STAKEHOLDERS' KNOWLEDGE, AWARENESS AND OCCURRENCE OF *ASPERGILLUS* SPECIES ON STORED MAIZE IN MOROGORO MUNICIPALITY AND MAKAMBAKO DISTRICT, TANZANIA

FADHILI MABRUKI

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN APPLIED MICROBIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2022

EXTENDED ABSTRACT

Maize is an important staple food crop grown in all regions of Tanzania. In the country, the crop is used as a source of income to reduce poverty and ensure food security in various regions. The crop is susceptible to *Aspergillus* and aflatoxin contamination during production and storage stages. Such contamination puts the health and well-being as well as economic status of Tanzanian people at risk by reducing the nutritional value and palatability of the crop. This study was conducted to determine the occurrence of aflatoxigenic fungi as well factors predisposing maize stored in Morogoro municipality and Makambako district to their subsequent contamination. The study also assessed knowledge and awareness on aflatoxins as well. A total of 226 maize samples were collected from warehouses from six wards, three from each study area for analysis of aflatoxigenic fungi. Isolates obtained from the maize samples were identified to species level by observing morphological characteristics with aid of taxonomic keys. Descriptive statistical analysis was used to describe the proportional occurrence of *A. parasiticus* and *A. flavus* with respect to areas where the samples were collected. The study found that only 34 (15%) of the collected samples were contaminated with aflatoxigenic fungal species particularly *Aspergillus flavus* and *Aspergillus parasiticus*. Incidence of *A. flavus* in the collected samples was observed to be relatively higher compared to that of *A. parasiticus*, whereby 28 samples were contaminated with *A. flavus* while only six samples were contaminated with *A. parasiticus*. A semi-structured questionnaire was administered to 226 respondents to assess their knowledge and awareness on aflatoxins and factors predisposing stored maize to aflatoxin contamination. Analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16.0 was performed to determine the statistical significance of responses reflecting factors predisposing stored maize to aflatoxin contamination by comparison of means among the study groups.

Descriptive statistics were presented to describe knowledge and awareness of the studied population on aflatoxins. The study revealed that majority of the studied areas had little knowledge and awareness in relation to aflatoxins contamination on stored maize. The results also showed that other factors such as storage with other crops, mode of storage and storage duration positively influenced infestation of aflatoxigenic fungi in the stored maize. The inadequacy of knowledge and awareness reflects the demand of stakeholder training to provide them with education on ways to mitigate and minimize the chance of aflatoxin contamination on the crop during storage.

DECLARATION

I, Fadhili Mabruki, 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.

_____ _____

Fadhili Mabruki Date

(MSc. Candidate)

The above declaration is confirmed by;

_____ _____

Dr. Isaac Makundi Date

(Supervisor)

_____ _____

Dr. Benigni A. Temba Date

(Supervisor)

COPYRIGHT

ACKNOWLEGMENTS

I would like to take this opportunity to acknowledge all the people who helped me to produce this dissertation. First and foremost, I would like to thank the Almighty God for the strength, wisdom and good health that enabled me to accomplish this work.

I also thank my supervisors Dr. Isaac Makundi of the Department of Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences and Dr. Benigni A. Temba of the Department of Veterinary Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine and Biomedical Sciences at Sokoine University of Agriculture (SUA) for their guidance to the accomplishment of this work. Thank you for taking time to read through the draft reports and all constructive suggestions, which helped me to improve this work.

Last special thanks go to my family, my father and my mother for their support while I was pursuing this degree. Further thanks go to my classmates for their support during pursuit of my postgraduate degree studies. It is not possible to mention everyone who assisted me in one way or another, but I am immensely indebted for their help and may they all be blessed.

DEDICATION

This work is dedicated to my young brothers Nasri Mabruki and Ally Mabruki, My father Mabruki Ally Abdallah and my mother Hadija Selemani Kipande for being supportive while I carried out this study. “Nasri and Ally Mabruki you need to achieve better than I in your life”. Furthermore, I also dedicate this work to all people passing through difficult challenges in one way or another while struggling for the betterment of their families and the Nation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABREVIATIONS AND ACRONYMS

ABPA	Allergic bronchopulmonary aspergillosis
AFB_1	Aflatoxins B_1
AFB_2	Aflatoxins B_2
AFG_1	Aflatoxins G_1
AFG_2	Aflatoxins G_2
AFM_1	Aflatoxins M_1
AFM_2	Aflatoxins M_2
ANOVA	Analysis of Variance
BDP	Bile Duct Proliferation
HCC	Hepatocellular carcinoma
IARC	International Agency for Research on Cancer
MC	Moisture content
MS	Malnutrition syndrome
NaCl	Sodium chloride
PDA	Potato dextrose ager
PICS	Purdue improved crop storage
POP	Polypropylene
Ppb	Parts per Billion
SPSS	Statistical Package for Social Sciences
TBS	Tanzania Bureau of Standard
TLC	Thin-layer chromatography
TNC	Tanzania Nation Census
UNCRC	United Nation’s Convention on the Rights of the Children
URT	United Republic of Tanzania President's Office
UV	Ultraviolet

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Fungal contamination is a worldwide problem facing agricultural products. This problem may occur at all stages of agricultural production and storage ultimately affecting the economy, food safety as well as human and animal health. Studies conducted earlier revealed that about 25% to 50% of world agricultural products are contaminated with mycotoxins (Mahmoud *et al.,* 2014; Priyanka *et al.,* 2014; Mtega *et al.,* 2020). Mycotoxins are naturally occurring toxins produced as secondary metabolites in agricultural products by fungi belonging to various genera such as *Aspergillus*, *Penicillium* and *Fusarium* (Chemining'wa *et al.,* 2009; Nyangi *et al.,* 2016; Shitu *et al.,* 2018). Literature shows that during the pre-harvest, processing, transportation and storage stages, the toxins can grow in a wide variety of agricultural products including cereal grains (Seetha *et al.,* 2017; Balendres *et al.,* 2019). Apart from agricultural products, mycotoxins also occur in animal-derived foods such as meat, milk, eggs and milk derivatives if the animals consume contaminated food (Yiannikouris and Jouany, 2002; Odhiambo *et al.,* 2013).

Mycotoxins are considered potential carcinogenic, teratogenic and mutagenic due to their deleterious effect in human and animal bodies. Furthermore, the toxins are associated with impaired growth in children, neural tubes defects in unborn children and immunosuppression (Nyangi *et al.,* 2016; Khan *et al.,* 2020). Recently conducted studies showed that high temperature, humidity, poor harvesting practices, unsuitable storage conditions, insect damage, improper transportation and improper processing are the major factors influencing fungal infestation and mycotoxins contamination in agricultural products (Suleiman and Kurt, 2015; Shitu *et al.,* 2018).

Currently more than 400 compounds are classified as mycotoxins worldwide, but five groups of mycotoxins are considered potentially dangerous to human and animal health. These are Aflatoxins, Ochratoxins, Fumonisins, Nivalenol and Zearalenone (Shitu *et al.,* 2018; Balendres *et al.,* 2019; Khan *et al.,* 2020). Such toxins when ingested or inhaled by humans or domestic animals are capable of causing death and diseases such as hepatocellular carcinoma (HCC) and kidney cancer (Kibe, 2015; Zulkifli and Zakaria, 2017).

Direct exposure of mycotoxins to humans can be through consumption of plant products contaminated with the toxins while indirect exposure occurs through consumption of animal products containing residual amounts of the mycotoxin ingested by the food-producing animals (Bankole and Adebanjo, 2003). Balendres *et al.* (2019) reported that occurrence of mycotoxins in agricultural products and foodstuff is associated with the climatic conditions of given areas, agronomical practices and the type of crops grown. Among the five potentially dangerous groups of mycotoxins, human and domesticated animal health is more vulnerable to aflatoxins exposure globally due to their widespread prevalence and high toxicity. Members belonging to the *Aspergillus* genus in particular produce the aflatoxins.

Aspergillus species are filamentous, cosmopolitan and ubiquitous fungi found naturally in different components of environments including soils, air and contaminated food. They are diverse in terms of habitat and are versatile in terms of their ability to produce toxins as secondary metabolites. Many of them are pathogenic to plant and animals while other are useful in industrial human food production (Palencia *et al.,* 2010). Under favourable conditions, various *Aspergillus* species can grow in maize grains during pre-harvesting, processing or storage stage and lead to maize spoilage by aflatoxins production (Odhiambo *et al.,* 2013).

Other studies reported that humidity due to leakage of stores and insect damage are prerequisite for the growth of aflatoxins producing fungi on well-dried grains (Kukom, 2017; Shitu *et al.,* 2018). *Aspergillus* species particularly *Aspergillus flavus* and *Aspergillus parasiticus* occur in wide varieties of agricultural commodities mainly in maize (Ellis *et al.,* 1991; Odhiambo *et al.,* 2013; Shitu *et al.,* 2018). Odhiambo *et al.* (2013) and Khan *et al*. (2020) reported that aflatoxins produced by *A. flavus* and *A.parasiticus* mainly infect maize from pre-harvesting stage in the field to post harvesting stage in the stores.

Aflatoxins that occur naturally on a wide variety of agricultural products and foodstuffs are mainly produced by *A. flavus* and *A. parasiticus* (Abbas *et al.,* 2004; Odhiambo *et al.,* 2013). Currently aflatoxin contamination of agricultural commodities has gained global significance because of their deleterious effects on human and animal health as well as their impact in international trade. The toxins are carcinogenic; they may also cause liver and kidney damage to both human and animal health, immuno suppression, mutagenesis and teratogenesis (Odhiambo *et al.,* 2013; Wu, 2015).

Aflatoxins have also been associated with effects such as exacerbation of energy, malnutrition syndrome (MS) in children, vitamin A malnutrition in animals and greater economic losses (Odhiambo *et al.,* 2013). Chronic exposure to high level of aflatoxin can cause various clinical problems such as bile duct proliferation (BDP), edema, anorexia, hepatitis, kidney malfunctioning, acute jaundice and fatigue, which may subsequently result in death (Odhiambo *et al.,* 2013; Khan *et al.,* 2020).

Currently, over five million people worldwide are at the risk of chronic exposure to uncontrolled aflatoxins in their diet annually (Wu and Guclu, 2012; Wu, 2015). Unnevehr

and Grace (2013) reported that about 26 000 Africans living in Sub-Saharan countries die annually due to liver cancer associated with aflatoxin exposure. In Tanzania, recently conducted studies in Kilimanjaro, Iringa and Tabora regions showed that about 40% of children under five years have stunted growth as a results of consumption aflatoxin contaminated food mainly maize (Seetha *et al.*, 2017; Kamala *et al.,* 2018). Other studies conducted in Tanzania reported an outbreak of aflatoxicosis with significant mortalities (Kamala *et al*., 2018). Aflatoxin contamination mostly occurs during storage due to poor storage practices. This form of contamination in food grains remains a big problem to farmers and traders in the world especially in African countries because no precautionary measures are routinely taken to inspect crops for fungal infections (Sukmawati *et al*., 2018). This study aimed to assess the occurrence of aflatoxigenic *Aspergillus* species and factors predisposing stored maize to their subsequent contamination. Findings from this study will reveal the status of *Aspergillus* species and factors associated with fungal infestation and aflatoxin contamination in stored maize in selected study areas. The information will help in designing intervention strategies towards reducing aflatoxin contamination in stored maize.

1.2 Description of the genus *Aspergillus*

1.2.1 Occurrence and mode of nutrition

Aspergillus is an important genus comprised of filamentous fungi found in a wide variety of substrates under diverse range of environmental conditions globally (Egbuta *et al.,* 2015). Most of the species grow at latitudes above 25^{o}N and 25^{0}S, with high occurrence between latitudes 26^{o} and 35^{o} and uncommonly at latitudes above 45^{o} (Bailly *et al.,* 2018). *Aspergillus* species belong to the family *Trichomaceae* within phylum Ascomycota. Most of these fungi reproduce asexually while few others reproduce sexually (Krishnan *et al.,* 2009; Dhanasekaran *et al.,* 2011). *Aspergillus* spp. posses versatile features, which enable

them to survive in various environmental conditions (Mousavi *et al.,* 2016). They are able to tolerate situations of high temperatures above 37^{0}C, high humidity and water scarcity (Thathana *et al.,* 2017).

Majority of the species in this genus are saprophytic as they tend to grow on decomposing organic substances while others that cause diseases in human beings and animals exhibit parasitic mode of nutrition (Paulussen *et al.,* 2017). There are over 185 species with diverse economic significances known to belong to the genus *Aspergillus,* some of them being opportunistic pathogens that produce toxins as secondary metabolites while others are useful in various industrial productions (Priyanka *et al.,* 2014). Among these species, more attention is given to opportunistic pathogens because of their deterioration effects on wide variety of agricultural commodities and foodstuffs.

1.2.2 Toxigenicity and industrial application

Under favourable conditions, *Aspergillus* spp. can infest a wide range of agricultural commodities and foodstuffs to produce mycotoxins. (Nazir *et al*., 2014; Egbuta *et al.,* 2015). The important mycotoxins produced by these species are aflatoxins, fumonisins and ochratoxins. These toxins are responsible for deterioration effects on contaminated agricultural commodities and foodstuffs (Egbuta *et al.,* 2015).The prevalence of these toxins in food and food-products not only affects human and animal health but also cause significant negative impact on its domestic and international trade of crops (Iheanacho *et al.,* 2014).

Aspergillus contamination in agricultural products and foodstuffs depends on various factors such as temperature, moisture content and humidity at storage as well as the method used during processing to pre and post-harvest practices (Gautam and

Bhadauria, 2012). Other studies reported that the presence of *Aspergillus* species in food commodities does not imply high levels of mycotoxins; indicating that not all species of *Aspergillus* are produce mycotoxins (Varga *et al.*, 2004). In addition, some *Aspergillus* spp. are able to produce mycotoxins only under special conditions in fact even those with the ability to produce the mycotoxins may not necessary do that at all times.

Currently a wide variety of *Aspergillus* species are reported to be associated with mycotoxins production but *A. flavus*, *A. parasiticus*, *A.nomius* and *A. niger* are considered to be main producers of mycotoxins in agricultural products and foodstuffs (Varga *et al.*, 2004; Gautam and Bhadauria, 2012). Apart from agricultural products, opportunistic *Aspergillus* spp. can affect other products including wood, paint, kerosene, textiles, cements, rubber, plastic materials and pharmaceutical items (Gourama and Bullerman, 1995). In addition, some species of *Aspergillus* can grow in a living animal body and resultantly cause aspergillosis.

Apart from food deterioration, *Aspergillus* species can be used in various industrial applications including production of citric acid, extracellular enzymes, antibiotics, traditional fermented foods and other biotechnological applications (Te Biesebeke and Record, 2008; Samson *et al.*, 2014).

1.2.3 Pathogenicity and clinical manifestation

The genus *Aspergillus* is widespread and ubiquitous; its members utilize a variety of substrates and adapt well to a broad range of environmental conditions. Paulussen *et al*. (2017) reported that even though there are several hundred species of the genus *Aspergillus*, about only 20 species including, *A. flavus*, *A. parasiticus*, *A. fumigatus*, *A. niger*, *A. nidulans*, *A. terreus* have been confirmed to be causative agents of infections

in human beings and animals. However, the pathogenicity of *Aspergillus* species that cause aspergillosis is influenced by the race and immune status of the individual affected (Paulussen *et al*., 2017). It has been reported that immunosuppression is the major factor predisposing a person to various clinical forms of aspergillosis, including allergic bronchopulmonary aspergillosis (ABPA), mycotic keratitis, otomycosis, nasal sinusitis and invasive infections (Gupta *et al.,* 2020). Furthermore, both fungus and host-related factors such as strain virulence and host immune status play an important role in disease development (Krishnan *et al.,* 2009; Paulussen *et al*., 2017).

Direct exposure to the pathogenic *Aspergillus* conidia of immunocomprised hosts, can lead to allergic manifestations, lethal infection of respiratory system followed by other important organs (Paulussen *et al*., 2017). Gupta *et al.* (2020) reported that conidia of pathogenic *Aspergillus* species dispersed in the environment are able to remain dormant until they encounter an environment that allows metabolic activation. In addition, invasive aspergillosis is rare in immunocompetent hosts but contributes significance morbidity and mortality in immunocomprised victims. Literature showed that *Aspergillus fumigatus* and *Aspergillus flavus* are the major pathogens for invasive aspergillosis in humans and animals; where over 80% of invasive aspergillosis is caused by A. *fumigatus* and 15-20% of infections caused by *A. flavus* (Krishnan *et al.,* 2009; Paulussen *et al*., 2017). In plants, *Aspergillus* species have the ability to cause diseases in economically important crops (Pasqualotto, 2009).

Currently, invasive fungal infections are probably one of the major life threatening causing significant mortality in the world. Globally, over 1.5 million people die annually due to these infections, where by invasive aspergillosis in particular plays a crucial role in this mortality. Recently conducted studies revealed an increase in the prevalence of

aspergillosis due to the rise in the proportion of immunocompromised populations because of cancer treatment and others factors (Gupta *et al.,* 2020). Furthermore, about 10% of bronchopulmonary cases reported in the world were caused by *Aspergillus* species as reported by Rudramurthy *et al.* (2019). Another study reported that *Aspergillus* species is responsible for about 50% of allergic fungal infections developed by people during their lifetime (Mousavi *et al.,* 2016).

1.3 Aflatoxins

Aflatoxins are secondary toxic metabolites produced by filamentous fungi mainly *Aspergillus parasiticus* and *Aspergillus flavus* (Ellis *et al.,* 1991; Karthikeyan *et al.,* 2013). Aflatoxins were first discovered in 1960s in England in the form of Turkey X disease, which attacked chickens and turkeys, that consumed feed enriched with Brazilian groundnut meal (Ellis *et al.,* 1991; Dhanasekaran *et al.,* 2011; Guchi, 2015). A later discovery revealed that the disease was caused by aflatoxins, which could also affect humans and other animals. Ellis *et al.* (1991) and Odhiambo *et al.* (2013) reported that *Aspergillus* species can produce these toxins on a wide variety of substrates under specific conditions of temperature, humidity and water activity. However, the presence of aflatoxins on a substrate is not determined by the presence or absence of aflatoxigenic mould on that substrate since the toxins may persist long after the mould growth has disappeared (Ellis *et al.,* 1991).

Currently, several aflatoxins have been discovered but four major types of aflatoxins are considered more potentially dangerous to humans and domestic animals health. These are aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2) (Ellis *et al.,* 1991; Balendres *et al.,* 2019; Khan *et al.,* 2020). Apart from these, aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) are other significant members of the aflatoxin family

commonly found in milk, dairy products, meat and eggs. (Guchi, 2015; Wu, 2015; Khalid *et al.,* 2018). Literature showed that AFB_1 and AFB_2 are produced only by *Aspergillus flavus* while AFB_1, AFB_2, AFG_1 and AFG_2 are produced by *Aspergillus parasiticus* (Ellis *et al.,* 1991; Balendres *et al.,* 2019; Guchi, 2015). The "B" and "G" refer to the blue and green fluorescent colours produced under ultraviolet (UV) light on the thin-layer chromatography (TLC) plates, and the subscript numbers 1 and 2 indicate major and minor compounds, respectively (Ellis *et al.,* 1991; Guchi, 2015).

Literature show that AFB_1 and AFG_1 are the most frequent, where AFB_1 is considered to be of most public health significance among the four major aflatoxins due its comparably higher toxicity (Ellis *et al.,* 1991; Guchi, 2015). Furthermore, the International Agency for Research on Cancer (IARC) classified AFB_1 as group one human carcinogen (Guchi, 2015). In addition, IARC arranges types of aflatoxins in descending order based on carcinogenicity potential; AFB_1> AFG_1> AFB_2>AFG_2 (Guchi, 2015).

1.3.1 Properties and chemical structure of aflatoxins

1.3.1.1 Aflatoxin B_1 and B_2

Aflatoxin B_1 is the most toxic compound among aflatoxins and it has potential carcinogenicity, cytotoxicity, hepatotoxicity, genotoxicity and immunotoxicity (Wogan, 1966; Ellis *et al.,* 1991). AFB_1 has a molecular weight of 312 g/mol, melting point of 268C-269^{0}C, emits fluorescence at 425nm and has a chemical formula of $C_{17}H_{12}O_2$ (Wogan, 1966; Kumar, 2018). Under UV light, AFB_1 emits strong blue fluorescence lights. On the other hand, AFB_2 has a chemical formula of $C_{17}H_{14}O_6$, with a molecular weight of 314 g/mol, emits fluorescence at 425nm and melts at 286-289^{0}C. Under UV AFB_2, exhibit slightly blue fluorescence (Wogan, 1966; Kumar, 2018). In addition, both

AFB_1 and AFB_2 are soluble in water and polar organic solvent (Dhanasekaran *et al.,* 2011).

1.3.1.2 Aflatoxin G_1 and G_2

Both AFG_1 and AFG_2 emit yellow-green fluorescence under UV light. The molecular formula for AFG_1 is $C_{17}H_{12}O_7$, with a molecular weight of 328 g/mol, emits fluorescence at 450nm and melting point of 244-246^{0}C. On the other hand, AFG_2 has the chemical formula of $C_{17}H_{14}O_7$, with a molecular weight of 330 g/mol, emits fluorescence at 450nm and melting point of 237-240^{0}C (Wogan, 1966; Kumar, 2018). In addition, both AFG_1 and AFG_2 are soluble in water and polar organic solvent **(**Dhanasekaran *et al.,* 2011).

1.3.1.3 Aflatoxin M_1 and M_2

AFM_1 and AFM_2 are natural oxidative metabolic products of AFB_1 and AFB_2, respectively (Khalid *et al.*, 2018; Shehab *et al.*, 2019). AFM_1 has a chemical formula of $C_{17}H_{12}O7$ and molecular weight of 328.3 g/mol while AFM_2 has the chemical formula $C_{12}H_{14}O_7$ and molecular weight of 330.3 g/mol. Like other aflatoxins, AFM_1 and AFM_2 are mutagenic, immunosuppressive, teratogenic and carcinogenic (Purchase, 1967; Bianco *et al.*, 2012).

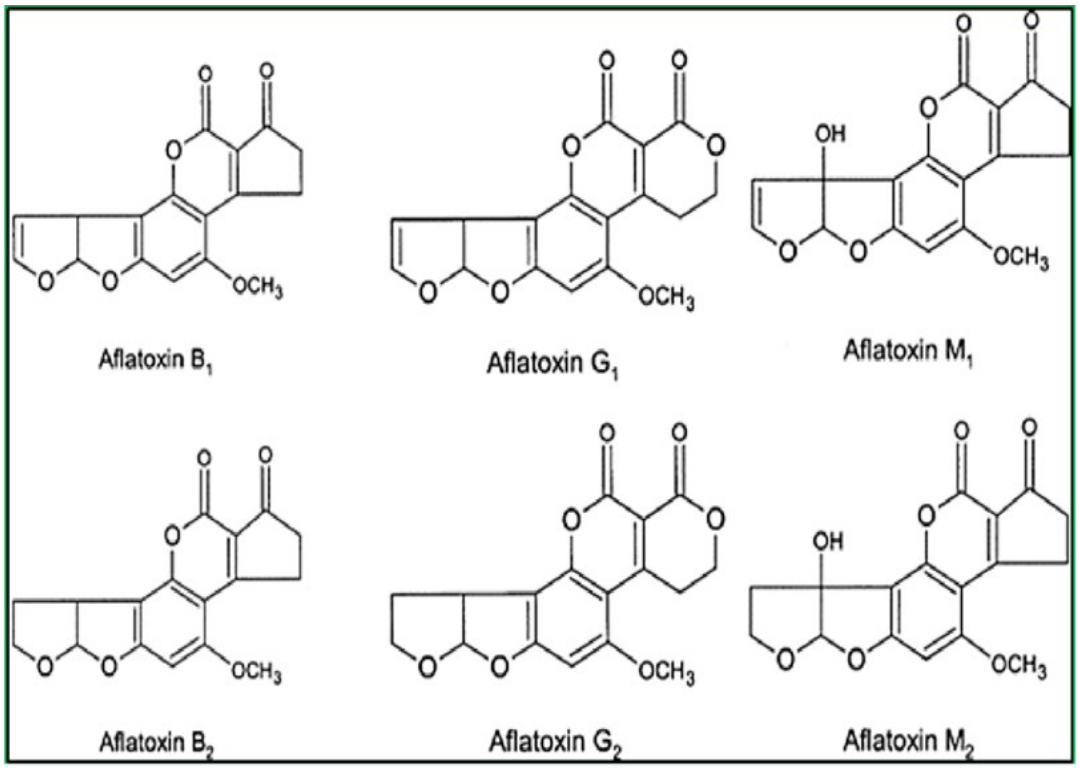


Figure 1.1: Chemical structure of Aflatoxins (B_1, B_2, G_1, G_2, M_1, M_2)

Source: Wogan, 1966; Dhanasekaran *et al.*, 2011.

1.3.2 Health effects of aflatoxins to humans and animals

It is estimated that 4.5 to 5 billion people in the developing countries located along 40^0N and 40^0S of the equator worldwide are chronically affected with aflatoxins (Bbosa *et al.*, 2013). The World Health Organization (WHO) hence recognizes aflatoxins as a global food safety concern. In addition, WHO reported that developing countries with rural subsistence farming communities are most vulnerable to aflatoxins exposure (Gong *et al.*, 2016). The contamination of food and seeds with aflatoxins can cause serious effects to human and animal health. Furthermore, research conducted recently estimated that aflatoxins destroyed more than 25% of global food crops produced annually (Kew, 2013; Guchi, 2015).

Aflatoxicosis is a disease caused by consumption of aflatoxins in both humans and animals. The disease can take two forms namely acute and chronic aflatoxicosis, these occur upon consumption of moderate to high and low to moderate level of aflatoxins respectively (Bbosa *et al.,* 2013). Acute and chronic exposure to aflatoxins may cause various serious effects to human and animal health. Arapceska *et al.* (2015) reported that various factors such as species, age, sex and nutrition influence susceptibility of individual animals to aflatoxins. Acute exposure to the high levels of aflatoxin leads to aflatoxicosis; characterized by acute liver damage, alteration in digestion, edema and hemorrhage that can eventually cause death (Dhanasekaran *et al.,* 2011; Bbosa *et al.,* 2013). In addition, acute dietary exposure to AFB_1 has been implicated in epidemics of acute hepatic injury (Bbosa *et al.,* 2013). Evidence of acute aflatoxicosis in humans has been reported worldwide, especially in third world countries such as Taiwan, Kenya, Uganda and many others (Dhanasekaran *et al.,* 2011; Bbosa *et al.,* 2013).

Exposure to low levels of aflatoxins causes chronic aflatoxicosis that is difficult to recognize due to its subclinical effects. Dhanasekaran *et al.* (2011) and Benkerroum (2020) reported that chronic exposure to aflatoxins is associated with several effects including; teratogenic effects, mutagenic effects due to changes in the genetic code and carcinogenic effects. Furthermore, aflatoxins are associated with malnutritional disorders such as kwashiorkor and growth faltering by interfering with the absorption of micronutrients (Sowley, 2016; Benkerroum, 2020).

The occurrence of liver cancer as one of the most common and deadly types of cancer disease has been strongly correlated with dietary exposure to aflatoxins enhanced by the presence of other risk factors such as the individuals immune status. Literature show that chronic infection with hepatitis B virus amplifies the carcinogenic potency of AFB_1 up to 60 times (Sowley, 2016; Benkerroum, 2020). AFB_1, AFG_1 and AFM_1 have been proved to

cause cancer in the liver and other various organs in humans and animals (Bbosa *et al.*, 2013; Sowley, 2016; Benkerroum, 2020). Furthermore, chronic effects of aflatoxins impair the normal body immune function by reducing phagocytic activity or reducing the number of T cells, or their function (Bbosa *et al.*, 2013).

In domesticated animals, exposure to aflatoxins induce impaired productivity and reproduction, increase susceptibility to diseases and reduce the quality of the food they produced (Bbosa *et al.*, 2013; Benkerroum, 2020). The aflatoxicosis syndrome in animals is characterized by vomiting, abdominal pain, pulmonary edema, coma, blood coagulation defects and death with cerebral edema. In dairy and beef cattle, the signs of acute toxicosis include anorexia, dramatic drop of milk production, weight loss, lethargy and jaundice (Bbosa *et al.*, 2013; Arapceska *et al.*, 2015).

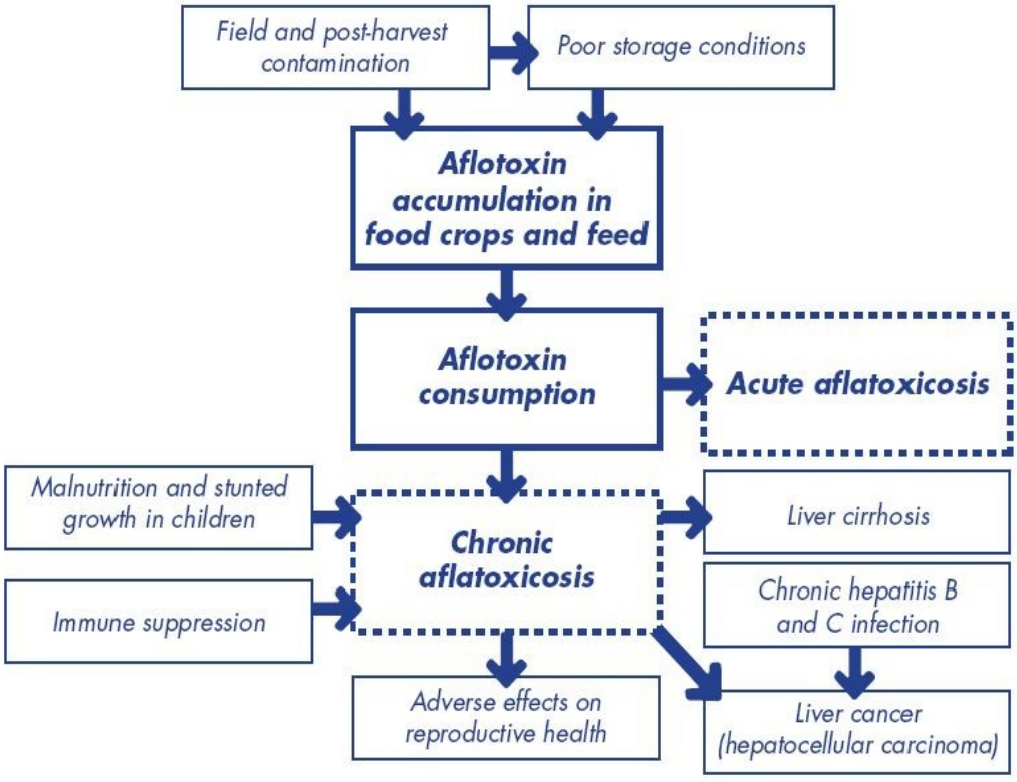


Figure 1.2: Health effects of aflatoxins in human and animal

Source: Bbosa *et al.* (2013)

1.3.3 Regulatory limits for aflatoxins

Various countries in the world have established regulations to limit the consumption levels of aflatoxins and other important mycotoxins in food products to protect consumers from their harmful effects. These levels are expressed in parts per billion (ppb) or µg/kg (Nabwire *et al.,* 2020). Furthermore, regulations to limit the tolerable levels of aflatoxin exposure depends on the types of food and its destined primary consumer (humans or animals).Various factors including availability of toxicological and survey data, knowledge about the distribution of mycotoxins in commodities, availability of analytical methods as well as other economic and political factors influence the decision making process of setting limits for specific aflatoxins (Gilbert, 1991; Van Egmond and Jonker, 2004). In addition, literature showed that the maximum limit for tolerable levels of aflatoxins on food products varies from country to country, depending on the cereal consumption pattern in those countries (Mtega *et al.,* 2020).

Xie *et al.* (2016) and Wu and Guclu (2012) reported that the maximum limit levels for AFB_1 and other aflatoxins in food were 5 and 10 μgkg^{-1} respectively in over 75 countries worldwide. Furthermore, regulatory limit levels for aflatoxins in most countries range from 5 to 20 μgkg^{-1} in food destined for human consumption (Nabwire *et al.,* 2020). In East Africa, the maximum safe level for aflatoxins in selected cereals and other food products is 10 μgkg^{-1} and 0.05 μgkg^{-1} is the set exclusively for AFM_1 in milk (Nabwire *et al.,* 2020). In Tanzania, the Tanzania Bureau of Standard (TBS) has set the maximum tolerable level for total aflatoxins to be 10 μgkg^{-1} while for AFB_1 the maximum tolerable level is to be 5 μgkg^{-1} (Mtega *et al.,* 2020).

1.3.4 Control of aflatoxins

Control of aflatoxins is important for public health and improvement of agricultural productivity in the world. Researchers have developed strategies to reduce and control of aflatoxins in order to curb economic losses and adverse health effects caused by their subsequent contamination on food products during pre and post-harvesting (Wu, 2015).

Pre-harvest management practices that prevent aflatoxin contamination in maize and other cereal crops at farm level include planting of breeds resistant to conditions that favour fungal infection and mycotoxin production, use of insecticides and proper harvesting. These practices are supported by consideration of other factors such as sowing season and proper plant nutrition (Hell and Mutegi, 2011; Wu, 2015; Nyangi *et al.*, 2016). Post-harvesting practices that prevent aflatoxin contamination in maize include hand sorting, drying of maize on mats/raised platforms, adequate sun drying and smoking, proper transportation and packaging, application of insecticides during storage and de-hulling of maize before milling (Hell and Mutegi, 2011; Wu, 2015; Kamala *et al.*, 2018).

1.4 Problem Statement and Justification of the Study

Aflatoxin contamination in agricultural products and foodstuffs is a global problem particularly in food safety perspectives. A recently conducted survey showed that agricultural commodities produced in over 20 African countries were contaminated with aflatoxins (Shephard, 2008).

In East Africa, outbreaks of aflatoxicosis that involved significant mortality cases were reported in various countries including Kenya and Tanzania in the year 2004 and 2016 respectively (Bandyopadhyay *et al.*, 2016). In spite of many efforts done by the Tanzanian

government to combat the problem, aflatoxin contamination in maize remains high in many parts of the country. Recently conducted studies on the contamination of pre-harvested, marketed and stored maize by aflatoxins in Tanzania showed relatively high contamination with the toxin (Kimanya *et al.*, 2008; Seetha *et al.*, 2017). Other studies reported that 18% of maize produced in the country detected to be contaminated with aflatoxins (Seetha *et al*., 2017).

Aflatoxin contamination of food endangers the health as well as the economy of Tanzanian people by reducing the nutritional value of the maize crop and its palatability (Seetha *et al*., 2017). Furthermore, aflatoxins adversely affect human health and productivity of domestic animals by mutagenesis, teratogenesis, carcinogenesis and immunosuppression (Nyangi *et al.,* 2016). Stunted growth and sometimes death can occur in children exposed to aflatoxins due to excessive malnutrition disorder and loss of weight. Apart from greatly influencing health perspectives, aflatoxins ultimately aggravate economic losses by reducing the trade potential of maize at national, regional and international level (Seetha *et al*., 2017).

The fact that maize remains a major staple food for the majority of Tanzanians, signifies the need for further research on aflatoxin contaminations of these crops. This study aims to assess the occurrence of aflatoxigenic *Aspergillus* species and practices predisposing the maize stored within Morogoro Municipality and Makambako district to the fungal contamination. The findings from this study can potentially reveal the status of aflatoxins on maize stored within the study areas and can therefore be utilized to develop effective control measures to reduce aflatoxicosis.

1.5 Study Objectives

1.5.1 Overall objective

To assess the stakeholders' knowledge, awareness and occurrence of *Aspergillus* species on stored maize in Morogoro municipality and Makambako district.

1.5.2 Specific objectives

i. To determine the proportional occurrence of *A. flavus* and *A. parasiticus* on stored maize in Morogoro municipality and Makambako district.

ii. To assess post-harvest practices predisposing stored maize to infestation with *Aspergillus* spp. and aflatoxin contamination in Morogoro municipality and Makambako district.

CHAPTER TWO

MANUSCRIPT ONE

2.0 Occurrence of *Aspergillus flavus* and *Aspergillus parasiticus* on Stored Maize in Morogoro Municipality and Makambako District, Tanzania.

Fadhili Mabruki[1]*, Isaac Mkundi[1], Benigni Alfred Temba[2]

[1] Department of Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, P.O.BOX 3019. Morogoro, Tanzania. Tel No +255232604557.

[2] Department of Veterinary Physiology, Biochemistry and Pharmacology, Sokoine University of Agriculture, P.O.BOX 3017. Morogoro, Tanzania. Tel No + 255764328939.

* Corresponding Author: Fadhili Mabruki. E-mail: mabrukifadhili@gmail.com

Submitted to Journal of Microbiology, Biotechnology and Food Sciences

2.1 Abstract

Maize is an important cash and food crop grown in Tanzania and other parts of the world. The crop is susceptible to fungal infestation and subsequent mycotoxins contamination that negatively affects human and animal health as well as the socio-economic status of the farmers and respective stakeholders. The study was conducted to determine occurrence of aflatoxigenic fungi (*A. flavus* and *A. parasiticus*) on maize stored in warehouses found in Morogoro municipality and Makambako district. A total of 226 maize samples were collected from six wards in the selected study areas then analysed for aflatoxigenic fungal infestation. Potato Dextrose Agar (PDA) was used for isolation of the fungi which were then identified to species level via observation of morphological characteristics with aid of taxonomic keys as described by McClenny (2005). The proportional occurrence of *A. parasiticus* and *A. flavus* with respect to areas where samples were collected was presented using descriptive statistics. The result showed that only 15% (34/226) of the collected samples were contaminated with *Aspergillus flavus* and *Aspergillus parasiticus*. In the collected samples, the incidence of *A. flavus* was higher than that of *A. parasiticus* whereby 28 samples were contaminated with *A. flavus* and 6 samples with *A. parasiticus*. The study elucidated that the methods used in the storage of this major staple food used by majority of Tanzanians makes it vulnerable to infestation and subsequent contamination by aflatoxigenic fungi. We therefore recommend that responsible sectors should implement appropriate intervention strategies designed to reduce occurrence of aflatoxigenic *Aspergillus* species on stored maize.

Keywords: Maize, *Aspergillus flavus*, *Aspergillus parasiticus*, Aflatoxins, Tanzania

2.2 Introduction

Aspergillus species are fungal species which occur on a wide variety of agricultural commodities and foodstuffs with ability to produce a number of toxins (Palencia *et al.,* 2010; Karthikeyan *et al.,* 2013). The species infest starchy foods and feeds at several stages of food production (pre-harvest, processing and transportation or storage stages) where they produce mycotoxins (Nazir *et al*., 2014; Egbuta *et al.,* 2015). The growth as well as toxins producing ability of the species is favoured by important environmental conditions particularly high moisture and temperature (Karthikeyan *et al.,* 2013; Balendres *et al.,* 2019). Aflatoxins, fumonisins and ochratoxins are important mycotoxins produced by the species. These toxins have gained global public health significance due to their deleterious effects on both human and animal health (Palencia *et al.,* 2010; Chilaka *et al.,* 2012). Aflatoxins have gained more attention compared to other mycotoxins produced by *Aspergillus* species due to their harmful effects in human and animal health.

Aspergillus flavus and *Aspergillus parasiticus* are members of the *Aspergillus* genus, commonly known to produce aflatoxins on a wide range of agricultural commodities especially on cereals grains (Dhanasekaran *et al.,* 2011; Guchi, 2015). Previously conducted studies highlighted that aflatoxin-contamination in crops is associated with ill-health effects in humans and animals including kidney and liver infections, immuno suppression, mutagenesis and teratogenesis (Bbosa *et al.,* 2013: Wu, 2015). On the broader sense, aflatoxin-contamination leads to greater economic losses by reducing the trade potential of the crops exported for international trade (Seetha *et al*., 2017).

Maize production assures a source of income, poverty reduction and food security as it is grown and used as a staple food crop in all regions of Tanzania (Lyimo *et al.,* 2014; Suleiman and Kurt, 2015). The contamination of this crop by aflatoxins prevents maize

producers from accessing international market, suppress economic opportunities and unfortunately affects consumer health (Bbosa *et al.,* 2013; Wu, 2015). The present study was conducted to determine the occurrence of aflatoxigenic fungi (*A. parasiticus* and *A.flavus*) on this important food crop stored inside facilities in Morogoro municipality and Makambako district, Tanzania.

2.3 Materials and Methods

2.3.1 Study Area

The study was carried out in six wards selected from two regions; three out of these were Kihonda, Uwanja wa Taifa and Kingo from Morogoro municipality in Morogoro region while the other three were Mwembetogwa, Mjimwema and Utengule from Makambako district in Njombe region. The wards were selected purposefully based on the availability of maize warehouses to maximize the chances of obtaining stored maize samples. Significant variations in some climatic conditions was the major criteria considered in selection of the two study areas. Variation in temperature between the two selected regions was most crucially considered as the climatic condition greatly influences the existence and survival of the pathogen under study.

Morogoro municipality is one of the six districts of Morogoro region, it is the region's headquarters covering an area of 531 km^2 with a population of 396 481, according to population projection of 2019. The municipality is located at about 195 kilometres West of Dar es Salaam and situated on the lower slope of Uluguru Mountains whose peak is about 1600 feet above the sea level (URT, 2017). The municipality lies at the crossings of longitudes $37^0$33' and $37^0$51'East of the Greenwich Meridian and between latitudes $6^0$37' and $6^0$55' South of the Equator. It is characterised by an annual average temperature ranging between 16^0C to 28^0C in the cold dry season and 21^0C and 33^0C in the warm wet

season. The municipality has an average annual rainfall range of between 821mm and 1505mm (URT, 2017). The average relative humidity is 63% to 83% in March through May and 46% to 82% from July to September. The major economic activities in Morogoro municipality include primary and secondary industrial activities, commercial farming, small-scale enterprises and commercial wholesale and retail (URT, 2017).

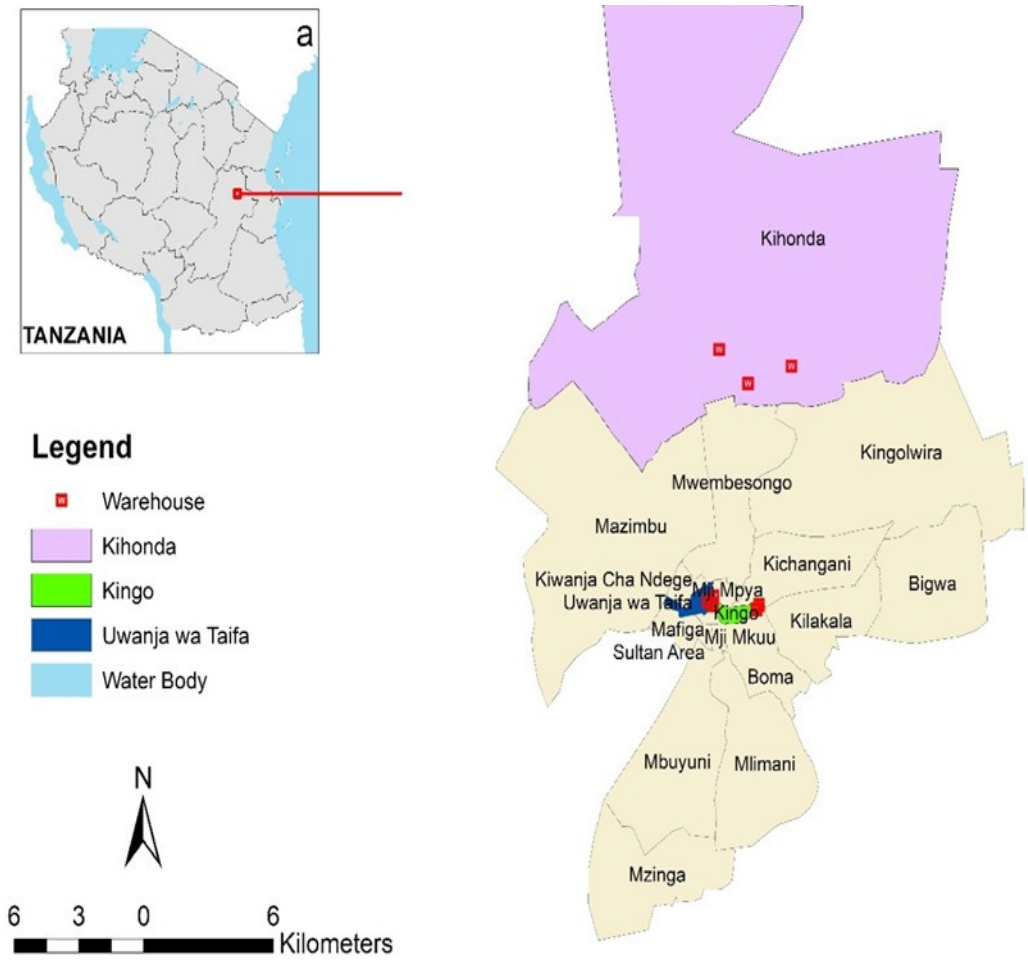


Figure 2.1: Map of Morogoro municipality showing the study sites

Makambako district is one among the six districts in Njombe region located at a junction between Njombe, Iringa and Mbeya region. The district lies approximately 40 miles North of the district capital of Njombe. The district is bordered Mufindi, Njombe and Wanging'ombe districts in the North and East, South and West respectively (URT, 2017). It lies at the crossings Longitudes $33^0$05' and $35^0$08' East of the Greenwich Meridian and Latitude $8^0$08' and $9^0$08' South of the Equator. The district covers an area of 884km^2 with a population of 93 827 according to the 2012 Tanzania Nation Census (TNC) (URT, 2017). The district displays an annual temperature range of between 15^0C and 25^0C and experiences mild and sunny weather throughout the year, with maximum temperature observed in September and December while minimum temperature occurs in June and August when the temperatures fall to 15^0C. The rainy season starts between October and November and ends in March and April with an annual average rainfall range of between 600mm and 1000mm (URT, 2017). The average relative humidity is 80-91% in March through June and 58-75% from July to October. The major economic activities in Makambako district are agriculture and trade (URT, 2017).

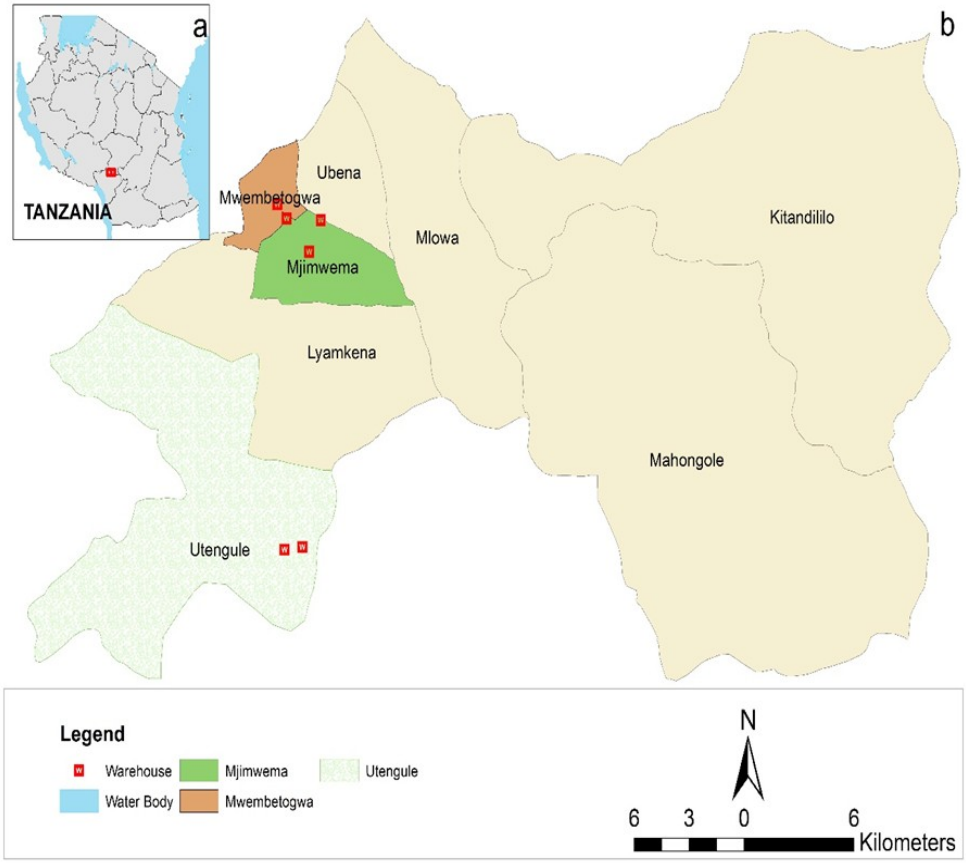


Figure 2.2: Map of Makambako district showing the study sites

2.3.2 Study design

A cross-sectional study design was adopted in this study. A simple random sampling technique was employed to collect maize samples from warehouses for further laboratory analysis.

2.3.3 Sample size calculation

The sample size for the study was calculated based on previously established prevalence by Seetha *et al*. (2017) using the formula;

$$n= [Z^2 P (1-P)]/ d^2$$

Whereby;

n = Sample size.

Z= Statistic for level of confidence (1.96 at confidence level of 95%)

P= Expected prevalence (18% based on study by Seetha *et al.*, 2017)

d= Precision (5%)

Therefore; n=1.96x1.96 x0.18 (1-0.18)/ 0.05 x 0.05= 226.

Therefore, the calculated sample size was 226, and for each sample about 200g of stored maize grains was collected from warehouses in different wards.

2.3.4 Sample collection

Samples collection sites were selected based on the information provided by Ward Executive Officers and District Agriculture and Livestock Development Officers. A total of 226 samples, each containing 200g of stored maize grains were collected randomly from warehouses in different wards of Morogoro municipality and Makambako district in Tanzania between March and May 2021. A total of 113 samples were collected from Morogoro municipality and the remaining 113 samples were collected from Makambako district. The distribution of samples collected from warehouses in Kihonda, Uwanja wa Taifa and Kingo wards within Morogoro municipality was 38, 38 and 37 respectively. Also, 33, 46 and 34 samples were collected from warehouses in Mwembetogwa, Utengule and Mjimwema wards respectively in Makambako district. These samples were collected from a total 14 warehouses, eight of these from Morogoro municipality and the remaining six from Makambako district. It was observed that most of the collected samples were stored between three to six months.

In the warehouses, maize was commonly stored in polypropylene bags/sacks each, weighing an average of 100kgs. The sampling procedure was determined by the total stock of sacks in a particular warehouse. A sample was taken from each sack in a warehouse with a total stock of 10 or fewer sacks. In warehouses with more than 10 total stock sacks, only 10 sacks were randomly selected for sampling. The number of samples drawn from each sack was determined by the position and posture of the sack. The sample from the sacks were taken by using local vendor tools (Locally made probes in Kiswahili known as "Bambo") or hands covered by sterile gloves. The collected samples were packed in sterile zipper bags, sealed and labelled appropriately, then transported to the laboratory at Sokoine University of Agriculture. In the laboratory, the samples were stored in the cold room with a temperature below 4^{0}C prior to analysis.

2.3.5 Chemicals and reagents

All chemicals and reagents used in this study were properly handled with adherence to proper laboratory practices to avoid accidents and contamination. In the laboratory some of the tools and machines used in during study include an Incubator, Refrigerator, Wire loop, Bunsen burner, Test tubes, Test tube rack, Beakers, Slide, Cover slip, Petri dishes, Washing bottle, Analytical balance, Autoclave, Spatula, Hot air oven, Flat bottom flask, Measuring cylinder, Disposable latex gloves, Electronic grain mill grinder, Forceps, Micropipettes and tips, Pirate conical flask, Inoculation needle and Microscope. Some of the materials used include Cotton wool, Aluminium foil, Lactophenol Catton blue, Sodium chloride (NaCl), Distilled water, Chloramphenicol, 70% Ethanol, Normal saline and Potato dextrose ager (PDA).

2.3.6 Determination of Moisture Content (MC)

Moisture content of each collected sample of stored maize grains was measured by using oven-drying method prior to analysis. Ten grams (10g) of each collected sample was placed in the aluminium foil, sealed and then labelled properly. The sealed samples were then kept in the hot air oven at 80^0C for 24 hours after which they were removed from the oven and their weight was re-measured by the analytical balance to determine the MC. The MC of each sample was calculated based on previously formula described by Reeb *et al.* (1999) as:

$$\text{MC (\%)} = \frac{Initial\ weight\ (g) - Oven\ dry weight\ (g)}{Initial\ weight\ (g)} \text{ x } 100\%$$

Where by:

Initial weight= Weight of sample before drying

Oven dry weight= Weight of sample after drying

2.3.7 Preparation of maize samples

In the laboratory, maize grains were randomly taken from each of the 200g packages of collected maize samples and then weighed on an analytical balance to obtain 30g. The 30g obtained from each package was ground using an electronic grain mill grinder to obtain a homogenous flour mixture. The grinder was cleaned with distilled water then sterilized with 70% of ethanol by using cotton wool after grinding each of 30g portions to overcome the problem of sample contamination. The ground samples were then packed in sterile zipper bags, labelled appropriately and stored in room temperature for further study.

2.3.8 Isolation and identification of the isolates (*A. flavus* and *A. parasiticus*)

2.3.8.1 Isolation of *A. flavus* and *A. parasiticus* from maize samples

Isolation of *A. flavus* and *A.parasiticus* from samples was conducted using a procedure as described by Shitu *et al.* (2018). Five gram of each ground samples was weighed separately on an analytical balance and then mixed with 20ml of normal saline in the test tubes. The mixture was shaken vigorously and allowed to settle for 10 minutes. One millilitre of the mixture was pipetted from each test tube, using a micropipette then poured and spread on plates containing sterile PDA to support growth of primary fungal cultures. The plates were then incubated at 37^0C for one up to three days. The incubated plates were examined daily for *Aspergillus* growth and spore formation. Colonies with morphological features of *Aspergillus* were transferred onto new PDA media plates using sterilized inoculation needles and forceps for sub-culturing to obtain pure culture. The sub-cultured plates were then incubated at 37^0C for one up to seven days for further processing.

2.3.8.2 Identification of the isolates (*A. flavus* and *A. parasiticus*)

Identification of *A. flavus* and *A. parasiticus* species from a pure culture of isolates obtained from the samples was conducted based on macroscopic and microscopic features. The macroscopic features used in identification of the isolates were; the colour of the colony, diameter of the colony, colony textures, colony reverse colour, production of sclerotia and presence of exudates in plates as explained by Sukmawati *et al.* (2018) and Khan *et al.* (2020). On the other hand, microscopic identification of the isolates was conducted utilizing a staining technique described by Egwurochi *et al.* (2015). The staining procedure was conducted as follows; A few drops of lactophenol cotton blue

were placed on a microscopic slide. A small portion of the colony was taken from the pure culture using sterilized forceps and then placed on the smeared side of the slide. A clean cover slip was then placed over the portion of the colony on the slide that was then placed on the stage of a light microscope to be observed by switching objectives to obtain convenient resolution. Presence and parameters of microscopic features such as conidia head, conidiophore, phialides, matulae and vesicles were observed. The identification the isolates to species level by observing macro and micro morphological characteristics was conducted with aid of taxonomic keys as described by McClenny (2005).

2.3.9 Statistical analysis

Descriptive analysis was conducted using Microsoft Excel 2013. Tables and graph were presented to describe moisture content of the samples and proportional occurrence of *A. flavus* and *A. parasiticus* with respect to areas of collection.

2.4 Results

2.4.1 Moisture contents of the samples with respect to site of collection

There was a statistical significance in variation of moisture content of the samples taken from warehouses in all wards (Appendix 2). This study found that the MC of the samples ranged from 10% to 19%. Out of the 226 samples collected, 54 (23.9%) samples had a MC above 14% while 172 (76.1%) had a MC below 14%. The proportion of samples observed with MC level above 14% were Mwembetogwa (55%), Utengule (26%), Uwanja wa Taifa (23.7%), Kingo (18.9%), Mjimwema (17.6%) and Kihonda (5.3%) wards (Table 2.1).

Table 2.1: Moisture content levels of the samples with respect to site of collection

Study sites	Moisture Level N (% of the samples)		
	Below 14%	**Above 14%**	**Total**
Kingo	30(81.1)	7(18.9)	37
Kihonda	36(94.7)	2(5.3)	38
Uwanja wa Taifa	29(76.3)	9(23.7)	38
Mjimwema	28(82.4)	6(17.6)	34
Mwembetogwa	15(45.5)	18(54.5)	33
Utengule	34(73.9)	12(26.1)	46
Total	**172(76.1)**	**54(23.9)**	**226**

2.4.2 Screening for fungal contamination from collected samples

Among the total 226 samples collected from the six wards for fungal screening, only 48(21.2%) samples revealed fungal contamination in their primary cultures. The 48 contaminated samples were then subjected to further screening of aflatoxigenic fungi of interest (*A. flavus* and *A. parasiticus*) on pure cultures where only 34 (15%) samples were positive. The results show that there was at least one sample that was contaminated with *A. flavus* and *A. parasiticus* from each of the six wards. The prevalence of samples that were contaminated with *A. flavus* and *A. parasiticus* from each ward are summarized on Table 2.2.

Table 2.2: *A. flavus* and *A. parasiticus* contamination among the collected samples

Sites	Screened Samples	Contaminated samples	Prevalence (%)
Uwanja wa Taifa	38	5	13.2
Kihonda	38	1	2.6
Kingo	37	10	27.0
Mjimwema	34	5	14.7
Mwembetogwa	33	6	18.2
Utengule	46	7	15.2
Total	**226**	**34**	**15.2**

2.4.3 Incidences and distribution of *A. flavus* and *A. parasiticus* isolated from contaminated samples

The results shows that *A. flavus* and *A. parasiticus* were isolated from 34 out of 226 maize samples collected from all wards. Out of 34 samples that were contaminated, 28 samples were due to *Aspergillus flavus* while *Aspergillus parasiticus* were found in 6 samples. Co-occurrence between *A. flavus* and *A. parasiticus* was also observed in two samples, one came from Makambako district and the other came from Morogoro municipality. Variation in the number of contaminated samples was also observed where Makambako district had the highest number of contaminated samples compared to Morogoro Municipality (Table 2.3).

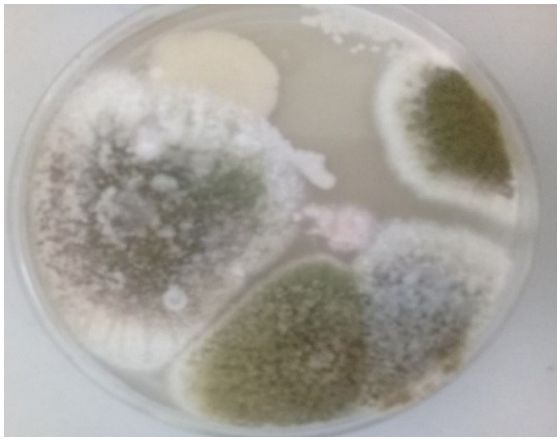
Table 2.3: Incidences and distribution of *A. flavus* and *A. parasiticus* isolated from all study sites

Study sites	Isolated aflatoxigenic fungi N (% of the isolates)		
	A. flavus	***A. parasiticus***	**Total**
Uwanja wa Taifa	5(100)	–	5
Kihonda	–	1(100)	1
Kingo	8(80)	2(20)	10
Mjimwema	4(80)	1(20)	5
Mwembetogwa	6(100)	–	6
Utengule	5(71.4)	2(28.6)	7
Total	**28(82.4)**	**6(17.6)**	**34**

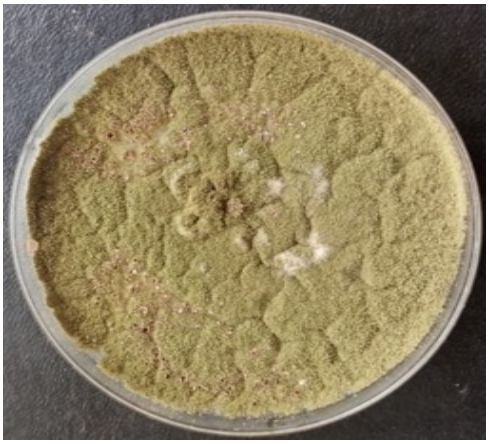
2.4.4 Macroscopic identification of *Aspergillus flavus* on PDA media

Macroscopic features of *Aspergillus flavus* on PDA media was almost similar in all 28 pure culture plates. Similarly, the colonies on the plates had powdered texture with slight variations in their diameters ranging between 45mm and 70mm after four days of incubation. In most cases, the colonies were flat at their borders and raised in the middle. All the plates were dominated by olive green surface color of the colony with whitish margin while their reverse sides were creamish. However, some of the plates had colonies

lacking the whitish margin. Some of the *A. flavus* isolates produced colourless exudates while others did not. In addition, some of the isolates produced a compact mass of the mycelia (sclerotia) that appeared dark brown in colour.



(a)



(b) (c)

Figure 2.3: Macroscopic features of *Aspergillus flavus* on PDA media: (a) Fungal growth on PDA plates after two days of incubation at 37^0C, (b) Appearance of the colony surface observed after six days of incubation at 37^0C and (c) Reverse side of the growth in (b)

2.4.5 Microscopic identification of *Aspergillus flavus*

Microscopic identification of *Aspergillus flavus* under the microscope showed that the conidiophores bearing the vesicles were colorless and thick walled. The conidial heads were biseriate or uniseriate with vesicles, which varied in size and appeared with globose or sub-globose shape. In the biseriate conidial heads, the phialides grew from the matulae while in the uniseriate the phialides grew on the vesicle. In addition, the conidial head of the species had a globose shape with variable size and aseptate hyphae.

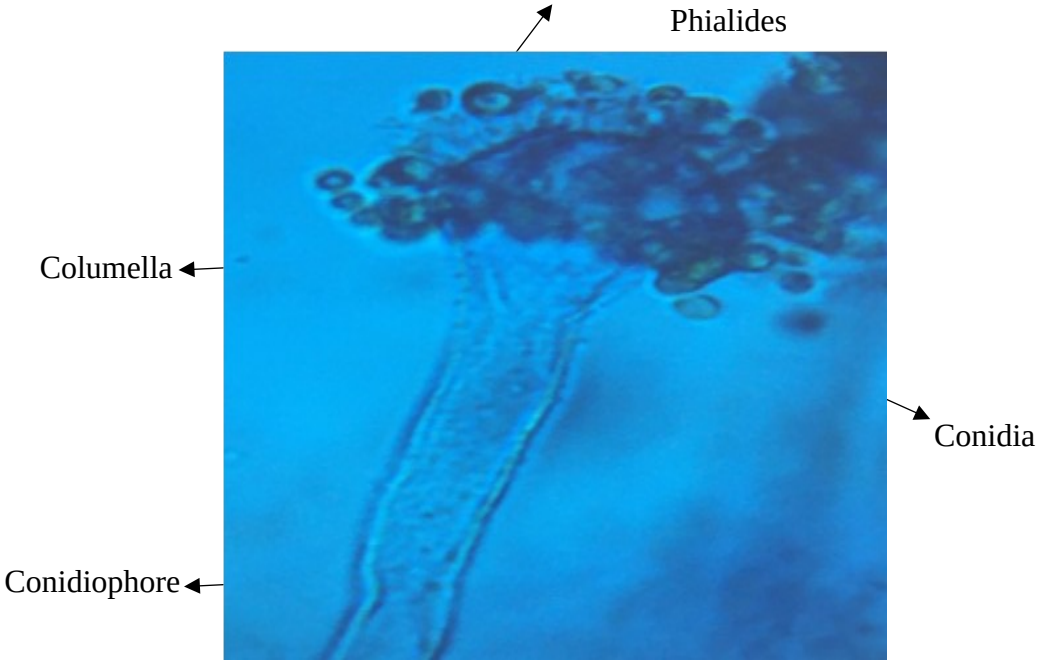
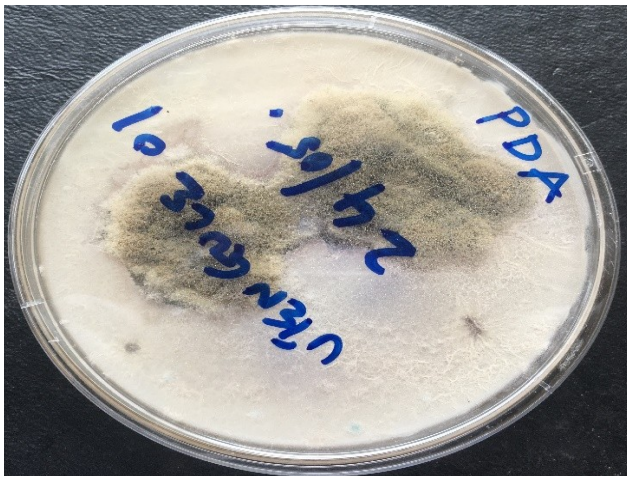


Figure 2.4: Microscopic observation of *Aspergillus flavus* under the light microscope

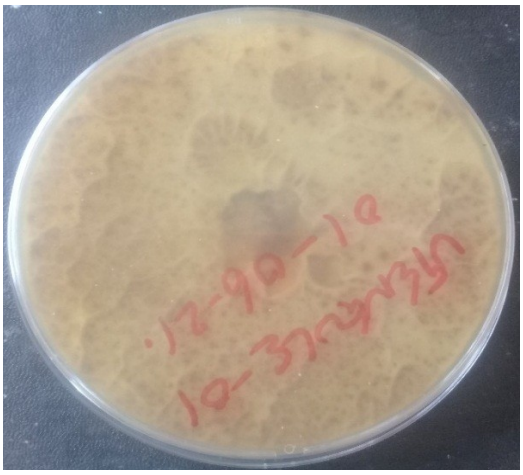
2.4.6 Macroscopic identification of *Aspergillus parasiticus* on PDA media

Macroscopic features of *Aspergillus parasiticus* on PDA media was almost similar in all six pure culture plates. Similarly, the colonies on the plates had powdered texture with slight variations in their diameters ranging between 35mm and 65mm after four days of incubation. All the plates were dominated by dark green surface color of the colony while their reverse sides were yellowish-cream. *A. parasiticus* isolates lacked exudates and

some produced pigments with brown colour on the reverse side of the growth. In addition, some of the isolates produced a compacted mass of mycelia (sclerotia) with a dark colour.



(a)



(b) **(c)**

Figure 2.5: Macroscopic observation of *Aspergillus parasiticus* on PDA media: (a) Fungal growth on PDA plates after two days of incubation at 37^0C, (b) Appearance of the colony observed after six day of incubation at 37^0C and (c) Reverse side of the growth in (b)

2.4.7 Microscopic observation of *A. parasiticus*

Microscopic identification of *Aspergillus parasiticus* under the microscope showed that the conidiophores bearing the vesicles were colorless, rough, aseptate and thin walled. At the tip of the conidiophores, there were columella with various sizes and globose shape. The conidial heads were biseriate and appeared with globose shape. In addition, the conidial head of the species had a globose shape with variable size and aseptate hyphae.

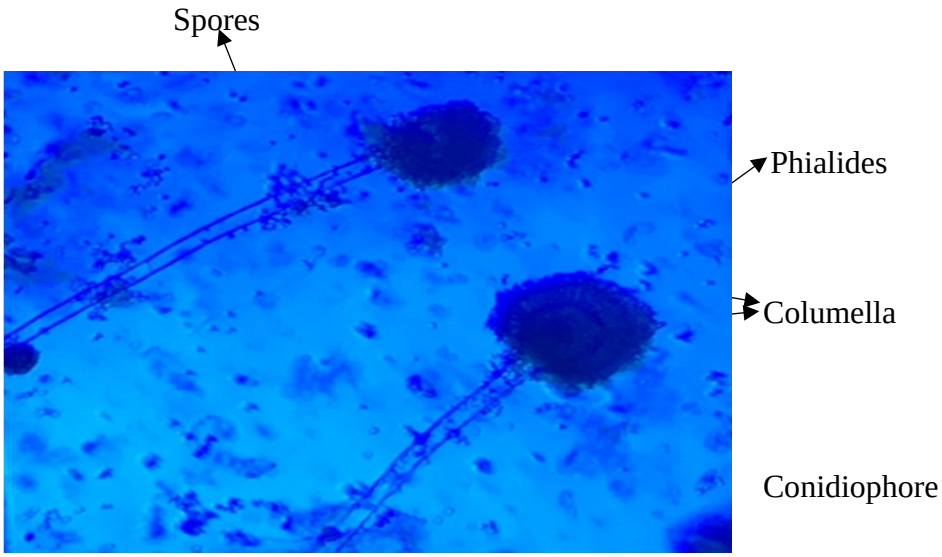


Figure 2.6: Microscopic observation of *A. parasiticus* under the light microscope

2.5 Discussion

The present study found that about 23.9% of all samples collected from both study areas had their moisture content (MC) above 14%. MC below 14% inhibits the growth of aflatoxigenic fungi while that above 14% favours their growth on stored crops at various temperatures according to Shekhar *et al*. (2018) and Shitu *et al.* (2018). The

two study areas have varying degrees of temperature and humidity where Makambako is relatively more humid and cool compared to Morogoro municipality.

Therefore, most of study samples (with MC above 14%) were from Makambako district (36) while the remaining (18) were from Morogoro municipality. With the fact that temperature and humidity are climatic conditions known to influence the behaviour and survival of fungi that infest stored crops (Weinberg *et al.*, 2008; Achaglinkame *et al.*, 2017), this indicates that the significant variation of these conditions between the two study areas could be the reason why a higher number of samples that had a MC above 14% were from Makambako district. This study corresponds to the study conducted by Mpuchane *et al.* (1997) in Botswana who found that some of the maize samples collected from warehouses had a MC above 14%. Similarly, the study conducted by Danso *et al.* (2019) in Ghana found that some of the maize samples collected from warehouses had a MC above 14%. This implies that the measures taken to reduce the moisture content in the maize prior to storage in many African countries are inadequate. The MC of the stored maize could also be affected by other factors including variations of temperatures and humidity within the warehouses during storage.

This study also revealed that about 21.2% of maize samples collected from warehouses were contaminated with fungal species. This percentage of contaminated samples is significantly low compared to that revealed by other studies such as that conducted by Olagunju *et al.* (2018) in Durban, South Africa where 70% of maize samples collected from stores were contaminated with fungal species. Similarly, another study conducted in South Africa by Chilaka *et al.* (2012) found that about 87% of commercial maize samples collected from stores were contaminated with fungal species. This variation in percentage of contaminated samples could reflect a parallel variation in the practices employed prior

to storing maize. Furthermore, the variations of the percentage of contaminated samples is also a consequence of the great variations in the climatic conditions of the two areas (Tanzania and South Africa).

Furthermore, the study also revealed that *Aspergillus* species were the most dominant fungal species contaminating the maize in storage facilities. This finding is in line with the findings from another study conducted by Saleemi *et al.* (2012) in Pakistan who also found that *Aspergillus* species were the most dominant fungal species compared to others capable of contaminating stored maize. Similarly, the study conducted by Balendres *et al.* (2019) in Philippines also found that *Aspergillus* species were most dominant fungal species contaminating agricultural commodities. This predominance of *Aspergillus* species in the contaminated maize samples is a result of the significantly greater ability of the species to survive in a wide range of environmental conditions as well as their capacity to infest a wide range of agricultural commodities as reported by Mousavi *et al.* (2016) and Thathana *et al*. (2017).

The study also found that there was unequal distribution *A. flavus* and *A. parasiticus* species among the isolates from the maize samples. Furthermore, the study found that the frequency of isolation of *A. flavus* (82.4%) was high compared to *A. parasiticus* (17.6%). This finding is comparable to that from a study conducted by Iheanacho *et al.* (2014) in South Africa who similarly found that *A. flavus* was more prevalent than *A. parasiticus* in agricultural crops. A study conducted in Zaria, Nigeria by Shitu *et al.* (2018) also found that there was High prevalence of *A. flavus* compared to *A. parasiticus* in millet and maize samples collected from markets. This might be influenced by resilience and ability of *A. flavus* to grow in substrates under a diverse range of environmental conditions as reported by Nazir *et al.* (2014).

2.6 Conclusion and Recommendation

The study revealed that *A. flavus* was the major aflatoxigenic fungi contaminating stored maize collected from warehouses in both Morogoro municipality and Makambako district. The study also indicated that humidity and temperature conditions of Makambako district favour the growth of aflatoxigenic fungi on stored maize compared to Morogoro Municipality. In view of the fact that it is not easy to control climatic conditions of an area, we recommend that stakeholders should adopt good storage practices to minimize fungal infestation and subsequent aflatoxin contamination on stored maize. Moreover, further studies should be conducted to determine the status of other mycotoxins contaminating stored crops in different regions of Tanzania. However, education on the impacts of fungal and mycotoxins contamination on stored crops should be provided to storage facility owners and other stakeholders.

References

Achaglinkame, M. A., Opoku, N. and Amagloh, F. K. (2017). Aflatoxin contamination in cereals and legumes to reconsider usage as complementary food ingredients for Ghanaian infants: A review. *Journal of Nutrition and Intermediary Metabolism* 10: 1– 7.

Balendres, M. A. O., Karlovsky, P. and Cumagun, C. J. R. (2019). Mycotoxigenic fungi and mycotoxins in agricultural crop commodities in the Philippines: A review. *Foods* 8(249): 1 – 12.

Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W. and Kyegombe, D. B. (2013). Review of the biological and health effects of aflatoxins on body organs and body systems. *Aflatoxins-Recent Advances and Future Prospects* 12: 239 – 265.

Chilaka, C. A., De Kock, S., Phoku, J. Z., Mwanza, M., Egbuta, M. A. and Dutton, M. F. (2012). Fungal and mycotoxin contamination of South African commercial maize. *Journal of Food Agric. Environ* 10(2): 296 – 303.

Danso, J. K., Osekre, E. A., Opit, G. P., Arthur, F. H., Campbell, J. F., Mbata, G. and McNeill, S. G. (2019). Impact of storage structures on moisture content, insect pests and mycotoxin levels of maize in Ghana. *Journal of Stored Products Research* 81: 114 – 120.

Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N. and Panneerselvam, A. (2011). Aflatoxins and aflatoxicosis in human and animals. *Aflatoxins-Biochemistry and Molecular Biology* 12: 221 – 254.

Egbuta, M. A., Mwanza, M., Njobeh, P. B., Phoku, J. Z., Chilaka, C. A. and Dutton, M. F. (2015). Isolation of filamentous fungi species contaminating some Nigerian food commodities. *Journal of Food Research* 4(1): 38 – 50.

Egwurochi, W. I., Nwosuocha, G. C., Olugbue, V. C., Uchendu, D. and Anyaegbunam, B. C. (2015). Isolation and identification of fungal association with stored maize grain in afikpo. *The Melting Pot* 1(1): 56 – 70.

Guchi, E. (2015). Aflatoxin contamination in groundnut (Arachis hypogaea L.) caused by Aspergillus species in Ethiopia. *Journal of Applied and Environmental Microbiology* 3(1): 11 – 19.

Iheanacho, H. E., Njobeh, P. B., Dutton, F. M., Steenkamp, P. A., Steenkamp, L., Mthombeni, J. Q. and Makun, A. H. (2014). Morphological and molecular identification of filamentous Aspergillus flavus and Aspergillus parasiticus isolated from compound feeds in South Africa. *Food Microbiology* 44: 180 – 184.

Karthikeyan, M., Karthikeyan, A., Velazhahan, R., Madhavan, S. and Jayaraj, T. (2013). Occurrence of aflatoxin contamination in maize kernels and molecular characterization of the producing organism, Aspergillus. *African Journal of Biotechnology* 12(40): 5839 – 5844.

Khan, R., Mohamad Ghazali, F., Mahyudin, N. A. and Samsudin, N. I. P. (2020). Morphological characterization and determination of aflatoxigenic and non-aflatoxigenic aspergillus flavus isolated from sweet corn Kernels and soil in Malaysia. *Agriculture* 10(450): 1 – 13.

Lyimo, S., Mdurum, Z. and De Groote, H. (2014). The use of improved maize varieties in Tanzania. *African Journal of Agricultural Research* 9(7): 643 – 657.

Mousavi, B., Hedayati, M. T., Hedayati, N., Ilkit, M. and Syedmousavi, S. (2016). Aspergillus species in indoor environments and their possible occupational and public health hazards. *Current Medical Mycology* 2(1): 36 – 42.

Mpuchane, S. F., Taligoola, H. K., Gashe, B. A., Zinzombe, I. and Matsheka, M. (1997). A mycological study of stored maize and sorghum grains. *Botswana Notes and Records* 29(1): 81 – 92.

Nazir, K. H. M. N. H., Hassan, J., Durairaj, P. and Yun, H. (2014). Isolation and identification of Aspergillus flavus from poultry feed samples using combined traditional molecular approach and expression of cyp64a1 at mRNA level. *Pakistan Journal Agriculture Science* 51(2): 287 – 291.

Olagunju, O., Mchunu, N., Venter, S., Guibert, B., Durand, N., Metayer, I. and Ijabadeniyi, O. (2018). Fungal contamination of food commodities in Durban, South Africa. *Journal of Food Safety* 38(6): 1 – 10.

Palencia, E. R., Hinton, D. M. and Bacon, C. W. (2010). The black Aspergillus species of maize and peanuts and their potential for mycotoxin production. *Toxins* 2(4): 399 – 416.

Reeb, J. E. and Milota, M. R. (1999). *Moisture Content by the Oven-Dry Method for Industrial Testing.* Oregon State University Corvailis, USA. 9pp.

Saleemi, M. K., Khan, M. Z., Khan, A., Javed, I., Ul Hasan, Z., Hameed, M. R. and Mehmood, M. A. (2012). Occurrence of toxigenic fungi in maize and maize-gluten meal from Pakistan. *Phytopathologia Mediterranea* 51(1): 219 – 224.

Seetha, A., Munthali, W., Msere, H. W., Swai, E., Muzanila, Y., Sichone, E. and Okori, P. (2017). Occurrence of aflatoxins and its management in diverse cropping systems of central Tanzania. *Mycotoxin Research* 33(4): 323 – 331.

Shekhar, M., Singh, N., Bisht, S., Singh, V. and Kumar, A. (2018). Effects of climate change on occurrence of aflatoxin and its impacts on maize in India. *International Journal Current Microbiology Applied Science* 7(6): 109 – 116.

Shitu, S., Macchido, D. A. and Tijjani, M. B. (2018). Detection of aflatoxigenic moulds and aflatoxins in maize and millet's grains marketed in Zaria Metropolis. *Journal of Advances in Microbiology* 13(4): 1 – 9.

Sukmawati, D., Setyaningsih, A., Rahayu, S., Rustam, Y., Moersilah, M., Wahyudi, P. and Husna, S. N. A. (2018). Isolation and characterization of aflatoxigenic Aspergillus spp. from maize of livestock feed from Bogor. In: *3rd Annual Applied Science and Engineering Conference Series.* Institute of Physics Publishing, UK. pp. 1 – 9.

Suleiman, R. A. and Kurt, R. A. (2015). *Current* Maize Production, Postharvest Losses and the Risk of Mycotoxins Contamination in Tanzania. *Presentation at the 2015 American Society of Agricultural and Biological Engineers Annual International Meeting*. New Orleans, Louisiana. 127pp.

Thathana, M. G., Murage, H., Abia, A. L. K. and Pillay, M. (2017). Morphological characterization and determination of aflatoxin-production potentials of Aspergillus flavus isolated from maize and soil in Kenya. *Agriculture* 7(80: 1 – 14.

United Republic of Tanzania (2017). *Morogoro Municipal Council Strategic Plan 2016/2017 – 2020/2021.* President's Office/Regional Administration and Local Government, Morogoro. 213pp.

United Republic of Tanzania (2017). *Makambako Town Council Strategic Plan* 2016/ 17 – 2020/21. Town Directors Office, Makambako, Njombe. 189pp.

Weinberg, Z. G., Yan, Y., Chen, Y., Finkelman, S., Ashbell, G. and Navarro, S. (2008). The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic storage conditions in vitro studies. *Journal of Stored Products Research* 44(2): 136 – 144.

Wu, F. (2015). Global impacts of aflatoxin in maize: trade and human health. *World Mycotoxin Journal* 8(2): 137 – 142.

CHAPTER THREE

MANUSCRIPT TWO

3.0 Knowledge, Awareness and Post-harvest practices Predisposing Stored Maize to Aflatoxin Contamination in Morogoro Municipality and Makambako District, Tanzania

Fadhili Mabruki[1*], Isaac Mkundi[1], Benigni Alfred Temba[2]

[1] Department of Microbiology, Parasitology and Biotechnology, Sokoine University of Agriculture, P.O.BOX 3019, Morogoro, Tanzania.

[2] Department of Veterinary Physiology, Biochemistry and Pharmacology, Sokoine University of Agriculture, P.O.BOX 3017, Morogoro, Tanzania.

* Corresponding Author: Tel: +255-785-772 625. E-mail: mabrukifadhili@gmail.com

Published by American Journal of Public Health Research

Link: http://www.sciepub.com/ajphr/content/10/4

CHAPTER FOUR

4.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 Discussion

This section discusses the results of this study with reference to the main objective that address the stakeholders' knowledge, awareness and occurrence of *Aspergillus* species on stored maize in Morogoro municipality and Makambako district. The discussion also addresses specific objectives by providing an insight on the practices predisposing stored maize to aflatoxins contamination as well as proportional occurrence of *Aspergillus flavus* and *Aspergillus parasiticus* isolated from the stored maize under the study areas.

Generally, the present study indicated that minority of the respondent (29.1%) had high knowledge and awareness on aflatoxins contamination on stored maize while majority of the respondent (70.1%) did not. This finding agrees with the findings of other studies conducted in various parts of Tanzania by Suleiman *et al*. (2017) and Fundikira *et al.* (2021) who found that majority of the stakeholders (Farmers, traders and Consumers), over 80%, were unaware of aflatoxins that contaminate crops. Similarly, a study conducted by Guchi *et al.* (2015) in Ethiopia also found that over 80% of the farmers and traders had little knowledge and awareness on aflatoxins contamination in groundnuts. Therefore, low knowledge and awareness found in this study and other reported studies suggest that the education level of the respondents towards aflatoxin contamination in crops is relatively low and could be reflected by inadequate dissemination of information on aflatoxins by the responsible stakeholders.

Based on the factors predisposing stored maize to aflatoxins contamination, this study found that aspects such as storage duration, storage with other crops, condition of storage

building and storage mode significantly influenced fungal infestation and subsequent contamination on stored maize. This study is in line with the findings conducted by Akowuah *et al.* (2015) in Ghana who found that practices such as storage method and storage structures employed by the farmers and traders positively influenced fungal infestation and aflatoxins contamination on stored crops. Similarly, the study conducted by Maina *et al.* (2016) in Kaiti district, Kenya also found that practices such as storage mode, storage duration and storage structure influenced fungal infestation and their subsequent contamination on stored maize. This implies that the majority of agricultural stakeholders involved in this study and other reported studies were unaware of the good storage practices that reduce fungal infestation and aflatoxins contamination on stored crops.

The present study revealed that about 23.9% of the total collected samples from both study areas had high moisture content above 14%. This implies that the measures taken to reduce moisture content in maize prior storage are inadequate. The study also found that about 34 (21.2%) of the total maize samples were contaminated with aflatoxigenic fungi particularly *Aspergillus flavus* and *Aspergillus parasiticus*. The study revealed that out of 34 contaminated samples, 16 (47.1%) samples came from Morogoro municipality while 18 (52.9%) came from Makambako district. Therefore, the slighter variation in contamination between Morogoro municipality and Makambako district might be influenced by variation of climatic conditions among the study areas. In addition, this slighter variation in contaminated samples could also reflect that poor storage practices are employed by stakeholders to store maize in warehouses under the study areas.

This study also found that *Aspergillus flavus* are more prevalent compared to *Aspergillus parasiticus* in all contaminated samples collected from both study areas. This finding is

comparable to the study conducted by Iheanacho *et al.* (2014) in South Africa who similarly found that *Aspergillus flavus* was more prevalent than *A.parasiticus* on stored crops. Similarly, a study conducted by Shitu *et al.* (2018) found that there was high prevalence of *A.flavus* than *A.parasiticus* in millet and maize samples collected from markets in Nigeria. This might be influenced by the ability of the *Aspergillus* species to grow and survive in substrates in diversity range of environmental conditions as reported by Nazir *et al.* (2014).

4.2 Conclusions

Maize cultivation is an important socio-economic activity that ensures the financial sustainability and food security to the Tanzanian community. More emphasis should be made on the management and production of the crop due to its great socio-economic sustainability potential. Infestation from pests and other pathogens, particularly aflatoxigenic fungi that consequently lead aflatoxin contamination is one of the major challenges facing the management of the crop especially during storage. This study revealed that some of the aspects including storage mode, nature of storage (alone /with other crops), storage duration and condition of storage building significantly influenced fungal infestation and subsequent contamination on stored maize. The study also clearly revealed the influence of climatic conditions on the subsequent fungal contamination on stored maize. Furthermore, the study revealed that responsible stakeholders in Morogoro municipality and Makambako district had relative little knowledge and awareness regarding aflatoxin contamination on stored maize.

Moreover, this study identified *A. flavus* was the major fungal species contaminating stored maize samples collected from both Morogoro municipality and Makambako district. This information reveals the necessity of creating a monitoring, surveillance and

intervention program on fungal infestation to prevent aflatoxin contamination. It is important to note that the crop is prone to infestation by aflatoxigenic fungi and other mycotoxigenic fungi that were not assessed in this study. This implies that there is high consumers' risk of exposure to other multiple mycotoxins with ill-health effects.

4.3 Recommendations

According to the findings from this study, it is recommended that;

i. The Government of Tanzania in collaboration with private sectors should provide agricultural stakeholders with adequate education to enhance knowledge and awareness regarding the impact of aflatoxins on humans and animal health through seminars and workshops.

ii. The Government of Tanzania should emphasize the stakeholders to employ the use of Purdue improve crop storage (PICS) with the aim of reducing fungal infestation in stored crops.

iii. Storage facility owners should regularly maintain their warehouses to reduce the chance of fungal infestation and subsequent aflatoxin contamination stored crops.

iv. Appropriate technology for the prevention and control of fungal infestation and aflatoxin contamination in stored crops, should be applied by storage facilities owners to reduce aflatoxin contamination on stored crops.

v. Further research focusing on different pre and post- harvest crop management system against fungal infestation and subsequent aflatoxin contamination should be undertaken in different regions of Tanzania.

References

Abbas, H. K., Zablotowicz, R. M., Weaver, M. A., Horn, B. W., Xie, W. and Shier, W. T. (2004). Comparison of cultural and analytical methods for determination of aflatoxin production by Mississippi Delta Aspergillus isolates. *Canadian Journal of Microbiology* 50(3): 193 – 199.

Akowuah, J. O., Mensah, L. D., Chan, C. and Roskilly, A. (2015). Effects 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.

Arapceska, M., Jovanovska, V., Jankuloski, Z., Hajrulai Musliu, Z. and Uzunov, R. (2015). Impact of aflatoxins on animal and human health. *International Journal of Innovative Science, Engineering and Technology* 2(2): 156 – 161.

Bailly, S., Mahgubi, A. E., Carvajal-Campos, A., Lorber, S., Puel, O., Oswald, I. P. and Orlando, B. (2018). Occurrence and identification of Aspergillus section flavi in the context of the emergence of aflatoxins in french maize. *Toxins* 10(525): 1 – 18.

Balendres, M. A. O., Karlovsky, P. and Cumagun, C. J. R. (2019). Mycotoxigenic fungi and mycotoxins in agricultural crop commodities in the Philippines: A review. *Foods* 8(249): 1 – 12.

Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Mutegi, C., Atehnkeng, J., Kaptoge, L. and Cotty, P. J. (2016). Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. *World Mycotoxin Journal* 9(5): 771 – 789.

Bankole, S. A. and Adebanjo, A. (2003). Mycotoxins in food in West Africa: Current situation and possibilities of controlling it. *African Journal of Biotechnology* 2: 254 – 263.

Bbosa, G. S., Kitya, D., Lubega, A., Ogwal-Okeng, J., Anokbonggo, W. W. and Kyegombe, D. B. (2013). Review of the biological and health effects of aflatoxins on body organs and body systems. *Aflatoxins-Recent Advances and Future Prospects* 12: 239 – 265.

Benkerroum, N. (2020). Chronic and acute toxicities of aflatoxins: Mechanisms of action. *International Journal of Environmental Research and Public Health* 17(423): 1 – 28.

Bianco, G., Russo, R., Marzocco, S., Velotto, S., Autore, G. and Severino, L. (2012). Modulation of macrophage activity by aflatoxins B1 and B2 and their metabolites aflatoxins M1 and M2. *Toxicon* 59(6): 644 – 650.

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

Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N. and Panneerselvam, A. (2011). Aflatoxins and aflatoxicosis in human and animals. *Aflatoxins-Biochemistry and Molecular Biology* 12: 221 – 254.

Egbuta, M. A., Mwanza, M., Njobeh, P. B., Phoku, J. Z., Chilaka, C. A. and Dutton, M. F. (2015). Isolation of filamentous fungi species contaminating some Nigerian food commodities. *Journal of Food Research* 4(1): 38 – 50.

Ellis, W. O., Smith, J. P., Simpson, B. K., Oldham, J. H. and Scott, P. M. (1991). Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and methods of control. *Critical Reviews in Food Science and Nutrition* 30(4): 403 – 439.

Fundikira, S. S., De Saeger, S., Kimanya, M. E. and Mugula, J. K. (2021). Awareness, handling and storage factors associated with aflatoxin contamination in spices marketed in Dar es Salaam, Tanzania. *World Mycotoxin Journal* 14(2): 191 – 200.

Gautam, A. K. and Bhadauria, R. (2012). Characterization of Aspergillus species associated with commercially stored triphala powder. *African journal of Biotechnology* 11(104): 16814 – 16823.

Gilbert, J. (1991). Regulatory aspects of mycotoxins in the European community and USA. *Fungi and Mycotoxins in Stored Products* 36: 194 – 197.

Gong, Y. Y., Watson, S. and Routledge, M. N. (2016). Aflatoxin exposure and associated human health effects, a review of epidemiological studies. *Food safety* 4(1): 14 – 27.

Gourama, H. and Bullerman, L. B. (1995). Aspergillus flavus and Aspergillus parasiticus: Aflatoxigenic fungi of concern in foods and feeds: A review. *Journal of Food Protection* 58(12): 1395 – 1404.

Guchi, E. (2015). Aflatoxin contamination in groundnut (Arachis hypogaea L.) caused by Aspergillus species in Ethiopia. *Journal of Applied and Environmental Microbiology* 3(1): 11 – 19.

Gupta, S. K., Srivastava, M., Osmanoglu, O., Xu, Z., Brakhage, A. A. and Dandekar, T. (2020). *Aspergillus Fumigatus Versus Genus Aspergillus: Conservation, Adaptive Evolution and Specific Virulence Genes*. Functional Genomics and Systems Biology Group, Wuerzburg, Germany. 39pp.

Hell, K. and Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African Journal of Microbiology Research* 5(5): 459 – 466.

Iheanacho, H. E., Njobeh, P. B., Dutton, F. M., Steenkamp, P. A., Steenkamp, L., Mthombeni, J. Q. and Makun, A. H. (2014). Morphological and molecular identification of filamentous Aspergillus flavus and Aspergillus parasiticus isolated from compound feeds in South Africa. *Food Microbiology* 44: 180 – 184.

Kamala, A., Kimanya, M., De Meulenaer, B., Kolsteren, P., Jacxsens, L., Haesaert, G. and Lachat, C. (2018). Post-harvest interventions decrease aflatoxin and fumonisin contamination in maize and subsequent dietary exposure in Tanzanian infants: cluster randomised-controlled trial. *World Mycotoxin Journal* 11(3): 447 – 458.

Kamala, A., Shirima, C., Jani, B., Bakari, M., Sillo, H., Rusibamayila, N. and Simba, A. (2018). Outbreak of an acute aflatoxicosis in Tanzania during 2016. *World Mycotoxin Journal* 11(3): 311 – 320.

Karthikeyan, M., Karthikeyan, A., Velazhahan, R., Madhavan, S. and Jayaraj, T. (2013). Occurrence of aflatoxin contamination in maize kernels and molecular characterization of the producing organism, Aspergillus. *African Journal of Biotechnology* 12(40): 5839 – 5844.

Kew, M. C. (2013). Aflatoxins as a cause of hepatocellular carcinoma. *Journal of Gastrointestinal and Liver Diseases* 22(3): 305 – 310.

Khalid, S., Hussain, N. and Imran, M. (2018). Detection of aflatoxigenicity of Aspergillus flavus, based on potential gene marker, from food and feed samples. *Journal of Food Safety* 38(3): 1 – 9.

Khan, R., Mohamad Ghazali, F., Mahyudin, N. A. and Samsudin, N. I. P. (2020). Morphological characterization and determination of aflatoxigenic and non-aflatoxigenic aspergillus flavus isolated from sweet corn Kernels and soil in Malaysia. *Agriculture* 10(450): 1 – 13.

Kibe, E. N. (2015). Occurrence of mycotoxigenic fungi in maize from food commodity markets in Kenya. *Bioscience Engineering* 21: 1 – 18.

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

Krishnan, S., Manavathu, E. K. and Chandrasekar, P. H. (2009). Aspergillus flavus: an emerging non-fumigatus Aspergillus species of significance. *Mycoses* 52(3): 206 – 222.

Kukom, E. O. (2017). Assessment of rodents' postharvest losses in on-farm maize storage in Kenya. Dissertation for Award of Jomo Kenyatta University, Nairobi, Kenya, 141pp.

Kumar, V. V. (2018). Aflatoxins: Properties, toxicity and detoxification. *Nutrition and Food Science International Journal* 6: 555 – 696.

Mahmoud, M. A., Ali, H. M., El-Aziz, A. R. M., Al-Othman, M. R. and Al-Wadai, A. S. (2014). Molecular characterization of aflatoxigenic and non-aflatoxigenic Aspergillus flavus isolates collected from corn grains. *Genetic Molecular Research* 13: 9352 – 9370.

Maina, A. W., Wagacha, J. M., Mwaura, F. B., Muthomi, J. W. and Woloshuk, C. P. (2016). Postharvest practices of maize farmers in Kaiti District, Kenya and the impact of hermetic storage on populations of *Aspergillus* spp. and aflatoxin contamination. *Journal of Food Research* 5(6): 53 – 66.

Mousavi, B., Hedayati, M. T., Hedayati, N., Ilkit, M. and Syedmousavi, S. (2016). Aspergillus species in indoor environments and their possible occupational and public health hazards. *Current Medical Mycology* 2(1): 36 – 42.

Mtega, M., Mgina, C. A., Kaale, E., Sempombe, J. and Kilulya, K. F. (2020). Occurrence of aflatoxins in maize and maize products from selected locations of Tanzania and the effects of cooking preparation processes on toxin levels. *Tanzania Journal of Science* 46(2): 407 – 418.

Nabwire, W. R., Ombaka, J., Dick, C. P., Strickland, C., Tang, L., Xue, K. S. and Wang, J. S. (2020). Aflatoxin in household maize for human consumption in Kenya, East Africa. *Food Additives and Contaminants* 13(1): 45 – 51.

Nazir, K. H. M. N. H., Hassan, J., Durairaj, P. and Yun, H. (2014). Isolation and identification of Aspergillus flavus from poultry feed samples using combined traditional molecular approach and expression of cyp64a1 at mRNA level. *Pakistan Journal Agriculture Science* 51(2): 287 – 291.

Nyangi, C., Mugula, J. K., Beed, F., Boni, S., Koyano, E. and Sulyok, M. (2016). Aflatoxins and fumonisin contamination of marketed maize, maize bran and maize used as animal feed in Northern Tanzania. *African Journal of Food, Agriculture, Nutrition and Development* 16(3): 11054 – 11065.

Odhiambo, B. O., Murage, H. and Wagara, I. N. (2013). Isolation and characterisation of aflatoxigenic Aspergillus species from maize and soil samples from selected counties of Kenya. *African Journal of Microbiology Research 7*(34): 4379 – 4388.

Palencia, E. R., Hinton, D. M. and Bacon, C. W. (2010). The black Aspergillus species of maize and peanuts and their potential for mycotoxin production. *Toxins* 2(4): 399 – 416.

Pasqualotto, A. C. (2009). Differences in pathogenicity and clinical syndromes due to Aspergillus fumigatus and Aspergillus flavus. *Medical Mycology* 47(1): 261 – 270.

Paulussen, C., Hallsworth, J. E., Álvarez-Pérez, S., Nierman, W. C., Hamill, P. G., Blain, D. and Lievens, B. (2017). Ecology of aspergillosis: Insights into the pathogenic potency of Aspergillus fumigatus and some other Aspergillus species. *Microbial Biotechnology* 10(2): 296 – 322.

Priyanka, S. R., Venkataramana, M., Kumar, G. P., Rao, V. K., Murali, H. C. S. and Batra, H. V. (2014). Occurrence and molecular detection of toxigenic Aspergillus species in food grain samples from India. *Journal of the Science of Food and Agriculture* 94(3): 537 – 543.

Purchase, I. F. H. (1967). Acute toxicity of aflatoxins M1 and M2 in one-day-old ducklings. *Food and Cosmetics Toxicology* 5: 339 – 342.

Rudramurthy, S. M., Paul, R. A., Chakrabarti, A., Mouton, J. W. and Meis, J. F. (2019). Invasive aspergillosis by Aspergillus flavus: epidemiology, diagnosis, antifungal resistance, and management. *Journal of Fungi* 5(55): 1 – 23.

Samson, R. A., Visagie, C. M., Houbraken, J., Hong, S. B., Hubka, V., Klaassen, C. H. and Frisvad, J. C. (2014). Phylogeny, identification and nomenclature of the genus Aspergillus. *Studies in Mycology* 78: 141 – 173.

Seetha, A., Munthali, W., Msere, H. W., Swai, E., Muzanila, Y., Sichone, E. and Okori, P. (2017). Occurrence of aflatoxins and its management in diverse cropping systems of central Tanzania. *Mycotoxin Research* 33(4): 323 – 331.

Shehab, L. M., El-Leboudy, A. A. and Abo El-Makarem, H. S. (2019). Prevalence of Aflatoxins M1 and M2 in some curd dairy products. *Alexandria Journal for Veterinary Sciences* 61(1): 140 – 145.

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

Shitu, S., Macchido, D. A. and Tijjani, M. B. (2018). Detection of aflatoxigenic moulds and aflatoxins in maize and millet's grains marketed in Zaria Metropolis. *Journal of Advances in Microbiology* 13(4): 1 – 9.

Sowley, E. N. K. (2016). Aflatoxins: A silent threat in developing countries. *African Journal of Biotechnology* 15(35): 1864 – 1870.

Sukmawati, D., Setyaningsih, A., Rahayu, S., Rustam, Y., Moersilah, M., Wahyudi, P. and Husna, S. N. A. (2018). Isolation and characterization of aflatoxigenic Aspergillus spp. from maize of livestock feed from Bogor. In: *3rd Annual Applied Science and Engineering Conference Series.* Institute of Physics Publishing, UK. pp. 1 – 9.

Suleiman, R. A. and Kurt, R. A. (2015). *Current* Maize Production, Postharvest Losses and the Risk of Mycotoxins Contamination in Tanzania. *Presentation at the 2015 American Society of Agricultural and Biological Engineers Annual International Meeting*. New Orleans, Louisiana. 127pp.

Suleiman, R. A., Rosentrater, K. A. and Chove, B. (2017). Understanding postharvest practices, knowledge, and actual mycotoxin levels in maize in three agro-ecological zones in Tanzania. *Journal of Stored Products and Postharvest Research* 8(7): 73 – 84.

Te Biesebeke, R. and Record, E. (2008). Scientific advances with Aspergillus species that are used for food and biotech applications. *Microbes and Environments* 23(3): 177 – 181.

Thathana, M. G., Murage, H., Abia, A. L. K. and Pillay, M. (2017). Morphological characterization and determination of aflatoxin-production potentials of Aspergillus flavus isolated from maize and soil in Kenya. *Agriculture* 7(80: 1 – 14.

Unnevehr, L. and Grace, D. (2013). *Aflatoxins: Finding Solutions for Improved Food Safety*. Focus No. 20. International Food Policy Research Institute, Washington DC. 62pp.

Van Egmond, H. P. and Jonker, M. A. (2004). Worldwide regulations on aflatoxins – the situation in 2002. *Journal of Toxicology: Toxin Reviews* 23(3): 273 – 293.

Varga, J., Juhász, Á., Kevei, F. and Kozakiewicz, Z. (2004). Molecular diversity of agriculturally important Aspergillus species. *European Journal of Plant Pathology* 110: 627 – 640.

Wogan, G. N. (1966). Chemical nature and biological effects of the aflatoxins. *Bacteriological Reviews* 30(2): 460 – 470.

Wu, F. (2015). Global impacts of aflatoxin in maize: trade and human health. *World Mycotoxin Journal* 8(2): 137 – 142.

Wu, F. and Guclu, H. (2012). Aflatoxin regulations in a network of global maize trade. *PloS One* 7(9): 1 – 8.

Xie, L., Chen, M. and Ying, Y. (2016). Development of methods for determination of aflatoxins. *Critical Reviews in Food Science and Nutrition* 56(16): 2642 – 2664.

Yiannikouris, A. and Jouany, J. P. (2002). Mycotoxins in feeds and their fate in animals: A review. *Animal Research* 51(2): 81 – 99.

Zulkifli, N. A. and Zakaria, L. (2017). Morphological and molecular diversity of Aspergillus from corn grain used as livestock feed. *HAYATI Journal of Biosciences* 24(1): 26 – 34.

APPENDICES

Appendix 1: **Questionnaire to assess stakeholders' knowledge and awareness on aflatoxins as well as factors predisposing stored maize to aflatoxin contamination**

Part A: Respondent Data

Date of interview (dd/mm/yy).....

Village/Street..... Ward..... District.....

Region.....

Sex: Male ( ) Female ( ): Put √ for correct answer.

Age (years).....

Education level: Non-formal ( ) Primary ( ) Secondary ( ) College ( ): Put √ for correct answer.

Questionnaire number .....

Part B: Information on Maize Storage Practices

- **Maize Storage (Circle the appropriate Answer)**

NOTE: you can circle more than one if applicable

1. Do you store maize in the store every season?

(a) Yes

(b) No

2. What type of storage do you use among the following?

a) Floor storage

(b) Sack storage

(c) Open field storage

(d) Hermatic storage

3. If sack storage is used what type of storage bag is used?

(a) Polypropylene bag

(b) Sisal-woven bag

4. Method of drying maize before storing;

(a) Bare ground

(b) Mats/Tarpaulin

(c) Platform

(d) Smoking

(e) Others

5. In what form do you preferably store maize?

(a) Husked maize

(b) De-husked maize cobs

(c) Shelled grains

6. Do you store other products in the store, together with maize?

(a) Yes

(b) No

7. If “Yes”, specify.

(a) Beans

(b) Rice

(c) Millet

(d) Groundnuts

(e) Cassava

(f) Others

- **Condition of building/Store (Circle the appropriate Answer)**

8. For how many seasons have you used the store?

(a) One year

(b) Two years

(c) Three years

(d) Four years

(e) More than four years

9. How often do you subject your storage structure to routine maintenances?

(a) Once every 3 months

(b) Once every 6 months

(c) Once per year

(d) Whenever necessary

(e) Never done

10. General storage condition;

(a) Good

(b) Fair

(c) Poor

- **Storage time state of maize grains (Circle the appropriate Answer)**

11. For how long the grains have been stored since harvested/ purchased?

(a) Less than a month

(b) 1 to 3 months

(c) 3 to 6 months

(d) 6 to 9 months

(e) 9 to 12 months

(f) Over 12 months

12. Describe the state of stored grains;

    (a) Clean

    (b) Spoilage

    (c) Dried

    (d) Moist

13. Any sorting before storage?

    (a) Yes

    (b) No

14. If yes, how do you sort?

    (a) Manually (hand picking)

    (b) Others method

15. What criteria do you use when sorting?

    (a) Colour

    (b) Size

    (c) Shape

    (d) Physical damaged

16. Do you use any method to treat grains before storing?

    (a) Yes

    (b) No

17. If “yes”, Specify.

    (a) Pesticides

    (b) Smoking

    (c) Others

Part C: Assessment of Knowledge and Awareness on Aflatoxin

Note: (Circle the appropriate Answer)

18. Have you ever heard about mycotoxins/aflatoxins?

    (a) Yes

    (b) No

19. If yes, what do you understand about aflatoxins? .....

20. If yes from question number (19), where did you get this information?

    (a) School

    (b) Social Media

    (c) Radio/ Television (TV)

    (d) Pear group/Friends

    (e) Other sources

21. Are you aware of aflatoxins that contaminate maize during storage?

    (a) Yes

    (b) No

22. Aflatoxins that contaminated in food crops is caused by?

    (a) Fungi

    (b)Parasite

    (c) Bacteria

    (d) Virus

    (e) Don’t know

23. Are you aware of ill-health effects of aflatoxins in human beings and animals?

    (a) Yes

    (b) No

24. Can you identify the presence of fungi in cereals by your naked eyes?

    (a) Yes

    (b) No

25. If yes, which features will you ascertain about presence of fungi contamination in the food materials/cereals?

    (a) Colour

    (b) Spoilage

    (c) Mouldy

    (d) All of the above

26. Which conditions among the following ones do you think favours growth of fungi that produce aflatoxins?

    (a) Moisture and conducive temperature

    (b) Dust and very cold condition

    (e) All of the above

    (d) Don’t know

.....THE END.....

Appendix 2: **Significance variation in moistures contents of the samples with respect to the site of collection**

Surveyed Wards		**Significance**	**95% Confidence Interval**	
			Lower Bound	**Upper Bound**
Mwembetogwa	Kingo	.000	.545	2.680
	Kihonda	.000	1.635	3.757
	Uwanja wa Taifa	.000	.912	3.033
	Mjimwema	.081	-.057	2.121
	Utengule	.019	.102	2.136