigeonpea grains (*Cajanus cajan* [L.] Millsp) in hermetic triple-layer bags stops losses caused by *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Products Research* 58: 39 – 47.
- Mwakalinga, H. A. and Massawe, W. (2007). Output market support. Agricultural Council of Tanzania. [http://www.tap.or.tz/documents/200704_OUTPUT_MARKETING_SUPPORT.pdf] site visited on 04/06/2016.
- Mwihia, J. T., Straetmans, M., Ibrahim, A., Njau, J., Muhenje, O., Gulacha, A., Gikuchi, S., Mutonga, D., Tetteh, C., Likiman, S., Breiman, R. F., Njenga, K. and Lewis, L. (2008). Aflatoxin levels locally grown maize from Makeni District, *East Africa Medical Journal* 85: 311 – 317.
- National Sample Census of Agriculture (2008). *Small Holder Agriculture, Regional Report Dodoma Region*. United Republic of Tanzania, Dar es Salaam, Tanzania. 319pp.
- Ni, X. and Streett, D. A. (2005). Modulation of water activity on fungicide effect on *Aspergillus niger* growth in Sabouraud dextrose agar medium. *Journal in Applied Microbiology* 41: 428 – 433.
- Njoroge, A. W., Affognon, H. D., Mutungi, C. M., Manono, J., Lamuka, P. O. and Murdock, L. L. (2014). Triple bag hermetic storage delivers a lethal punch to *Prostephanus truncates* (Horn) (Coleoptera: Bostrichidae) in stored maize. *Journal Stored Products Research* 58: 12 – 19.
- Nukenine, E. N. (2010). Stored product protection in Africa: Past, present and future. *Julius-Kühn Archive* 425: 23 – 26.
- Nyangi, C., Mugula, J. K., Beed, F., Boni, F., Koyano, E. and Sulyok, M. (2016). Aflatoxin and Fumonisin contamination of marketed maize, maize and maize

- used as animal feed in northern Tanzania. *African Journal of Food, Agriculture, Nutrition and Development* 16(3): 11054 – 11065.
- Nyangi, J. C. (2014). Aflatoxin and Fumonisin contamination of maize and beans along the food and feed value chain in Babati district, Tanzania. Dissertation for Award of MSc Degree at Sokoine University of Agriculture. Morogoro, Tanzania, 129pp.
- Ogunsanwo, B. M., Faboya, O. O. P., Idowu, O. R., Lawal, O. S. and Bankole, S. A. (2004). Effect of roasting on the aflatoxin contents of Nigerian peanut seeds. *African Journal of Biotechnology* 9: 451 – 455.
- Okion, M. N. (2005). The 2014 and 2015 aflatoxin tragedies in Kenya. [<https://www.researchgate.net/publication>]site visited on 10/7/2017.
- Okori, P. (2014). *Report of the Kongwa Kiteto Action Sites Innovation Platform Launch*. International Institute of Tropical Agriculture, Nairobi, Kenya. 18pp.
- Okoth, S. A., Nyongesa, B., Joutsjoki, V., Korhonen, H., Ayugi, V. and Kang'ethe, E. K. (2016). Sclerotia Formation and Toxin Production in Large Sclerotial *Aspergillus flavus* Isolates from Kenya. *Advances in Microbiology* 6: 47 – 56.
- Oladele, D. (2014). The effect of aflatoxin on animals partnership for africa control in Africa. *Aflatoxin Partnership Newsletter* 3(4): 1 – 6.
- Olanya, O. M. and Hoyos, G. H. (1997). Waste corn as point source of inoculum for *Aspergillus flavus* in corn agroecosystem *Plant Disease* 81(6): 576 – 581.
- Oliveira, C. A. F., Goncalves, N. B., Rosim, R. E. and Fernandes, A. M. (2009). Determination of aflatoxin in peanut products in the Northeast Region of Sao Paulo, Brazil. *International Journal Molecular science* 10(1): 174 – 183.
- Omar, H. E. M. (2013). Mycotoxins-Induced Oxidative Stress and Disease. In: *Mycotoxin and Food Safety in Developing countries*. (Edited by Makun, H. A.), In Tech Janeza Trdine, Rijeka, Croatia. pp. 75 – 104.

- Ostry, V., Malii, F. and Pfohl-Leszkowicz, A. (2015). Comparative data concerning aflatoxin contents in Bt maize and non-Bt isogenic maize in relation to human and animal health. *Acta Veterinaria Brno* 84: 47 – 53.
- Passone, M. A., Rosso, L. C., Ciancio, A. and Etcheverry, M. (2010). Detection and quantification of *Aspergillus* section *Flavi* spp. in stored peanuts by real-time PCR of nor-1 gene, and effects of storage conditions on aflatoxin production. *International Journal Food Microbiology* 138: 276 – 281.
- Patel, S. V., Bosamia, T. C., Bhalani, H. N., Singh, P. and Kumar, A. (2015). Aflatoxins: causes and effects. *Monthly Magazine of Agricultural and Biological Science* 13(9): 140 – 142.
- Peraica, M., Radić, B. and Lucić, A. (1999). Toxic effects of mycotoxins in humans. *Bulletin World Health Organization* 77(9): 754 – 766.
- Pirgozliev, S. R., Edwards, S. G., Hare, M. C. and Jenkinson, P. (2003). Strategies for the control of *Fusarium* head blight in cereals. *European Journal of Plant Pathology* 109(7): 731 – 742.
- Pratiwi, C., Rahayu, W. P., Lioe, H. N., Herawati, D., Broto, W. and Ambarwati, S. (2015). The effect of temperature and relative humidity for *Aspergillus flavus* BIO 2237 growth and aflatoxin production on soybeans. *International Food Research Journal* 22(1): 82 – 87.
- Pratiwi, C., Rahayu, W., Lioe, H., Herawati, D., Broto, W. and Ambarwati, S. (2015). The effect of temperature and relative humidity for *Aspergillus flavus* BIO 2237 growth and aflatoxin production on soybeans. *International Food Research Journal* 22(1): 82 – 87.
- Proctor, D. L. (1994). Grain storage techniques-evolution and trends in developing countries. [<http://www.fao.org/docrep/T1838E/T1838E00.htm>] site visited on 20/7/2017.

- Quezada, M. Y., Moreno, J., Vázquez, M. E., Mendoza, M., Méndez-Albores, A. and Moreno-Martínez, E. (2006). Hermetic storage system preventing the proliferation of *Prostephanus truncatus* Horn and storage fungi in maize with different moisture contents. *Postharvest Biology and Technology* 39(3): 321 – 326.
- Raphael, M. W., Prosper, K. D., Dismas, L. M., Catherine, P. M., Dorothy, M. M., Lilian J. M. and George, M. I. (2016). Promotion of agricultural innovation systems approach: policy implications for maize extension and advisory services in Tanzania. *Tanzania Journal of Agricultural Sciences* 14(2): 112 – 118.
- Reddy, K. R., Seepaul, R., Henry, W. B., Gajanayake, B., Lokhande, S. and Brand, D. (2014). Maize (*Zea mays L*) yield and aflatoxin accumulation responses to exogenous glycinebetaine application. *International Journal of Plant Production* 8(2): 1735 – 6814.
- Reverberi, M., Zjalic, S., Ricelli, A., Di Meo, C., Scarpari, M., Fanelli, C. and Fabbri, A. (2011). Mushrooms versus fungi: natural compounds from *Lentinula edodes* inhibit aflatoxin biosynthesis by *Aspergillus parasiticus*. *World Mycotoxin Journal* 4: 217 – 224.
- Robbins, C. A., Swenson, L. J., Nealley, M. L., Gots, R. E. and Kelman, B. J. (2000). Health effect of mycotoxin in indoor air. Critical review Apple. *Occupation Environmental Hygiene* 15(10): 773 – 784.
- Sanjarani, A. (2015). The study of Aflatoxin Types and Methods of Detection, Purification, Identification and their Effects on Human and Animal. *Science Road Journal* 3(1): 229 – 235.

- Sanon, A., Dabiré-Binso, L. C. and Ba, N. M. (2011). Triple-bagging of cowpeas within highdensity polyethylene bags to control the cowpea beetle *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). *Journal of Stored Products Research* 47(3): 210 – 215.
- Sardinas, N., Vazquez, C., Gil-Serna, J., González-Jaén, M. T. and Patiño, B. (2011). Specific detection and quantification of *Aspergillus flavus* and *Aspergillus parasiticus* in wheat flour by SYBR Green quantitative PCR. *International Journal Food Microbiology* 145: 121 – 125.
- Scheinfeld, N. S. and Mokashi, A. (2010). Protein energy malnutrition. [<http://emedicine.medscape.com>] site visited on 15/8/2017.
- Schmalle, D. G. and Munkvold, G. P. (2015). Mycotoxins in Crops: A threat to human and domestic. *Animal Health* 5: 45 – 83.
- Scully, B. T., Krakowsky, M. D., Ni, X., Wilson, J. P., Lee, R. D. and Guo, B. Z. (2009). Pre harvest aflatoxin contamination of corn and other grain crops grown on the US Southern Coastal Plain. *Tax Review* 28: 169 – 179.
- Setarnou, M., Cardwell, K. F., Schulthess, F. and Hell, K. (1998). Effect of insect damage to maize ears, with special reference to *Mussidia nigrivene* Ua (Lepidoptera: Pyralidae), on *Aspergillus flavus* (Deuteromycetes: Monoliales) infection and aflatoxin production in maize before harvest in the Republic of Benin. *Journal of Economic Entomology* 91: 433 – 438.
- Shaaban, I., Kimanya, M. E., Gichuhi, P. N., Bonsi, C. and Bovell-Benjamin, A. C. (2015). Maize storage and consumption practices of farmers in Handeni, District, Tanzania: Corollaries for mycotoxin contamination *Open Journal of Preventive Medicine* 5: 330 – 339.

- Shephard, G. S. (2008). Risk assessment of aflatoxins in food in Africa. *Food Additives and Contaminants: Part A: Chemistry, Analysis, Control, Exposure and Risk Assessment* 25(10): 1246 – 1256.
- Sheriff, S. O., Salama, E. E. and Abdel-Wahhab, M. A. (2009). Mycotoxin and child health: the need for health risk assessment. *International Journal Hygiene Environmental Heath* 212: 347 – 368.
- Shiferaw, B., Prasanna, B. M., Hellin, J. and Banziger, M. (2011). Crops that feed the World 6. Past successes and future challenges to the role played by maize in global food security. *Food Security* 3(3): 307 – 327.
- Shirima, C. P., Kimanya, M. E., Routledge, M. N., Srey, C., Kinabo, J. L., Humpf, H. U., Wild, C. P., Tu, Y. K. and Gong, Y. Y. (2014). A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. *Environment Health Perspective* 123(2): 173 – 178.
- Smith, L. and Subandoro, A. (2012). *Measuring Food Security Using Household Expenditure Surveys. Food Security in Practice Series*. International Food Policy Research Institute, Washington DC. 147pp.
- Smith, L. E., Stoltzfus, R. J. and Prendergast, A. (2012). Food chain mycotoxin exposure, gut health and impaired growth: A concepture framework. *Advances in Nutrition. An International Review Journal* 3(4): 526 – 531.
- Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R., Brune, M., De-Cock, K., Wagacha, J. M. and Muthomi, J. W. (2008). Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology* 124: 1–12.

- Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R., Brune, M. N., DeCock, K., Dilley, A., Groopman, J., Hell, K., Henry, S. H., Jeffers, D., Jolly, C., Jolly, P., Kibata, G. N., Lewis, L., Njapau, H., Ong, C. N., Onsongo, M. T. K., Page, S. W. and Wilson, D. (2006). Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspectives* 114(12): 1898 – 1903.
- Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R., Brune, M., DeCock, K., Dilley, A., Groopman, J., Hell, K., Henry, S. H., Jeffers, D., Jolly, C., Jolly, P., Kibata, G. N., Lewis, L., Liu, X., Lubber, G., McCoy, L., Mensah, P., Miraglia, M., Misore, A., Njapau, H., Ong, C., Onsongo, M. T. K., Page, S. W., Park, D., Patel, M., Phillips, T., Pineiro, M., Pronczuk, J., Schurz Rogers, H., Rubin, C., Sabino, M., Schaafsma, A., Shephard, G., Stroka, J., Wild, C., Williams, J. T. and Wilson, D. (2006). Workgroup Report: Public health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspectives* 114: 1989 – 1903.
- Suleiman, R. and Rosentrater, K. (2015). *Current Maize Production, Postharvest Losses and the Risk of Mycotoxin Contamination in Tanzania*. Iowa State University, USA. 128pp
- Suleiman, R., Rosentrater, K. and Bern, C. (2013). Effect of Deterioration Parameters on storage of maize: A review *Journal of Natural Sciences Research* 3(9):147 – 165.
- Summer, E. P. and Lee, D. (2009). Reducing aflatoxin in corn during harvest and storage. *Learning for Life Bulletin* 1231: 4 – 12.
- Tanaka, K., Sago, Y., Zheng, Y., Nakagawa, H. and Kushiro, M. (2007). Mycotoxins in rice. *International Journal of Food Microbiology* 119(2): 59 – 66.

- Tanzania Foods and Drugs Authority (2012). Aflatoxin contamination and potential solution for its control in Tanzania. *A Summary of the County and Economic Assessment Conducted in 2012 and the Aflatoxin Stakeholder Workshop*, held in 3 – 4 December, 2012 in Dar es Salaam. pp. 1 – 7.
- TBS (2004). *Rice Specification. TZS 592*. Bureau of Standards Tanzania, Dar es Salaam, Tanzania. 6pp.
- Tedihon, E., Olatinwo, R., Hell, K., Hau, B. and Hoogenboom, G. (2012). Effect of variety cropping systems and soil inoculation with *Aspergillus flavus* on aflatoxin levels during storage of maize. *Tropical Plant Pathology* 37: 25 – 36.
- Tedihou, E., Olatinwo, R., Hell, K., Hau, B. and Hoogenboom, G. (2012). Effects of variety, cropping system and soil inoculation with *Aspergillus flavus* on aflatoxin levels during storage of maize. *Tropical Plant Pathology* 37: 25 – 36.
- Udoh, J. M., Cardwell, K. F. and Ikotun, T. (2000). Storage structures and aflatoxin Content of maize in five agro-ecological zones of Nigeria. *Journal of Stored Products Research* 36: 187 – 201.
- Vayias, B. J., Athanassiou, C. G., Milonas, D. N. and Mavrotas, C. (2010). Persistence and efficacy of spinosad on wheat, maize and barley grains against four major stored product pests. *Crop Protection* 29: 496 – 505.
- Waaliyar, F., Osiru, M., Ntare, B. R., Kumar, V. K., Sudini, H., Traore, A., Dianna, B. (2014) Post- harvest management of aflatoxin contamination in groundnuts. *World Mycotoxin Journal* 8(2): 245 – 252.
- Wagacha, J. M. and Muthoni, J. N. (2008). Mycotoxin problems in Africa: Current status, implication to food safety, health and possible management strategies. *International Journal of Food Microbiology* 124: 1 – 12.

- Waliyar, F., Reddy, S. V., Subramaniam, K., Reddy, T. Y., Rama Devi, K., Craufurd, P. Q. and Wheeler, T. R. (2003). Importance of mycotoxins in food and feed in India. *Aspects of Applied Biology* 68: 147 – 154.
- Wicklow, D. T. and Pearson, T. C. (2014). Detection and removal of single mycotoxin contaminated maize grains following harvest. *9th International Working Conference on Stored Product Protection*, 15 – 18 October 2006, Campinas. pp. 109 – 119.
- Widstrom, N. W., Butron, A., Guo, B. Z., Wilson, D. M., Snook, M. E. and Cleveland, T. E. (2003). Control of pre harvest aflatoxin contamination in maize by pyramiding QTL involved in resistance to ear-feeding insects and invasion by *Aspergillus spp.* *European Journal Agronomy* 19: 563 – 572.
- Wild, C. and Gong. Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis* 31: 71 – 82.
- Wild, C. P. and Hall, A. J. (2000). Primary prevention of hepatocellular carcinoma in developing countries. *Mutation Research/Reviews in Mutation Research* 462(2): 381 – 393.
- Williams, P. W. and Windham, G. L. (2015). Aflatoxin accumulation in a maize Diallel Gross. *Journal Agriculture* 5: 344 – 352.
- Williams, S. B., Baributsa, D., and Woloshuk, C. (2014). Assessing Purdue Improved Crop Storage bags to mitigate fungal growth and aflatoxin contamination. *Journal of Stored Products Research* 59: 190 – 196.
- World Health Organization (2006). Publications of the world health organization can be obtained. [http://www.who.int/whr/2006/whr06_en.pdf<http://www.promedmail.org/post/4310887>] site visited on 13/9/2016.
- Wu, F. (2006). Mycotoxin reduction in Bt corn: potential economic, health, and regulatory impacts. *Transgenic Research* 15: 277 – 289.

- Wu, F. (2007). Bt Corn and impact on mycotoxins. *Perspective Agriculture Veterinary Science Nutrition Natural Resource* 2(060): 1 – 8.
- Wu, F. and Khlangwiset, P. (2010). Health economic impacts and cost-effectiveness of aflatoxin-reduction strategies in Africa: case studies in biocontrol and post-harvest interventions. *Food Additives and Contaminants* 27(4): 496 – 509.
- Wu, F. and Khlangwiset, P. (2010). Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: Case studies in biocontrol and postharvest interventions. *Chemistry Analysis Control Expo Risk Assess* 27(4): 496 – 509.
- Wu, F., Bhatnagar, D., Bui-Klimke, T., Carbone, I., Hellmich, R., Munkvold, G., Paul, P., Payne, G. and Takle, E. (2011). Climate change impacts on mycotoxin risks in US maize. *World Mycotoxicology Journal* 4: 79 – 93.
- Yin, L. Y., Yan, L. Y., Jiang, J. H. and Ma, Z. H. (2008). Biological control of aflatoxin contamination of crops. *Journal of Zhejiang University Science* 9: 787 – 792.
- Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society* 15: 129 – 144.
- Zorya, S., Morgan, N. and Rios, L. D. (2011). *Missing Food: The Case of Postharvest Grain Losses in Sub-Saharan Africa*. Report No. 60371. World Bank, Washington DC. 230pp.

APPENDICES

Appendix 1: Sampling protocol for maize from field, and household villages in Kongwa, Tanzania

This document was adapted from the International Institute of Tropical Agriculture (IITA) main sampling protocol and used as a guide for collection of dried maize samples for aflatoxin analyses three villages of Manungu, Mlanga and Kongwa. Samples will be collected from farmer fields and stores. Within a household, field and stored samples were collected separately. The samples collected were delivered to IITA in Dar es Salaam as soon as the work completed for grinding, sub-sampling and analysis of Aflatoxin.

Materials required in the field

- Protocols:
 - 1 copy for the District Agricultural Officer (DALDO)
 - 1 copy for extension officer of the village where samples are collected
 - 1 copy for the interviewer
- Questionnaires: the total number of questionnaires were 90 for crop (Maize) in three villages.
- Coloured-printed Aflatoxin factsheets in English for DALDO and extension officer
- Coloured-printed Aflatoxin factsheets in Kiswahili:
 - 1 copy for each farmer giving a sample
 - 4 copies for the village government office
- A GPS handsets

- Paper bags (A3 envelopes) for keeping collected samples from each sampling station (household or market). One envelope for one sample. There will be a need of 150 pieces of envelopes.
- Rubber bands to tight each collected sample
- Polyethylene bags of 50kg capacity to store the collected samples: About 4 pieces of such bags will be needed for the 150 samples
- A clip board for holding the documents during administering the questionnaires
- Pencils for data recording on the questionnaire. 5 pieces
- Pencil sharpeners, 1 pieces
- Marker pens, 12 pieces
- Sisal rope, to use a 10m long piece for tying up the polyethylene bags containing samples after collection
- 1 Umbrella

Villages Survey Planning Meeting

The DALDO were consulted to provide village map to facilitate planning for the villages to be visited. He/she will also provide phone numbers of the local Extension Officers located in the villages to be visited. The sampling unit will be randomly selected from the village register provided by the DALDO and extension officers.

Sampling methods and procedures

In order to provide representative samples and consistence in sampling method for all villages and all surveys (there will be several surveys to collect the required samples per village per crop), the following points should be taken into account:

- In the three villages a total of 90 samples will be collected from maize along food chain. Samples will be collected from different sampling unit production (farmers), household (storage).
 - i. Samples will be collected at pre-harvest in the field such as 0, and post-harvest (at storage) 90 and 180 days. They will be collected both from normal fields and improved methods of production (trial farms). A total of 90 samples will be collected in three villages such as 0 day 30 samples, 90 days 30 samples and 180 days 30 samples.
 - ii. When sampling from storage structures (static lots—bins, sacks, or containers), multiple probing will be used. Small numbers of samples will be collected from different areas of a container and then mixed to produce a representative sample. Samples will be taken with a probe, at three levels: top, middle, and bottom. At each level, samples of 1 kg are taken randomly and then mixed. Then, 1 kg is drawn at random from the mix. A total of 90 samples will be collected (10 unit x 1 sample x 3 villages x 3 months).
- Clear and detailed explanation will be given to farmers about what is needed and for which purpose. This will minimize farmers' suspicions. I will not collect only clean samples, the bad samples are more likely to have aflatoxin contamination
- Effective time use
 - Samples from store will be collected at the same time.

Sampling procedures

1. The interviewee will be briefly and clearly explained the intention and reason of collecting from him/her the sample of the crop in question.
2. The questionnaire provided should be filled with all information needed as indicated.
3. Coloured-printed photographs (fact sheet) of crops infected with aflatoxin will be shown to the interviewee and ask if he/she has seen such symptoms in his/her crop. Fill in his/her answer on the appropriate space in the questionnaire.
4. For samples from the field: samples will be taken following the two diagonals of the field and stop at regular intervals to pick sample to have as representative sample as possible. Estimate the distances of the diagonals to decide on the number of stops to have a sample of 1kg.
5. For stored sample: sub-samples will be drawn from each package, if there are more than one packages of the same lot as will be explained by the interviewee, then mix the sub-samples to have the required quantity of sample.
6. If the farmer has two lots of crop, say a good lot for human consumption as food and another lot of especially spoiled crop for livestock or other uses, two separate samples will be taken. In this case, the sample code will be the same for each sample except that the one for human food will be marked "A" and the one for livestock will be marked "B".
7. Put the sample in the paper bag (envelope) provided.
8. A pencil will be used to write a label by copying the sample code already filled in on the questionnaire on a piece of paper and put this label inside the envelope containing the sample.
9. The envelope containing the sample will be rolled and the labelled from the bottom upwards, and when reaching the flap remove the paper tissue from the flap to

expose its sticky side, then press the flap on the side of the envelope to hold on and prevent unrolling.

10. Correctly the same sample code will be written on the roll and wrap on a rubber band.

Note: Sample code writing on the questionnaire is very important and labels must be written onto outside of envelope and also onto a piece of paper placed inside the envelope. The label should be made up of the following sequence: **day, month, year / crop / village / farm code.**

The crop names will have to be abbreviated (MA. for maize) but the village names written in full. In the sample code everything will be written in capital letters, for example, 260915/MA/KONGWA/01 where 26 stands for the 26th day, 09 for the month of September and 15 for the year 2015. The next sample collected on the same day in this example will then be 260915/MA/KONGWA/02

The rolled envelope of the sample will be placed in the polyethylene sack and proceed for the next sampling station (household/market).

11. When the polyethylene sack has accommodated samples of approximately 50kg, it will be tied at the 'neck' using sisal rope and packed in the car. Start another empty sack in the next sampling station.
12. Keep all samples dry in the vehicle and all the time; avoid any moisture risk. For this reason a vehicles used in these surveys are preferably station wagons to avoid spoilage by rain if the vehicle is a pick-up.
13. Temporary storage of the samples waiting for dispatch to IITA Dar es salaam should also be done in a moisture-free environment.

14. The samples will be dispatched to IITA Dar es salaam, while ensuring moisture-free environment.

In case the collected sample is not completely dry (i.e. feels moist and cool to the touch) the sample must be air-dried. This should be done in either the village or ward or district office room or in the hotel room. This is to avoid keeping moist samples that may develop unwanted microbes and contaminants. This should be done carefully with clear labelling in order to avoid mixing up samples and in a way to prevent spoilage, theft, wind disruption or eaten by animals.

Appendix 2: Determination of aflatoxin in maize by Neogen ELISA

Storage length	Farm code	Village	Sex	Education	Aware of mycotoxin	Health problem	Storage pests	Do you remove old	Storage facilities treatment	Seasons the storage has been used	Drying methods	Storage type	Sorting	Criteria	Total aflatoxin (ppb)
0	06MA	1	M	Primary	No	No	Insect	Yes	Cleaning	>10	dry on field	Polypropylene bags	No	No	4
0	05MA	1	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Polypropylene bags	Yes	colour	5.5
0	04MA	1	M	Primary	No	No	Yes	Yes	Insecticides	1	dry on field	Polypropylene bags	No	No	1.8
0	03MA	1	F	Primary	No	No	Yes	Yes	Insecticides	1	dry on field	Polypropylene bags	No	No	11.5
0	07MA	1	M	Primary	No	No	Yes	Yes	Insecticides	>5	dry on field	Polypropylene bags	No	No	1.3
0	02MA	1	M	Primary	No	No	Yes	Yes	Insecticides	>10	dry on field	Polypropylene bags	No	No	0
0	01MA	1	F	Primary	No	No	Yes	Yes	Spray with actelic	>5	on bare ground	Polypropylene bags	No	No	0
0	08MA	1	M	Primary	No	No	Yes	Yes	Insecticides	>9	dry on field	Cribs	No	No	3.2
0	01MLA	2	M	Primary	No	No	Yes	Yes	Pesticides	>5	Platform	Polypropylene bags	No	No	0
0	02MLA	2	M	Primary	No	No	Yes	Yes	Insecticides	>9	on bare ground	Polypropylene bags	No	No	0
0	04MLA	2	M	Primary	No	No	Yes	Yes	Insecticides	18	on bare ground	Polypropylene bags	No	No	0.4
0	05MLA	2	M	Primary	No	No	Yes	Yes	Insecticides	>5	Platform	Polypropylene bags	No	No	0
0	06MLA	2	M	Primary	No	No	Yes	Yes	Insecticides	5	on bare ground	Polypropylene bags	No	No	0
0	07MLA	2	M	Primary	No	No	Yes	Yes	Insecticides	12	Platform	Polypropylene bags	No	No	0
0	08MLA	2	M	Primary	No	No	Yes	Yes	Insecticides	>5	Platform	Polypropylene bags	No	No	0
0	09MLA	2	M	Tertiary	No	No	Yes	Yes	Insecticides	>5	Platform	Polypropylene bags	No	No	0.3
0	10MLA	2	F	Primary	No	No	Yes	Yes	Insecticides/r odent trap	>5	Platform	Polypropylene bags	Yes	colour	0
0	01KO	3	M	Primary	No	No	Yes	Yes	Insecticides	>5	on bare ground	Polypropylene bags	No	No	0.3

Storage length	Farm code	Village	Sex	Education	Aware of mycotoxin	Health problem	Storage pests	Do you remove old	Storage facilities treatment	Seasons the storage has been used	Drying methods	Storage type	Sorting	Criteria	Total aflatoxin (ppb)
0	03KO	3	M	Primary	No	No	Yes	Yes	Insecticides	>10	Platform	Polypropylene bags	No	No	24.4
0	04KO	3	M	Primary	No	No	Yes	Yes	Burnt Goat Dung	>6	Platform	Polypropylene bags	Yes	colour	18.8
0	05KO	3	M	Primary	No	No	Yes	Yes	Insecticides	>10	Platform	Polypropylene bags	No	No	24.5
0	06KO	3	M	Primary	No	No	Yes	Yes	Insecticides	>10	on bare ground	Polypropylene bags	No	No	0
90	01MATRD	1	M	Primary	No	No	Yes	Yes	Insecticides	>10	Platform	Polypropylene bags	Yes	colour, size	43.8
90	01MAIMP	1	M	Primary	No	No	Yes	Yes	cleaning	>10	Platform	Super grain safe bags	No	N/A	9.8
90	02MATRD	1	M	Primary	No	No	Yes	Yes	cleaning	>6	on bare ground	Polypropylene bags	Yes	colour, Insect	0.7
90	02MAIMP	1	M	Primary	No	No	Yes	Yes	cleaning	>6	on bare ground	Super grain safe bags	Yes	Colour, Insect	0
90	03MATRD	1	M	Primary	No	No	Yes	Yes	cleaning	2	on bare ground	Polypropylene bags	Yes	colour, Insect	0.8
90	03MAIMP	1	M	Primary	No	No	Yes	Yes	cleaning	2	on bare ground	Super grain safe bags	Yes	colour, Insect	0.1
90	04MATRD	1	M	Primary	No	No	Yes	Yes	cleaning	1	on bare ground	Polypropylene bags	Yes	Colour, Insect	1.9
90	04MAIMP	1	M	Primary	No	No	Yes	Yes	cleaning	1	on bare ground	Super grain safe bags	Yes	colour, Insect	0.4
90	05MATRD	1	M	Primary	No	No	Yes	Yes	cleaning	>3	on bare ground	Polypropylene bags	No	colour, Insect	12.3
90	05MAIMP	1	M	Primary	No	No	Yes	Yes	cleaning	>3	on bare ground	Super grain safe bags	No	colour, Insect	0.7
90	06MATRD	1	M	Primary	No	No	Yes	Yes	cleaning	1	on bare ground	Polypropylene bags	No	No	9.9
90	06MAIMP	1	M	Primary	No	No	Yes	Yes	cleaning	1	on bare ground	Super grain safe bags	No	No	0.8
90	01MLATRD	2	M	Primary	No	No	Yes	Yes	Insecticides	1	Platform	Super grain safe bags	Yes	No	0.9
90	01MLAIMP	2	M	Primary	No	No	Yes	Yes	Insecticides	1	Platform	Polypropylene bags	No	No	0.4

Storage length	Farm code	Village	Sex	Education	Aware of mycotoxin	Health problem	Storage pests	Do you remove old	Storage facilities treatment	Seasons the storage has been used	Drying methods	Storage type	Sorting	Criteria	Total aflatoxin (ppb)
90	02MLATRD	2	M	Secondary	No	No	Yes	Yes	No	1	on bare ground	Polypropylene bags	No	-	16.8
90	02MLAIMP	2	M	Secondary	No	No	Yes	Yes	No	1	on bare ground	Super grain safe bags	No	No	6.9
90	03MLATRD	2	F	Primary	Yes	No	Yes	Yes	Insecticides	1	Mats	Polypropylene bags	No	No	0.3
90	03MLAIMP	2	M	Tertiary	Yes	No	Yes	Yes	Insecticides	1	Mats	Super grain safe bags	No	No	0
90	04MLA TRD	2	M	Primary	No	No	Yes	Yes	Insecticides	>5	on bare ground	Polypropylene bags	Yes	colour	0
90	04MLA IMP	2	M	Primary	No	No	Yes	Yes	No	>5	on bare ground	Super grain safe bags	Yes	colour	28.3
90	05MLA TRD	2	F	Primary	No	No	Yes	Yes	Insecticides	4years	on bare ground	Polypropylene bags	Yes	colour, damaged	2
90	05MLA IMP	2	F	Primary	No	No	Yes	Yes	Insecticides	4years	on bare ground	Super grain safe bags	Yes	colour, damaged	2.5
90	01KOTRD	3	M	Primary	Yes	No	Yes	Yes	Insecticides	3	Platform	Polypropylene bags	Yes	Damage	0
90	01KOIMP	3	M	Primary	Yes	No	Yes	Yes	No	3	Platform	Super grain safe bags	No	No	9.6
90	02 KOTRD	3	M	Primary	Yes	No	Yes	No	no cleaning	>5	on bare ground	Polypropylene bags	Yes	colour, mould	0.9
90	02KO IMP	3	M	Primary	Yes	No	Yes	Yes	Spray with bami force	1	on bare ground	Super grain safe bags	Yes	No	0.1
90	03KO TRD	3	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Polypropylene bags	No	No	8.1
90	03 KO IMP	3	M	Primary	No	No	Yes	Yes	No	1	on bare ground	Super grain safe bags	No	No	0.3
90	04 KO TRD	3	M	Primary	No	No	Yes	Yes	Insecticides	>10	on bare ground	Polypropylene bags	No	No	0.9
90	04 KOIMP	3	M	Primary	No	No	Yes	Yes	No	>10	on bare ground	Super grain safe bags	No	No	19.2
90	05 KO TRD	3	M	Primary	No	No	Yes	Yes	Animal dang	>10	Platform	Polypropylene bags	No	No	18.5
90	05 KOIMP	3	M	Primary	No	No	Yes	Yes	No	>10	Platform	Super grain safe bags	No	No	6.1

Storage length	Farm code	Village	Sex	Education	Aware of mycotoxin	Health problem	Storage pests	Do you remove old	Storage facilities treatment	Seasons the storage has been used	Drying methods	Storage type	Sorting	Criteria	Total aflatoxin (ppb)
180	01MAIMP	1	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Polypropylene bags	No	No	22.7
180	01MATRD	1	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Super grain safe bags	No	No	70.4
180	02MAIMP	1	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Polypropylene bags	No	No	13
180	02MATRD	1	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Super grain safe bags	No	No	0
180	03MAIMP	1	M	Primary	No	No	Yes	Yes	Animal dang	1	on bare ground	Polypropylene bags	No	No	70.5
180	03MATRAD	1	M	Primary	No	No	Yes	Yes	Animal dang	1	on bare ground	Super grain safe bags	No	No	9.4
180	04MAIMP	1	M	Primary	No	No	Yes	Yes	Insecticides	>10	on bare ground	Polypropylene bags	No	No	53.2
180	04MATRD	1	M	Primary	No	No	Yes	Yes	Insecticides	>10	on bare ground	Super grain safe bags	No	No	3.3
180	05MAIMP	1	M	Primary	No	No	Yes	Yes	Insecticides	1	Platform	Polypropylene bags	No	No	8.8
180	05MATRD	1	M	Primary	No	No	Yes	Yes	Insecticides	1	Platform	Super grain safe bags	No	No	3
180	06MAIMP	1	M	Primary	No	No	Yes	Yes	Insecticides	>3	on bare ground	Polypropylene bags	No	No	9.5
180	06MATRD	1	M	Primary	No	No	Yes	Yes	Insecticides	>3	Mats	Super grain safe bags	No	No	0
180	01MLA IMP	2	M	Primary	No	No	Yes	Yes	Insecticides	1	Mats	Polypropylene bags	No	No	13.2
180	01MLATRD	2	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Super grain safe bags	No	No	0
180	02MLAIMP	2	F	Primary	No	No	Yes	Yes	Insecticides	>10	on bare ground	Polypropylene bags	No	No	8.6
180	02MLATRD	2	F	Primary	No	No	Yes	Yes	Insecticides	>10	Platform	Super grain safe bags	No	No	0.1
180	03MLAIMP	2	M	Primary	No	No	Yes	Yes	Insecticides	1	Platform	Polypropylene bags	No	No	3.2
180	03MLATRD	2	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Super grain safe bags	No	No	1.4

Storage length	Farm code	Village	Sex	Education	Aware of mycotoxin	Health problem	Storage pests	Do you remove old	Storage facilities treatment	Seasons the storage has been used	Drying methods	Storage type	Sorting	Criteria	Total aflatoxin (ppb)
180	04MLAIMP	2	M	Primary	No	No	Yes	Yes	Insecticides	>2	on bare ground	Polypropylene bags	No	No	21.4
180	04MLATRD	2	M	Primary	No	No	Yes	Yes	Insecticides	>2	on bare ground	Super grain safe bags	No	No	2
180	05MLAIMP	2	F	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Polypropylene bags	No	No	4.6
180	05MLATRD	2	F	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Super grain safe bags	No	No	0.6
180	06MLAIMP	2	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Polypropylene bags	No	No	52
180	06MLATRD	2	M	Primary	No	No	Yes	Yes	Insecticides	1	Platform	Super grain safe bags	No	No	0.9
180	07MLAIMP	2	M	Primary	No	No	Yes	Yes	Insecticides	1	Platform	Polypropylene bags	No	No	23
180	07MLATRD	2	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Super grain safe bags	No	No	2.3
180	01KOIMP	3	M	Primary	Yes	No	Yes	Yes	Insecticides	>10	on bare ground	Polypropylene bags	Yes	No	22.7
180	01KOTRD	3	M	Primary	Yes	No	Yes	Yes	Insecticides	>10	on bare ground	Super grain safe bags	Yes	No	0.6
180	02KOIMP	3	M	Primary	Yes	No	Yes	Yes	Insecticides	1	Platform	Polypropylene bags	Yes	No	9.4
180	02KOTRD	3	M	Primary	Yes	No	Yes	Yes	Insecticides	1	Platform	Super grain safe bags	Yes	No	0
180	03KOIMP	3	M	Primary	No	No	Yes	Yes	Cleaning	>4	on bare ground	Polypropylene bags	No	No	13
180	03KOTRD	3	M	Primary	No	No	Yes	Yes	Cleaning	>4	on bare ground	Super grain safe bags	No	No	0
180	04KOIMP	3	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Polypropylene bags	No	No	2.7
180	04KOTRD	3	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Super grain safe bags	No	No	0.4
180	05KOIMP	3	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Polypropylene bags	No	No	22.1
180	05KOTRD	3	M	Primary	No	No	Yes	Yes	Insecticides	1	on bare ground	Super grain safe bags	No	No	3.4

Appendix 3: Determination of aflatoxin in maize by means of LC-MS/MS

Samples	Conc. (µg/kg) DON	Conc. (µg/kg) AFG2	Conc. (µg/kg) AFG1	Conc. (µg/kg) AFB2	Conc. (µg/kg) AFB1	Conc. (µg/kg) HT-2	Conc. (µg/kg) T-2 Toxin	Conc. (µg/kg) HT- 2 + T-2	Conc. (µg/kg) FB1	Conc. (µg/kg) FB2	Conc. (µg/kg) FB1+FB2	Conc. (µg/kg) OTA	Conc. (µg/kg) ZEN
12022016/MA/MA2-IMP	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	153.0	54.8	207.8	<CCα	<CCα
12032015/KO/MA2-TRD	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	432.7	187.2	619.8	<CCα	<CCα
13032016/MLA/MA/2-IMP	112.2	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	1005.5	479.3	1484.7	<CCα	<CCα
1240/MA/MA/1-IMP	361.9	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	1570.2	847.7	2417.9	<CCα	<CCα
12032015/MLA/MA/1-IMP	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	165.4	100.8	266.2	<CCα	77.8
12062015/MLA/MA/1-IMP	<CCα	<CCα	<CCα	4.2	39.6	<CCα	<CCα	<CCα	190.9	104.9	295.8	<CCα	<CCα
12022016/MA/MA/3-IMP	<CCα	<CCα	<CCα	16.4	85.2	<CCα	<CCα	<CCα	600.2	338.5	938.7	<CCα	<CCα
12072015/MLA/MA/2-TRD	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	1017.0	479.8	1496.7	<CCα	<CCα
12052015/MLA/MA/3-IMP	<CCα	<CCα	<CCα	6.5	59.0	<CCα	<CCα	<CCα	1400.5	660.1	2060.6	<CCα	<CCα
13022016/MLA/MA/3-IMP	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	685.6	381.0	1066.6	<CCα	<CCα
13022016/MLA/MA2-TRD	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	51.6	43.4	95.0	<CCα	<CCα
12022016/MLA/MA/4-IMP	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	556.9	310.7	867.7	<CCα	<CCα
13022016/MLA/MA/3-TRD	<CCα	2.6	<CCα	<CCα	8.3	<CCα	<CCα	<CCα	1064.1	625.7	1689.9	<CCα	<CCα
12022016/MLA/MA/4-TRD	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	42.0	42.0	<CCα	<CCα
12022016/MLA/MA/007	228.5	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	1022.9	452.5	1475.4	<CCα	21.1
13022016/MLA/MA/1-TRD	325.4	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	<CCα	1144.5	473.3	1617.9	<CCα	<CCα

Samples	Conc. (µg/kg) DON	Conc. (µg/kg) AFG2	Conc. (µg/kg) AFG1	Conc. (µg/kg) AFB2	Conc. (µg/kg) AFB1	Conc. (µg/kg) HT-2	Conc. (µg/kg) T-2 Toxin	Conc. (µg/kg) HT- 2 + T-2	Conc. (µg/kg) FB1	Conc. (µg/kg) FB2	Conc. (µg/kg) FB1+FB2	Conc. (µg/kg) OTA	Conc. (µg/kg) ZEN
12022016/MA/MA/1-TRD	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	899.8	509.5	1409.3	<CC α	<CC α
131215/MA/MA/3-TRD	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α
12052015/KO/MA/2-TRD	<CC α	<CC α	<CC α	<CC α	6.1	<CC α	<CC α	<CC α	670.0	252.8	922.9	<CC α	<CC α
12022016/KO/MA/4-IMP	<CC α	<CC α	4.6	15.0	5.3	<CC α	<CC α	<CC α	565.7	365.6	931.3	<CC α	<CC α
12062015/MLA/MA/2-TRD	278.1	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	1172.2	479.6	1651.8	<CC α	<CC α
12042015/KO/MA/2-TRD	<CC α	<CC α	<CC α	1.7	9.7	<CC α	<CC α	<CC α	827.1	351.4	1178.5	<CC α	<CC α
12022016/MA/MA/009	766.5	<CC α	9.9	2.7	13.2	<CC α	<CC α	<CC α	1894.2	800.2	2694.4	<CC α	44.6
12022016/MA/MA/4-IMP	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	337.6	73.5	411.1	<CC α	<CC α
12032015/MLA/MA/2-TRD	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	520.7	230.5	751.2	<CC α	<CC α
12032015/KO/MA/2-IMP	64.0	<CC α	7.9	<CC α	4.5	<CC α	<CC α	<CC α	710.7	293.2	1003.9	<CC α	<CC α
12022016/KO/MA/4-TRD	<CC α	<CC α	<CC α	1.7	6.7	<CC α	<CC α	<CC α	464.0	171.9	635.9	<CC α	<CC α
13022016/MA/MA/05-TRD	778.4	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	1870.2	770.5	2640.7	<CC α	40.9
13022016/MLA/MA/1-IMP	673.0	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	887.8	441.9	1329.7	<CC α	<CC α
12022016/MA/MA/008	479.9	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	<CC α	3085.4	1388.5	4473.8	<CC α	252.7
% Measument Uncertainty	29.2	40.15	39.37	36.39	37.38	37.72	38.1	51.03	11.75	8.01	9.42	11.7	29.04