AFLATOXIN AND FUMONISIN CONTAMINATION IN HOMEMADE AND COMMERCIAL CEREAL BASED COMPLEMENTARY FOODS WITH FORMULA IN MOROGORO MUNICIPALITY, TANZANIA

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ABSTRACT

Aflatoxins (AF) and fumonisins (FM) are major foodborne mycotoxins of public health concerns that infect maize and groundnuts which are the main components of cereal based complementary foods with formula (CBCF-F). The study was conducted to: determine and compare the quantity of total AF and FM among different types of CBCF-F, identify the factors that might influence high contamination of AF and FM in CBCF-F, and assess awareness and perceptions of the processors about AF and FM contamination in cereals. The study design was cross-sectional, involving 60 processors whose 70 CBCF-F were collected for laboratory analysis. High Performance Liquid Chromatography and Enzyme Linked Immunosorbent Assay methods were used to quantify total AF and FM in the 70 CBCF-F samples. Data analysis was done using SPSS, and comparisons of the total AF and FM among samples were done with Duncan's LSD. The results showed that 93 and 98% of CBCF-F samples were contaminated with AF and FM respectively; 32.9% of the samples exceeded the regulatory limit of 10 ppb set by TBS. There was no sample that exceeded the regulatory limit of 2 ppm for total fumonisin. The factors that increased AF contamination in CBCF-F were the use of groundnuts ($p < 0.05$) and living in rental houses ($p < 0.05$). Eighty-two (82%) and 95% of the respondents were not aware of AF and FM respectively; 90 had seen molds but 76.7% of them did not associate them with health implications while 28.3 and 48.3% perceived molds as crop diseases and decayed foods with no health implications to human being respectively. It is concluded that infants depending on CBCF-F are exposed to AF and FM contamination. Also, groundnuts and rental houses contribute significantly to increasing total AF in CBCF-F, and the majority of processors of CBCF-F are not aware of multiple occurrences of mycotoxins in cereal products. It is recommended that mycotoxins contamination should be viewed as a cross-cutting issue and given priority in Tanzania.

DECLARATION

I, TABU PASKAZIA KATENGESYA, 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.

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The above declaration is confirmed by;

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(Supervisor)

26/03/2018

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I dedicate this work to my Son Kisena-Goodluck Isack Kandola, from whom I derived motivation to carry out this study. Kisena you need to achieve better than me in your life. I also dedicate this work to all women passing through difficult situations in one way or another while struggling for important issues for betterment of themselves, their families and the nation. To all I say don’t give up until you reach your destiny.

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LIST OF ABREVIATIONS AND ACRONYMS

AF	Aflatoxin
a_W	Water activity
CBCF-F	Cereal Based Complementary Foods with Formula
CBCFs	Cereal Based Complementary Foods
CFM	Commercial Factory Made
CLM	Commercial locally made CBCF-F
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FM	Fumonisin
HM	Homemade CBCF-F
HPLC	High Performance Liquid Chromatography
IITA	International Institute of Tropical Agriculture
n	Number of people
NCHS	National Centre for Health Statistics
NGOs	Non-Government Organizations
pH	Hydrogen Iron Concentration
ppb	Parts per Billion
ppm	Parts per Million
SIDO	Small Scale Industries Organizations
SPSS	Statistical Package for Service Solution
TBS	Tanzania Bureau of Standard
TDHS	Tanzania Demographic and Health Survey
TDHS-MIS	Tanzania Demographic Health Survey and Malaria Indicator Survey
TFDA	Tanzania Food and Drug Authority

UNIDO	United Nations Industrial Development Organization
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Mycotoxins are groups of naturally occurring toxic secondary metabolites produced by certain molds, capable of causing death and diseases to human beings (Strosnider *et al*., 2006; Smith *et al*., 2012). They are produced on a variety of different crops and foodstuffs including cereals like maize, sorghum, rice and wheat, dried cassava, nuts, spices, dried fruits, apple juice and coffee, often under warm and humid environmental conditions (Groopman *et al*., 2014). They mostly affect people in developing countries located in 40° N and 40° S of the Equator; it is estimated that 4.5 billion people living in the equatorial countries have been affected by aflatoxins (Williams *et al.*, 2004).

1.1.1 Types of mycotoxins

There are approximately 300 to 400 compounds that are currently classified as mycotoxins, with 12 recognized to be important to animal and human health (Bennett and Klich, 2003). But five (5) toxins are most harmful from a food safety perspective for humans. These are: i) Aflatoxins (B1, B2, G1, G2,M1 and M2) produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi, ii) Fumonisins (B1, B2 and B3) produced by *Fusarium* molds, iii) *Ochratoxin A,* iv) *Patulin* and v) *Trichothecenes* (Robbins *et al.*, 2000). Aflatoxins especially B1 and fumonisins are two families of mycotoxins that are particularly important in the context of child health in sub-Saharan Africa, because they are highly prevalent in the food chain and have substantial negative health consequences (Simith *et al*., 2012). This is because both toxins are particularly common in maize and groundnuts which constitute a major portion of the diet in many developing countries (Smith *et al*., 2012).

1.1.2 Aflatoxin and fumonisin

Aflatoxins and fumonisins are the major mycotoxins that naturally contaminate maize worldwide. The highest contamination was recorded in maize from countries located in tropical and subtropical regions of the world (Williams *et al*., 2004; Kimanya *et al*., 2008a; Njobeh e*t al*., 2012). Aflatoxin contamination has been world-widely studied because of its role in liver cancer. Conversely, in sub-Saharan Africa, fumonisin contamination in maize has been extensively studied in South Africa and to a lesser extent in some other countries (Kimanya *et al*., 2009; Smith *et al*., 2012).

1.1.3 Tanzania's complementary foods

In Tanzania, majority people use homemade complementary foods based on cereals usually thin cereals porridge made from undehulled maize for their infants (Mamiro *et al*., 2005; Nyaruhucha *et al*., 2006; Kimanya *et al*., 2009). Other mothers use commercial locally produced blended cereal based complementary food commonly known as ''*Unga wa Lishe''* (observed from Tanzanian Urban societies). Mycotoxins such as aflatoxins and fumonisins have also been found in some home grown maize in Tanzania (Kimanya *et al*., 2008a; 2008b). Inadequate complementary feeding has been linked with poor growth among infants due to increased infections from contaminated weaning foods (Gong *et al*., 2003; Kimanya *et al*., 2010).

1.2 Problem Statement

Maternal and child health has become a public concern of government and other stake-holders all-over the world. In Tanzania, in spite of many efforts to combat the problem, yet malnutrition in children under five years is still high in many parts of the country. For example, the prevalence of stunting in the country is 34%, underweight is 14% and wasting is 5% (TDHS-MIS, 2015-2016). It has been observed that in Tanzania, infants

experience growth retardation during the period of introduction of complementary food (Mosha and Tarimo 2006; Kimanya *et al*., 2010; TDHS, 2010). It is possible that some of the factors contributing to growth retardation at this age are consumption of diets contaminated with mycotoxins (Lombard, 2014). For instance children less than 2 years old are fed with undehulled blended cereals (Maize, rice and groundnuts) and infants aged 6-8 months are fed with undehulled maize-porridge alone (observed from our societies). A study of aflatoxin B1 (AFB1-lysine adduct) in pregnant women's blood and cord in Kumasi, Ghana which was conducted by Shuaib *et al.* (2010) indicated that participants with very high AFB1-lysine level >11.34 pg/mg (AFB1-lysine equivalent/mg albumin) were more likely to have low birthweight babies compared to participants in the lowest quartile. Furthermore, another study which was conducted by Shirima *et al*. (2013) and Shirima (2016) in some parts of Tanzania to children under five years age indicated that poor children's growth was correlated with high aflatoxin and fumonisin levels in their plasma blood adduct and urine. In addition, Kimanya *et al*. (2014) studies in some parts of Tanzania on correlation between food intake and children nutritional status found that underweight among children was correlated with exposures to food contaminated by aflatoxin and fumonisin.

In Tanzania, there are many entrepreneurs dealing with processing of blended cereal products which are known as *Composite Flour* (‘‘*Unga wa Lishe”*) which are believed by many Tanzanians to be safe and nutritious for human consumption, specifically for infants and pregnant mothers. These complementary foods are mostly formulated from maize and groundnuts, foods that are susceptible to infestation by molds and hence are likely to be contaminated with mycotoxins. Several studies which have been conducted in different parts of Tanzania have revealed that mycotoxins (aflatoxins and fumonisins) exist in homemade cereals based complementary foods (Kimanya *et al*., 2009, 2010, 2014; Srey *et*

al., 2014). Also, Biomarkers studies testing infants' blood samples conducted by Shirima *et al*. (2013) and Shirima (2016) revealed that young children in Tanzania are chronically exposed to both aflatoxin and fumonisin through contaminated diet, although the level of exposure varies from one place to another place. Unlike in developed countries, in developing countries most of countries have no regulatory enforcement set to ensure regular checkup of the safety limit of mycotoxins in foods neither human nor animals. For example testing of raw agricultural products consumed locally in Tanzania is rare (Abt Associates Inc, 2012a). One approach to managing the risks associated with mycotoxin contamination is the use of an integrated system based on the Hazard Analysis and Critical Control Point (HACCP) approach. This approach involves strategies for prevention, control, good manufacturing practices, and quality control at all stages of production, from the field to the final consumer (Williams *et al*., 2004). There is limited information on mycotoxins content of complementary foods based on blended cereals made by home and local producers/ commercial complementary foods (Kimanya *et al*., 2009, 2010, 2014; Rushunju, 2012; Lombard, 2014). This study, therefore, intended to find out whether the processed composite flour from homemade and commercialized are within the recommended standard of contaminants according to Tanzanian set standards and also to identify factors influencing AF and FM in CBCF-F. Moreover, the study sought to assess awareness, attitude and perception of processors of CBCF-F on issues pertaining to aflatoxin and fumonisin contamination in cereals. Then suggest the recommendations to processors and government stakeholders on how to avoid such contamination.

1.3 Justification

The completion of this study therefore, will add information about the current status of aflatoxins and fumonisins in homemade and commercial processed CBCF-F. The study will also add knowledge on previous studies done on the level of awareness, attitude and

perception of farmers and handlers of cereal in relation to mycotoxins contamination. This will help to strengthen standards and regulation mechanisms on consumer safety and so contributing to formulating policies about safety of cereal based complementary foods in Tanzania.

The findings from this study can also be used for planning, designing and implementing effective nutritional intervention programs by policy makers and other stakeholders aiming at reducing malnutrition and related morbidity among infants and young children aged 6 to 23 months in Tanzania, particularly in Morogoro Region where stunting and underweight are still persistent in spite of high foods production.

1.4 Study Objectives

1.4.1 Overall objective

To determine the status of and factors influencing total aflatoxin and fumonisin in homemade and commercialized blended Cereal Based Complementary Foods with formulation (CBCF-F).

1.4.2 Specific objectives

i. To determine and compare the amount of total aflatoxin and fumonisin in homemade and commercialized blended cereal based complementary foods with formula.
ii. To identify factors which influence mycotoxins contamination levels in CBCF-F.
iii. To assess awareness and perception of both processors of CBCF-F on issues pertaining to aflatoxin and fumonisin contamination in cereals.

1.5 Research Questions

i) Is there any significant difference between Tanzania set standard levels of total aflatoxin and fumonisin contamination and the level of mycotoxins contamination in Formulated CBCF-F both (homemade and commercial) fed to Tanzanian infants?

ii) What are the factors influencing occurrence of high levels of AF and FM in CBCF-F?

iii) Given the existing knowledge of nutrition damage that can occur due to exposure of mycotoxins in foods, what is the extent of awareness and perception of processors for both homemade and commercial CBCF-F on issues related to aflatoxins and fumonisins contaminations of foods used for infants' and young children's feeding in Tanzania?

CHAPTER TWO

2.0 LITERATURE REVIEW

Mycotoxins are a group of naturally occurring chemicals produced by certain molds. They can grow on a variety of different crops and foodstuffs including cereals, nuts, spices, dried fruits, apple juice and coffee, often under warm and humid conditions (Groopman *et al*., 2014). Aflatoxins especially B1 and fumonisins are two families of mycotoxins that are particularly important in the context of child health in sub-Saharan Africa, because they are highly prevalent in the food chain and may have significant negative health consequences, and this is because both toxins are particularly common in maize and groundnuts which constitute a major portion of the diet in many developing countries (Smith *et al*., 2012).

2.1 Aflatoxin

Aflatoxins are fat soluble toxins produced by the *Aspergillus* species of fungi, principally *A*. *flavus*, and *A. parasiticus* (Smith *et al*., 2012). Aflatoxin was first discovered in 1961 in England as Turkey X diseases which attacked chickens and turkeys from feeds incorporated with peanut meal. Further they were discovered as aflatoxins effecting animals and human beings (Richard, 2012). It is estimated that 4.5 billion people in developing countries located along 40° N and 40° S of the Equator are chronically affected with aflatoxins (Williams *et al*., 2004). Crops that are frequently affected include cereals such as maize, oil seeds including peanuts (groundnuts), various spices, figs, dried fruits, and tree nuts. The toxins can also be derived in milk of animals that are fed with B1 and B2 aflatoxins' contaminated feeds, in the form of aflatoxin M1 and M2 respectively (Martin, 2008; Wacoo *et al*., 2014; Hernández-Camarillo *et al*., 2016).

2.1.1 Molecular structure of aflatoxin

Aflatoxins have similar structures and form a unique group of highly oxygenated, naturally occurring herocyclic compounds. Aflatoxins B2 and G2 have been identified as dihydroxy derivatives of B1 and G1 respectively (Fig. 1). In a condensed form Aflatoxins B1 is identified as $C_{17}H_{12}O_6$, aflatoxins B2 as $C_{17}H_{14}O_6$, aflatoxins G1 as $C_{17}H_{12}O_7$ and aflatoxins G2 as $C_{17}H_{14}O_7$, aflatoxins M1 as $C_{17}H_{12}O_7$ and aflatoxins M2 as $C_{17}H_{14}O_7$ (Leatherhead Food Research Association, n.d). Aflatoxins are crystalline substances, freely soluble in moderately polar solvents such as chloroform, methanol and dimethyl sulfoxide, and dissolve in water to the extent of 10-20 mg/liter. They fluoresce under UV radiation with maximum emission absorption of 360-362 wave length (methanol) (Leatherhead Food Research Association, n.d).

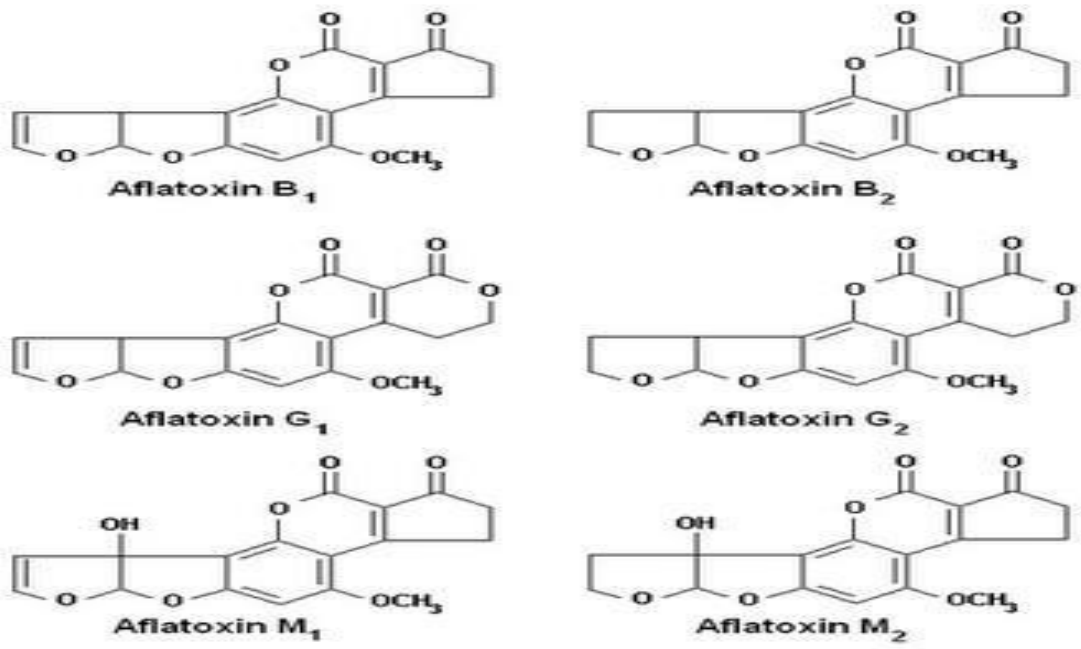


Figure 1: Molecular structures of aflatoxins (B_1, B_2, G_1, G_2, M_1 and M_2)

Source: Food Safety and Standards Authority of India (2016).

2.1.2 Conditions which favor growth of aflatoxin

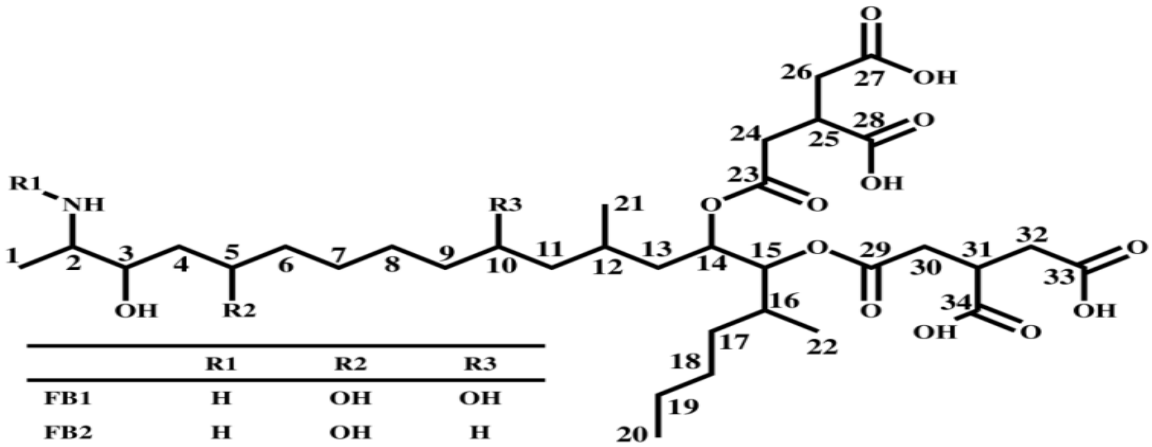
Conditions that provide conducive environments for fungal growth and multiplication are hot temperature (25 to 35 °C) and water activity (a_W) above 0.7 a_W or humidity ≥ 77% (Shuaib *et al*., 2010). *Aspergillus* fungi normally grow on dead organic matters, including fallen blossoms and leaves as well as other dead plant materials that may be found on ground in trees plantation (Codex Alimentarius Commission, 2003). *Aspergillus* fungi can rarely be able to infect healthy plants or nut tissues. *Aspergillus* infection and production of aflatoxin usually happen under stress condition and in seeds damaged by insects or pests, and may infect other crops in the farms through winds and bodies of insects activities in the farm and might continue to multiply during transportation, storage and handling of crops under conducive moisture and temperature for their survivor (Baku, 2011; Suleiman *et al*., 2013).

2.2 Fumonisins

Fumonisins are water soluble toxins produced by *Fusarium* molds, which were discovered in 1988 from laboratory corn culture in Southern Africa (Bacon and Nelson, 1994). Different *Fusarium* toxins can be associated more with certain types of cereal. For example, fumonisins with maize (corn), DON with wheat, T-2 and HT-2 toxins with oats. Currently, there are about four types of identified and characterized fumonisins (B1, B2, B3 and B4), of which B1 and B2 commonly affect corn (Jackson *et al*., 1996) also, there are other unknown compound attached to fumonisin B1, B2 and B3 (Bacon and Nelson, 1994; Tamura *et al*. (2014). In addition, FB1 and FB2 have been found to inhibit sphingolipid biosynthesis by blocking the conversion of sphingosine into ceramide. Fumonisins have been related to esophageal cancer in humans, and to liver and kidney toxicity in animals (Norred and Voss, 1994; Marasas, 1995; Myburg *et al*., 2002).

2.2.1 Molecular structure for fumonisins

Fumonisins are a structurally related group of which fumonisins B1 (FB1) are diesters of propane-1, 2, 3-tricarboxylic acid and various 2-amino-12, 16-dimethylpolyhydroxyeicosanes in which the C14 and C15 hydroxyl groups are esterified with the terminal carboxyl group of propane -1, 2, 3- tricarboxylic acid (Yazar and Omurtag, 2008). Other identified fumonisins are FB2, FB3 and with addition of unknown compound termed as fumonisin As (FA1, FA2 and FA3) (Tamura *et al*., 2014). Each fumonisin differs from others by lacking one of the free hydroxyl groups at either the C-5 or C-l0 position of the 20-carbon aminopental backbone (Bacon and Nelson, 1994) as shown in Fig. 2. In a condensed form fumonisins are $C_{34}H_{60}NO_{15}^{+}$, $C_{34}H_{60}NO_{14}^{+}$, $C_{34}H_{60}NO_{14}^{+}$, $C_{36}H_{62}NO_{16}^{+}$, $C_{36}H_{62}NO_{15}^{+}$, $C_{36}H_{62}NO_{15}CHO$ for fumonisin B1, B2, B3, FA1, FA2 and A3 respectively. The condensed formula was derived from mycotoxin reference material (MTC-9999E) by Tamura *et al*. (2014).



	R1	R2	R3
FB1	H	OH	OH
FB2	H	OH	H
FB3	H	H	OH
FA1	CH_3CO	OH	OH
FA2	CH_3CO	OH	H
FA3	CH_3CO	H	OH

Figure 2: Molecular structure of Fumonisins B1, B2 and B3 and unknown compounds (FA1, FA2 and FA3)

Source: Tamura *et al*. (2014)

2.2.2 Conditions which favours growth of fumonisin

Levels of fumonisins in corn are influenced by environmental factors such as temperature ranging from 20 to 30 ºC with high multiplication at 25ºC and very slow at 30 °C together with humidity of 22 to 42% with high multiplication at 42% (Dilkin *et al*., 2002) and rainfall during pre-harvest and harvest periods. High levels of fumonisins may also occur in corn that has been damaged by insects and birds (Fandohan *et al*., 2003). Water activity (a_W) also affects fumonisin production during seed maturation in the field and during storage. Moreover, *Fusarium* can grow from 0.90 a_W (Pitt and Hocking, 1997). However, *Fusarium* species do not colonize maize grain in isolation of other fungus including *Aspergillus* to be effective they compete with other colonizers. With this regard, a_W becomes an important factor in influencing production of fumonisins (Marine *et al.*, 1998). At any temperature, the growth rate of *Fusarium* increases at 0.93 to 0.98 a_W while at 0.93 to 0.95 a_W the colorizers inhibit each other (Marine *et al.,* 1998). The optimal a_W for fumonisin production by *Fusarium* in maize grain is found to be at the interval of 0.97 to 0.995 a_W (Mogensen *et al*., 2009).

Improper storage conditions different from the recommended moisture content of grain above 18-23% will lead to increase in fumonisin levels. This is because, generally, maize grain is harvested with moisture content around 18 to 20% and then dried (Suleiman *et al*., 2013). According to Codex Alimentarius Commission (2001), growth of *Fusarium* will stop when water content of grain is reduced at approximately 15%. With regards to Hydrogen Iron Concentration (pH), most fumonisins production happens at pH 5 and lowers at pH 3, but at neutral pH most fungi compete with each other, especially when there are high moisture contents in the cereals (Pitt and Hoking, 1997).

2.3 Regulatory Limit of Detection for Aflatoxin and Fumonisin

Different countries over the world have set varying levels of detection limit for total aflatoxins and other mycotoxins, depending on the types of foods and who is intended to use i.e., humans or animals. In addition, the detection limit varies from country to country, depending on the consumptions pattern of cereals in those countries (Abt Associates Inc, 2012b; Kimanya, 2008) and seed genotype prevailing to that country because they differ from country to country. Moreover, some seed genotypes are resistant to aflatoxins (Richard, 2012). The level of aflatoxins is measured in ppb or in µg/kg; the weight of one ppb or 1 µg/kg is proportional to the weight of one grain of rice in 50 kg bags. Because of its high level of toxicity, small measurements must be used to detect aflatoxins (Abt Associates Inc, 2012a). For example, human food is allowed 4 to 30 ppb (Williams *et al*., 2004), depending on the country involved. In Tanzania, according to Tanzania Bureau of Standard (TBS, 2014) the limit of detection of total aflatoxins for cereals and cereal products intended for human consumption including baby food is 10 parts per billion (ppb) or microgram per kilogram (µg/kg); in Nigeria the limit is 20 ppb (Sule *et al*., 2015). High levels up to 300 ppb are allowed in feeds for cattle, hogs and poultry (Fung and Clark, 2004). Tolerable intake of fumonisin in human food including baby food is 2µg/kg or 2 parts per million (ppm), according to standard set by Food and Drug Authority (Kimanya, 2008).

2.4 Public Health and Economic Implications of Aflatoxin and Fumonisins

Health implications of aflatoxins and fumonisins contamination to human-being depend on amount of toxins ingested. Consuming or ingesting large amounts of aflatoxins in a short period of time can cause acute toxicity leading to death. For instance in Kondoa District, Tanzania in 2016, more than 7 people died together with 8 pet dogs and their newly born puppies and 14 people got liver problems (Kiolongwe, 2016). Also, acute

aflatoxicosis happened in Eastern Kenya in 2004, resulting 125 deaths of people and 317 cases. Furthermore, this has happened in other parts of the world (Azziz-Baumgartner *et al*., 2005). No acute toxicity has occurred for fumonisins elsewhere in the world, while small doses over a long time will result in chronic effects to the consumer (Darwish *et al*., 2014). Ingestion of small doses of aflatoxin and fumonisin contamination to human-beings will induce conditions like environmental enteropathy (EE), targeting the intestinal tract (Smith *et al*., 2012). EE is a subclinical condition, characterized by chronic inflammation of the cell infiltrate along the gut and weaken villous, leading to modest malabsorption of nutrients ingested and chronic systemic immune activation (Watanabea and Petri, 2016).

In addition, aflatoxin and fumonisin cause i) reduced nutrient absorption such as Zinc ii) inhibition of protein synthesis, iii) inhibition of sphingolipid synthesis; and iv) food refusal (Smith *et al*., 2012). All those conditions have been proved to contribute to infants growth retardation (Kimanya *et al*., 2014 and Shirima, 2016), immunity impairments (Gong *et al*., 2003), carcinogenic effects (Fung and Clark, 2004), allergy symptoms, abdominal pain and diarrhea (Williams *et al*., 2004). Likewise, human and animal investigation on the effect of aflatoxins during pregnancy, showed positive correlation towards bad outcomes of pregnancy such as foetus growth retardation, especially animals have shown biological support mediating adverse pregnancy outcomes (Smith *et al*., 2017). Moreover, contaminated grains or crops have poor quality to consumer thereby reducing income (Suleiman *et al*., 2013; Xinhua News Agency, May 20, 2015). Furthermore, as a global economic concern, aflatoxin and fumonisin cost a lot in terms of regulatory enforcement which leads to rejection of specific export shipments and increased inspection and high sampling rates (Abt Associates, 2012b). Similarly, research cost employed increase in combatting them and also loss of humans and animals due to acute aflatoxicosis outbreak (Zain, 2011) (The conceptual frame work in Appendix 2).

2.5 Other Factors Influencing Growth of AF and FM in Cereals

Other factors influencing growth of AF and FM in cereals which have not been discussed in this report include: geographical location, season of the year, and environmental conditions on which cereals are grown and harvested and stored. Countries located between 40 ° C North and 40 ° C South of the Equator are characterized by tropic and subtropical conditions that favours growth of most of fungi influencing production of AF and FM (Williams *et al*., 2004). Season of the year also favours growth of fungi on cereals. For example, the dry wet season accompanied by temperatures ranging from 15 to 35 °C influences production of most AF and FM in the field and if harvested but not properly dried (Warfield and Gilchrist, 1999).

Environmental conditions on which cereals are grown and stored also play important roles in affecting production of AF and FM. For example, poor environment conditions such as lack of enough rainfall especially during maturation of grain, influence stress to plants making them unhealthy. Unhealthy plants harbour most of fungi and bacteria (Suleiman *et al*., 2013). Also, if plants are grown without enough nutrients for their survival, such as manure and also lack of insecticides to kills insects and pests that may cause infection to the plants as well as grain cereals (FAO, 2011). Grains infected by insects have been found to influence abnormalities of grains which influence molds, AF and FM production. Poor storage conditions such as poor storage facilities such as nylon bags (Nyangi, 2014) which allow accumulation of moisture contents in cereals, also that allow activities of storage insect and pests, facilitate growth and production of mycotoxins. Likewise, stores with high temperature and moisture contents surrounding the store without air circulation influence production of AF and FM as a result of mold activities in cereals stored. Furthermore, lack of crop rotation within the same farm accumulates fungi in the fields and recycles to the same crop each year planted (Codex Alimentarius Commission, 2001).

2.6 Prevention

Aflatoxin and fumonisin contamination is promoted by stress due to drought before harvest, damage to the crop due to insect activity, poor timing of harvest, heavy rains at and after harvest, and inadequate drying of the crop before storage (Strosnider *et al*., 2006). Levels of humidity, temperature, and ventilation during drying and storage are also important factors. Therefore, prevention of aflatoxin and fumonisins can be done through effective and integrated use of current agricultural knowledge and public health practice of food processing and handling. Agricultural practices include proper irrigation, fertilization and proper pest management. Also cultured practice like crop rotation, proper drying of pre-harvested and harvested crops and proper storage of foods in dry and cool conditions (Eeckhout *et al*., 2013; Suleiman *et al*., 2013).

Food preparation methods which may reduce levels of aflatoxin and fumonisins include sorting, washing, crushing, and dehulling, removal of contaminated portions of food and diluting contaminated food with uncontaminated food (Strosnider *et al*., 2006). Unlike other mycotoxins that can be prevented by heat, aflatoxins and fumonisins are relatively heat stable (Fung and Clark, 2004; Strosnider *et al*., 2006); so little or no destruction of many mycotoxins occurs under normal cooking conditions of food processing temperatures (80 – 121 °C), such as boiling, frying and pasteurization (Milićević *et al*., 2010). However, heat processing capable of destroying *C. botulinum* will also destroy all fungi spores (Pitt and Hocking, 1997). Other methods are use of Chemo-protection, which is the use of chemicals (e.g., oltipraz, chlorophylin) to reduce aflatoxins level for animal feeds (Williams *et al*., 2004) and dietary intervention (e.g, eating broccoli sprouts and drinking green tea) to reduce the susceptibility of humans to carcinogens (Kensler *et al*., 2004).

Another method is the use of non-toxigenic strain of *Aspergillus flavus* such as Aflasafe as biological control of aflatoxin by 80% during planting and storage of agricultural produces (Probst *et al*., 2011). The non-toxigenic strain has genetic ability to inhibit aflatoxin production by toxigenic strain, also non-toxigenic strain produce a factor in culture that alone also inhibits production of aflatoxin by toxigenic strains (Ehrlich, 2014; Grubisha and Cotty, 2015). In Tanzania, Aflasafe can be obtained from laboratory of International Institute of Tropical Agriculture (IITA) in Dar es Salaam. The application of these strategies in developing countries is difficult because of differences in food production, such as the existence of subsistence farming. Furthermore, these countries often lack the resources, technology, and infrastructure necessary for routine food monitoring as well as optimal drying and storage practices (Strosnider *et al*., 2006).

2.7 Tanzania's Blended Cereal based Complementary Foods with Formulas (CBCF-F)

In Tanzania, CBCF-F which in another name is termed as composite flour that refers to flour which consists of more than one cereal, blended together with legumes to add protein and nuts to add oil and fats. In other countries like Ghana CBCF-F is termed as *Weanimix* (Mastersa *et al*., 2011). It is usually prepared following certain ratios confirmed to bring good test, aroma, texture and color to the final user. CBCF-F is normally assumed to be more nutritious than the normal flour used as complementary foods, and as such it is mostly given to infants, pregnant mothers, nursing mothers and sick people. Based on that, in *Kiswahili* it is named as *Unga wa Lishe* ready for preparation of porridge.

CBCF-F can be processed by mothers or caregivers at home for home consumption alone, but not for selling (termed as Homemade (HM)) or can be locally prepared by local producers for selling as commercial locally made (CLM), or CBCF-F can be processed

from Industries (Factory) for selling and is termed as Industrial made (CFM) or Factory-made. Both the CLM and CFM can be purchased from local markets, supermarkets or retail shops.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Description of the Study Areas

3.1.1 Study area

The study was conducted in Morogoro Municipality which is the head-quarters of Morogoro Region. The municipality lies within Morogoro District, and is one of the six (6) districts of Morogoro Region. It is about 195 kilometers West of Dar es Salaam and situated on the lower slopes of Uluguru Mountains whose peak is about 1 600 feet above sea level (Morogoro Municipal Council, 2017a). It lies at longitudes 35° 25″ and 38° 30″ East of the Greenwich Meridian and between latitudes 5° 58″ and 10° 0″ South of the Equator. It is characterised by an annual average temperature ranging between 16 to 28 °C in the cold dry season and 21 and 31 °C in the warm wet season. The average relative humidity is 63 – 88% in March through May (the wet season) and 46 – 82% from July to September (the dry season) (Mkoma and Mjemah, 2011).

3.1.2 Population and economic activities

The current population of the municipality stands at 315 866 people in the ratio of 47.85% men (151 700) and 52.15% women (164 166) according to the population census of 2012. The population growth rate in the municipality is 4.7% per annum. Rapid growth of population creates a gap in service delivery hence inadequate health services, shortage of housing, inadequate safe and clean water supply, inadequate infrastructural expansion and maintenance (Morogoro Municipal Council, 2017a).

The major economic activities in Morogoro Municipality include industries of primary and secondary level, subsistence and commercial farming, small scale enterprises and

commercial retail as well as wholesale. The main agricultural cash crops are maize, rice, sesame and sisal, which are grown in the periphery and the neighbouring districts of the municipality. Food crops include maize, rice, vegetables, fruits and yams. Maize and paddy are the major staple food crops. Other crops include sorghum, sweet potatoes, beans, cassava, millet, groundnuts, pears, banana and a wide range of tropical and temperate fruits and vegetables (Morogoro Municipal Council, 2017a).

3.1.3 Health services

The Health Department is among the most crucial areas of concern in the provision of social services in the municipality. According to the Local Government Reform Programme which began in 2000, the vision of Health department is focused on the most risky/health hazardous areas in the municipality. The department is divided into two major sections which are preventive and curative that operates through the public, health centers, dispensaries and RCH clinics (Morogoro Municipal Council, 2017b). This department, together with the Agriculture Department is useful in mitigation of mycotoxins in cereals and cereal products sold in Morogoro Town (Fig. 3) Map of Morogoro Municipality and area studied.

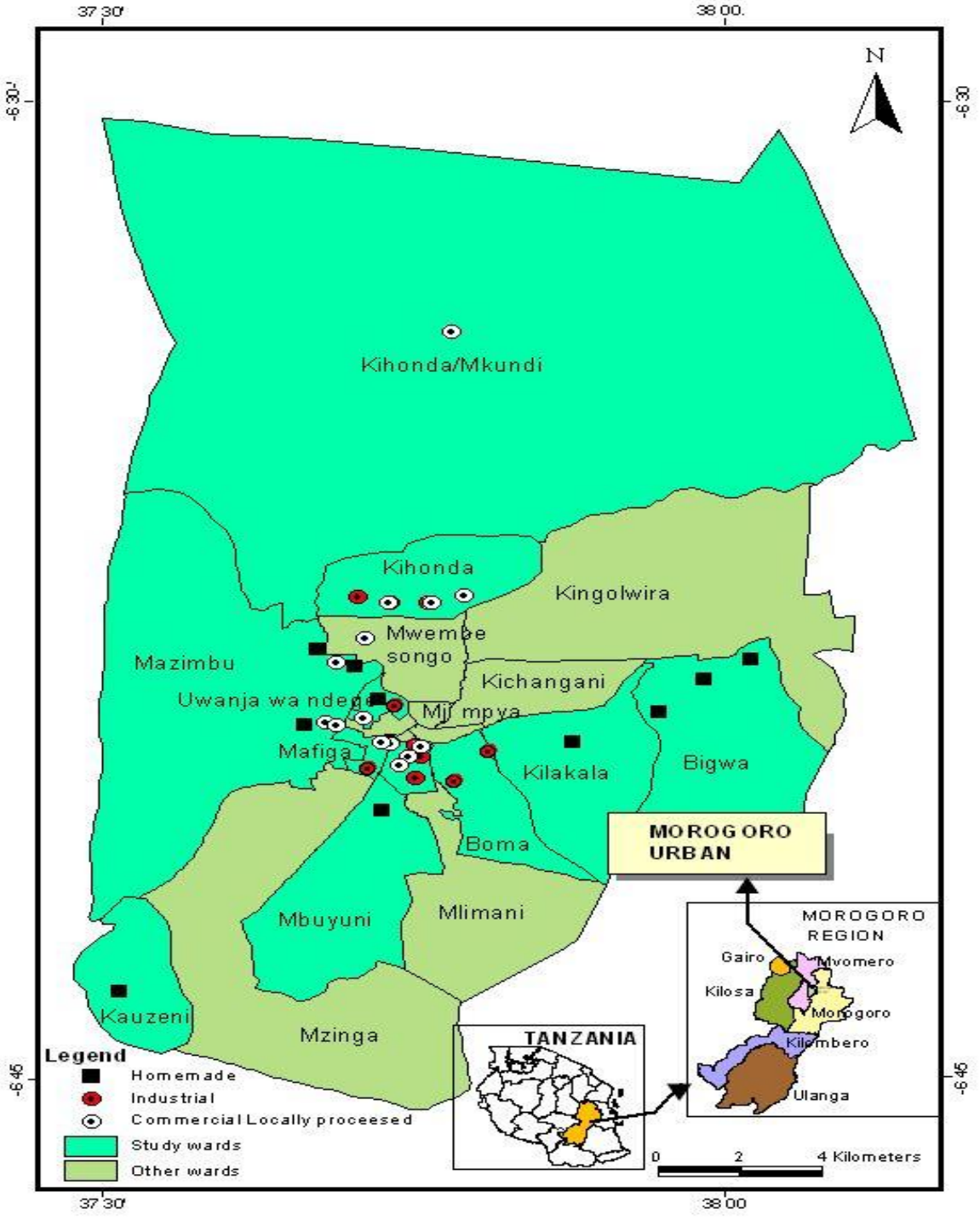


Figure 3: Map of Morogoro Municipality and areas studied as indicated by green color and icons (white, red and Black color)

Source: Drawn from Global Positioning System (GPS) Coordinates taken during data collection of this study using software programme of map construction (ArcView GIS 3.2)

3.2 Study Design

A cross-sectional design in this study was adopted because this design allows data collection at one point in a time. Hence it was adopted to cope with the limitation of time and other resources.

3.3 Sampling Procedure

3.3.1 Study population

The study population involved processors of CBCF-F whose product of CBCF-F was sampled for laboratory quantification of total aflatoxin and fumonisin. Both processors of CBCF-F from Home and Commercial (Local and Factory) processors of CBCF-F from Morogoro Urban District were involved. Home processors included mothers and care givers who attended milling machines for the purpose of milling their CBCF-F for home consumption alone but not for sale. A total of six (6) milling machines were surveyed for this purpose. Commercial local dealers included local processors dealing with processing and selling of CBCF-F whose products (CLM) were found at the local markets, retail shops or supermarkets. Commercial Factory dealers included processors of CBCF-F based in industries (factories) whose products (CFM) were obtained from supermarkets found within Morogoro Urban district. Products of commercial local and factory processors of CBCF-F were differentiated by the packaging material used and the mandatory requirements of labels for commercial prepacked food according to TFDA (2016). The commercial locally made products (CLM) most of them were found packed by transparent nylon bags alone and few of them not packed at all with no labels or with labels which had insufficient information regarding the product. On the other hand, the commercial factory products (CFM) most of them were packed by paper bags or nylon bags which were not transparent. Furthermore, the CFM products labels had barcodes and also conformed to TFDA (2016) regulatory mandatory for labels of commercial prepacked food which are;

Name of the food (Common name and brand name), list of ingredients, net content, name and address of the manufacturer, country of origin, batch or lot number, date of expiry, date of manufacture, and recommended storage condition.

3.3.2 Sampling techniques

A multi-stage sampling technique was employed in selecting respondents to be included in the study. According to Babbie (1990), the technique is used in a diverse population. The technique involves sampling in phases (stages) and more than one sampling method. In the first stage, Morogoro Municipal was purposefully selected within Morogoro Region because it is a center of Morogoro Region and also the region had higher (43%) prevalence of total aflatoxin contamination with an average of 50 ppb in maize sample than other regions, according to a survey conducted in Tanzania's regions by Abt Associates Inc (2012b) in collaboration with Tanzania Food and Drug Authority (TFDA). Then the municipality's wards were divided into five clusters, namely North, South, East, West and Center cluster. In the second stage, stratified sampling technique was used to select respondents (Homemade, Local and Factory commercial processors) who were processing CBCF-F. Finally, random sampling method from each cluster was used to get the representatives-respondents according to their strata (similarity i.e HM, CLM and CFM) and actual samples of CBCF-F were taken from each respondent who agreed to be interviewed.

3.3.3 Selection criterion of processors and CBCF-F

All parents/care givers found at the milling machine for the purpose of milling their CBCF-F were eligible for the HM study. Likewise, the commercial processors both Local and factory people dealing with CBCF-F (whose products of composite flour were found in Morogoro Municipality district) were eligible for the CLM and CFM study. The

mothers/care giver processors (HM) were differentiated from CLM processors at the community milling machine through their bulkiness of their ingredients/products; the HM products ranged from 2 kg to 14 kg but the CLM products encountered at the milling machine had more than 30 kg, also respondents were asked whether their products were for home consumption alone or for sale purpose. Also the LCM samples were not sampled at the milling machine; the respondents were requested to tell the researcher where do they normally put their products for sale, then after, the researcher followed the product to the market.

Composite flour in most cases is formulated from blended flour of many combinations: i) maize flour, finger-millet and groundnuts, ii) maize flour, rice, finger-millet and groundnuts. Also more than one protein is used: iii) maize, rice, groundnuts and soya-beans, and iv) maize flour, rice, finger-millet, groundnuts, simsim and soya-beans. Therefore, any type of composite flour comprising both maize and groundnuts were eligible for this study regardless of other ingredients added in it.

3.3.4 Sample size

The sample size calculations were based on Kirkwood and Steme (2003) as cited by Rushunju, (2012) as follows;

$$n = \frac{[\mu\sqrt{\pi(1-\pi)} + v\sqrt{\pi\text{null}\,(1-\pi\text{null})}]^2}{(\pi-\pi\text{null})2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where:

n = required minimum sample size

μ = one sided percentage point of the normal distribution corresponding to 100%- power, where power = 90%, μ=1.28

π = proportion of interest = 0.7 (known from previous study by Rushunju (2012) = 70% for total aflatoxin in commercial CBCF-F and Nyangi (2014) for total fumonisins in maize products to be 45%)

π_{null} = null hypothesis proportion = 0.5 (50% the researcher gave benefit of dought because it was not known for homemade CBCF-F for neither total aflatoxin nor fumonisin)

v= percentage of the normal distribution corresponding to the required (two-sided) significance with 5% level of significance, v = 1.96

$$n = \frac{[1.28\sqrt{0.7(1-0.7)}+1.96\sqrt{0.5\,(1-0.5)}]^2}{(0.7-0.5)2} = 61.4$$

Furthermore, it has been recommended by Bramley (2014) that incremental samples to be taken for mycotoxin, shall depend on the weight of the lot, a minimum of 10kg and a maximum of 100kg for very small lots (<100kg or ≤ 0.5 tonnes). A lower number of incremental samples may be taken, but the aggregate sample uniting all incremental samples shall also be in that case at least 1 kg.

Therefore the minimum number of sample size required was 62, but the researcher decided to take 90 (30 HM, 30 CLM and 30 CFM samples), however, it was not easy to get 30 manufacturers/products of factory-made CBCF-F; only five of them were available (5) from the scope of the study area which conformed by regulations for prepacked commercial food of Tanzania put by TFDA (2016). Therefore, two (2) samples were taken from each of the CFM. Hence, 70 samples of flour of CBCF-F were taken for the study (quantification of AF and FM) including 60 respondents whose products were taken (30 homemade and 30 commercials locally made) for the questionnaire part of this study.

3.4 Data Collection

Before data collection, the questionnaire was pretested by interviewing 15 processors of CBCF-F who were obtained by random sampling in two peripheral wards of the study area. The pretesting was conducted to ascertain validity of questions and then corrections were incorporated.

3.4.1 Sources of data

Both primary and secondary data were collected through standardized questions, consultation with key informants and personal observation. Primary data were obtained through:

i) Administering questionnaire which had open and closed ended questions to respondents for obtaining socio-demographics information of respondents, awareness, attitude and perception on issues related with aflatoxin and fumonisin and the factors which influence level of contamination as shown in Appendix 1.

ii) Samples for laboratory analysis of total aflatoxins and fumonisins were obtained in 2 areas: Homemade products (composite flour) were sampled at the milling machine and commercialized composite flour (1 kg capacity) were purchased from supermarkets, local markets and retail shops). Sampling protocol according to IITA (2009) (Appendix 3 and 4) and Mahuku *et al*. (2010) were used to get the actual sample materials for analysis, where by one kilogram (kg) sample from different parts of each package such as on the top, middle and bottom was taken and homogenised into single units. One kilogram was divided into four parts, finally 250g for laboratory analysis was taken from each respondent at the milling machine.

Secondary data were obtained from Municipal Office such as the sample framework of the local processor marketing the CBCF-F, and through reading various reports and records, Wards' Health Officers and district council offices. Other sources for secondary data were NGOs records such as Tanzania Women Food processors Trust (TWFT) dealing with processing of various foods commodity in Morogoro Municipality where information concerning commercial local processors of CBCF-F was easily obtained. Literature review was conducted from various sources; the regional library of Morogoro, Sokoine National Agricultural Library (SNAL) and the Internet service in order to get insight of the study objectives.

3.4.2 Ethical considerations

The research was conducted by the researcher herself, with the help of one trained SUA student under the researcher supervision. Permission to collect sample and data from respondents were sought from Morogoro Municipal Council before data collection and during data collection. Also, respondents were requested for their willingness to participate in the study. Moreover, the objectives and benefits of the study to themselves, to the researcher and to the nation as well as the procedures for selecting them were explained to them. Furthermore, after data collection, the respondents were given feedback concerning the findings of the study in respect to the status of aflatoxin and fumonisin contamination in their respective samples.

3.5 Sample Collection and Handling Prior to Laboratory Analysis

Samples were collected in February and at the beginning of April 2016. The samples collected from milling machines (Homemade) were left open from each container to allow any moisture contents obtained from the milling machine to evaporate for 12 hours at room temperature. Then they were packed into labelled clean virgin envelops (A4

capacity) tighten with rubber-bends. Also they were kept at room temperature in a moisture free environment (cool and dry), inside the insulated ice box before transferring them to IITA laboratory in Dar es Salaam. Samples purchased from the retail shops/markets (commercial made) were not opened but were labelled accordingly and kept in the same room with the homemade samples.

All samples were sent from Morogoro to IITA in an insulated ice box every week until the required sample size (70) was obtained. At IITA, each sample was repacked into new clean plastic bags special for food refrigeration. Then they were refrigerated until laboratory analysis at - 4 °C (Fig. 4).

Figure 4: **Pictures of the insulated ice box and envelop used in transportation of samples and also a freezer used in storage of samples at IITA laboratory.**

3.6 Aflatoxin and Fumonisins Quantification

Quantification of total aflatoxins and fumonisins contamination in Composite flour was performed in two phases. Phase 1: in May 2016 by Enzyme Linked Immunosorbent Assay (ELISA) method with AccuScan machine as used by Nyangi *et al*. (2016). Then, the remained sample was stored in a refrigerator (temperature set at below – 4 ºC). Phase 2: validation whereas the stored sample were analysed by use of HPLC machine with fluorescence detector to validate the results obtained from AccuScan in phase 1.

3.6.1 Sample preparation, extraction and analysis by AccuScan machine

Quantification for total aflatoxin and fumonisin were performed using Neogen's AccuScan Pro® lateral flow test reader at IITA-Dar es salaam. Collected sample (each 1kg capacity in ground form) were poured on clean virgin papers sheets, homogenized and sub-divided to obtain a representative sub-sample for analysis, according to method suggested by Maestroni, and Cannavan (2011) and used regularly by IITA (Fig. 5).

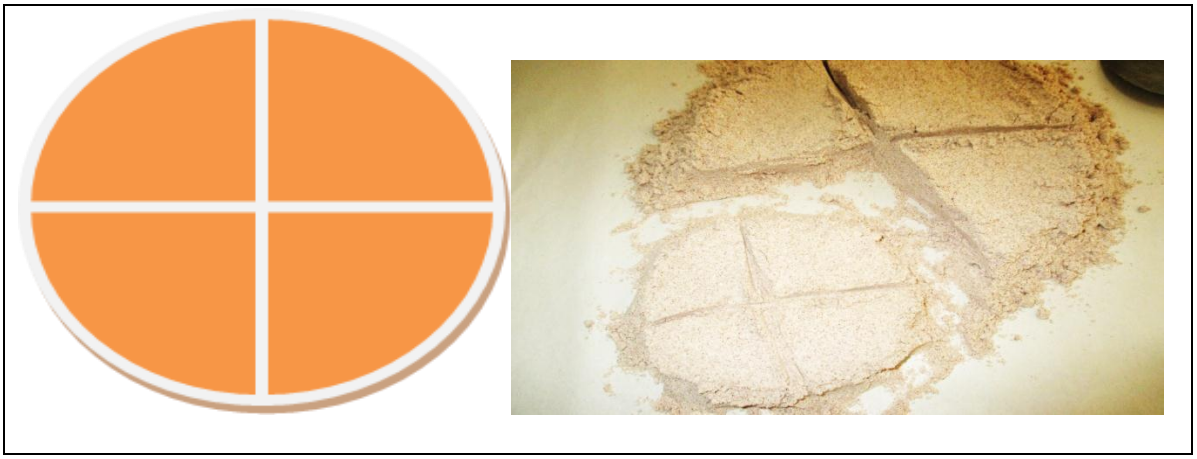


Figure 5: **Quartering sampling technique for sub-sampling for laboratory analysis of mycotoxins. It involved sequential quartering until an estimate of the mass of the required sub-sample was attained before using a weighing balance to measure the exact amount required for analysis.**

Source: IITA lab instructions (n.d.), picture left side and actual picture of study-right side

Then 50 g sub-sample were taken from one of the homogenized sub-divided samples and extracted with 250 mL mixture of ethanol/water (65:35, v/v) in an extraction mixing jar. From this, 50:250 (sub-sample/ethanol-water) were taken instead of 5:25 in order to reduce variability of the test procedure and increase precision in detecting mycotoxins as suggested by Maestroni and Cannavan (2011). Then all individual samples were covered and then shaken vigorously at 150 revolutions per minute (rpm) for 3 min using a laboratory shaker (IKA® Werke, Germany). The extracts were then allowed to settle and were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maid stone, UK). The filtered extracts (100 µl) from each individual sample were transferred to red cup containing sample diluent 500 µl for aflatoxins or 200 µl for fumonisin. The mixture was mixed by pipetting up and down five times, and then from it 100 µl was transferred to white cup ready for quantification of total aflatoxin or fumonisin.

New Reveal Q_+ strips for either aflatoxin or fumonisins were placed into the white cup containing individual diluted sample and were left for six (6) minutes to develop and become wick, then it was removed and inserted into black AccuScan III cartridge adopter. The reading was done at six to seven minutes only. Quantification for total aflatoxin and fumonisin was performed following information and test procedure provided by the manufacturer of the test kit (Neogen's AccuScan Pro®) III reader (Neogen, USA) (Fig. 6). Limit of detection of the Neogen's AccuScan Pro®) for aflatoxin was 2 to 150 ppb and for fumonisin it was 0.3 to 6 ppm. Dilution factor was done to samples containing more than the detection limits of the AccuScan machine, and this was performed to aflatoxins alone. The dilution factor were 1:5 (100 µL sample extract / 400 µL ethanol, v/v) and 1:10 or 900 µL ethanol, v/v for samples above 150 ppb). The reading displayed was manually multiplied by summations of the ratio used i.e 5 or 10. There was no sample above the detection limit for fumonisins.

Figure 6: Laboratory procedures used in this study for quantification of aflatoxins and fumonisins by AccuScan-Pro® III reader

3.6.2 Determination of aflatoxins by HPLC with fluorescence detector

Validation test of the ELISA method was performed using High Performance Liquid Chromatography (HPLC) with fluorescence detection at IITA laboratory on 31 samples previously analyzed using Reveal AccuScan Pro reader III at IITA-Dar es salaam in accordance with the method suggested by Stroka *et al*. (1999), Sobolev (2007) and AflaTest® Instruction Manual (1999) with minor modification of the method as suggested by Martin (2008) on the use of Phosphate Buffer Saline Solution (PBS) during the extraction stage. Validation test of the ELISA method by HPLC was important since HPLC is confirmed worldwide to detect fast and more accurate results for aflatoxins (B1, B2, G1 and G2) (Wacoo *et al*., 2014) not just total aflatoxins as the ELISA methods gives out. However, HPLC method is very expensive to perform; it requires extensive sample cleanup and very expensive equipment which can only be operated by highly trained and

skilled person. Besides that, HPLC method can only be performed in laboratory setting not in field condition as ELISA method does (Wacoo *et al*., 2014).

3.6.2.1 Sample preparation, extraction and analysis by HPLC

The composite flour was homogenized; then 50g from it was taken as a representative sample for analysis as it was done previously in AccuScan methods. To the 50g taken was added 5g of NaCl into a blender jar, then it was extracted with 100ml of 80% Methanol/Water (80:20, v/v). The mixture was blended for two (2) minutes by high-speed (13000 rpm) Waring ® commercial laboratory blender with 1 L glass jar and cover, then it was filtered through folded filter paper (VICAM-reorder No. 31240. Filtered extract was diluted with 20ml of Phosphate Buffer Saline Solution (PBS-pH 7.3 ± 0.2 at 25°C, NaCl 8.0 g/L). Ten mills of the diluted filtrate was taken and applied to 50ml of a volumetric flask and filled with distilled water up to the mark of the volumetric flask.

Diluted sample from the volumetric flask was filtered again using microfiber filter paper (VICAM – reorder No. 31955), then 10ml of the filtered sample was applied into a 10ml syringe of vacuum (4-Position Pump Stand w/2 Pumps 10mL – Vicam No. 21045) connected to the AflaTest min-column (VICAM reorder No. G1024). The outlet at the bottom of the min-column was released, and the sample was allowed to flow through without application of the vacuum or with slight force at a speed of six drop per minutes, then the column and syringe were washed with 10ml of water two times. Also, a slight vacuum was applied to remove any residue which remained in the bed of the syringe and column.

Aflatoxin was eluted from AflaTest min-column into a small test tube by passing through 1 mL of pure methanol (HPLC grade). One mL of distilled water (HPLC water) was added

into the eluted sample and vortexed for 10 seconds at 2500 rpm. All diluted sample (2ml) was filtered through syringe filter with a pore size of 0.22µm (Part number SLCA2522S) and then was transferred into HPLC vial ready for HPL analysis. The procedure was performed to all 31 samples in a duplicate form; in this regard 62 samples were prepared ready for analysis with HPLC.

3.6.2.2 HPLC analysis

The vials containing samples (62) were subjected to HPLC machine. Each day the HPLC machine was conditioned by 2.6 v/v of aflatoxins standard solution before running the HPLC sample of CBCF-F. The HPLC condition was as shown in Table. 1. Readings from the HPLC machine were transformed into peaks, and from the peaks to data through computer programmed with EMEPOWER (software version 3.0) connected to the HPLC machine. The limit of detection for the selected method for HPLC quantification of aflatoxin was 0.1 to 300 ppb.

Table 1: **HPLC Condition**

Item	**Particular**
Column	Waters XBridge C_{18} , 5µm 4.6 x 150mm Column
Part Number	186003116
Mobile Phase	65:35 v:v, Waters (B) : Methanol (C)
Seal Wash	90:10, v:v, Water : Methanol
Needle Wash	20 : 80, Water : Methanol
Flow Rate	0.6 ml/min
Injection volume	10µl
Column temperature	40 ºC
Detection	Λex: 365nm, Λem: 455nm
Emmition Wave length	455nm
Gain	1
Sampling Rate	2 Points / second
Instrument Type	Waters Alliance e2695 separation model, with Fluorescence Detector: Waters 2475 model

Λex-Excitation Wavelength, Λem-Emmition Wave length, v-volume, min-minutes, µ-microliter, µm-micromill

3.6.2.3 Recovery of the sample / Spiking levels

The recovery test values of HPLC method were determined by spiking known blank sample CBCF-F with different concertation of aflatoxin standard solution (50 µL, 100 µL and 200 µL) each applied to 50g of blank sample, in order to have contamination value close to 5, 10 and 20 ppb for total aflatoxins. The spiked sample was allowed to set 2 hours at ambient temperature under a fume hood to allow any residual solvent to evaporate. Two replicates were analyzed at each level (50, 100 and 200 µL). The recovery test for the blank sample for total aflatoxins at 5, 10, and 20 ppb showed 108.25, 102.73 and 103.47 % respectively, The recovery precision performance was within 80 to 110% which met the criteria for recovery of total aflatoxin > 10µ/kg for B1 B2 G1 and G2, according to European Commission regulation for mycotoxins No 401/ 2006 of 2006. This precision indicates that the HPLC method was detecting exactly what were injected by the machine.

3.7 Data Analysis

Data processing and analysis were computed by using Statistical Product & Service Solution software version 20.0 (IBM-SPSS). Before analysis, the data were verified, compiled, coded and summarized. T-test analysis (one sample T-test and analysis of variance (ANOVA) were used to determine status of products, to compare means and distributions of aflatoxin and fumonisins among the samples collected. The factors which influence contamination level of aflatoxin in CBCF-F were estimated by linear regression analysis. Also cross–tabulations (chi-square) and correlation were used to test association between different variables. All tests were performed at 0.05 confidence interval (CI). Ethical considerations or the multicollinearity of the linear regression model was considered; for example data of the dependent variable (aflatoxin) were transformed into

the inverse of logarithm 10 to make them normally distributed, and variables used in the linear regression were coded as shown in Table 2.

Table 2: **Variables and codes used in the linear regression model**

Independent variable	Meaning of variable	Coding used	
		0	**1**
Groundnuts (kg)	Groundnuts used in making CBCF-F	No coding used but put the exact figures used as continuous datas (kg) ie; 1, 2, - - - - - - - - - - 51 kg	
Maize (kg)	Maize used in making CBCF-F		
Fingermillet (kg)	Fingermillet used in making CBCF-F		
Rice (kg)	Rice used in making CBCF-F		
Millet (kg)	Millet used in making CBCF-F		
Gender	Gender of a processors	Male	Female
Age	Age of processors (years)	Continuous data	
Experience	Number of years spend as a processor of CBCF-F		
Education level	Years of school of the processors		
Household labour	Members working in processing CBCF-F		
Status of house	Status of house processors live and spend in processing CBCF-F	Rental house	Own house
Knowledge source	Where processor gained skill of making CBCF-F	Informal training	Formal training
Stored cereals	If cereals are stored for making CBCF-F	Yes	No
Packaging materials used	Materials used in packing CBCF-F	No packaging material used	Polythene bags

CHAPTER FOUR

4.0 RESULTS

This chapter presents findings of the study. It is divided into two sections: results from laboratory analysis for quantification of total aflatoxins and fumonisins, and results from direct interview on respondents' awareness, attitude and perception on issues concerning aflatoxin and fumonisin contamination in cereals used in processing blended Cereal Based Complementary Foods with Formula (CBCF-F).

4.1 Characteristics and Ingredients Used by Respondents

This section describes the socio-demographic characteristics of the respondents: sex, age, education level, household labour (number of family/group members involved in processing CBCF-F, duration of the respondents in processing blended CBCF-F and whether the respondents were involved in processing groups or not. Also, it highlights sources and ingredients used by the respondents in processing CBCF-F.

4.1.1 Socio-demographic characteristics of respondents

The sample comprised 70 samples of blended CBCF-F from different processors. These were subjected to laboratory analysis of aflatoxin and fumonisin, from which 60 some processors were enrolled for the interview part of the study of whom 83.3% were females. The majority of the respondents (63.3%) were of reproductive age, between 18 and 45 years. In general, ages of respondents ranged from 20 to 65 years. Educational levels of respondents ranged from no formal education to university; the majority (46.7%) of the respondents had primary education. Most of the respondents (73.3%) had 1 to 3 household members who were directly involved in the processing of blended CBCF-F. Few respondents (40%) belonged to small groups of CBCF-F processing. In general,

respondents' experience in processing CBCF-F ranged from 0.25 to 30 years. However, most of them (38.3%) had 1 to 4 years' experience (Table 3).

Table 3: **Distribution of respondents by socio-demographic characteristics**

Social demographic characteristics	Frequency (n=60)	Percent
Sex		
Male	10	16.7
Female	50	83.3
Total	**60**	**100**
Age		
18-45 years (Reproductive age)	38	63.3
46-60 years (Aged people)	20	33.3
61+ years (Retired people)	2	3.3
Total	**60**	**100**
Education		
No formal education	1	1.7
Primary education	28	46.7
Secondary education	18	30.0
Certificate and diploma	3	5.0
University	10	16.7
Total	**60**	**100**
Household labor (people involved in preparation of CBCF-F)		
1-3	44	73.3
4-6	15	25.0
7-9	0	0
10+	1	1.7
Total	**60**	**100**
Processors engaged into groups		
Yes	24	40.0
No	36	60
Total	**60**	**100**
Experience of processing blended CBCF-F (years)		
1-4	23	38.3
5-8	16	26.7
9-12	15	25.0
13-16	1	1.7
17-20	3	5.0
21+	2	3.3
Total	**60**	**100**

n= Total number of Processors of Blended CBCF-F visited with questionnaires.

4.1.2 Ingredients for CBCF-F and their sources

4.1.2.1 Ingredients used by processors for making blended CBCF-F

Two ingredients namely maize and groundnuts were the main components in making CBCF-F. An average of 2 kg of maize, which ranged from 0.5 to 7 kg and an average of 0.5 kg of groundnuts ranging from 0.25 to 1.5 kg were used by HM respondents, making an average ratio of 2:0.5 kg (maize: groundnut). On the other hand, the CLM used an average of 19 kg of maize ranged from 3 to 51 kg and an average of 5.5 of groundnuts which ranged from 1 to 10 kg, making an average ratio of 19:5.5 kg (maize: groundnut). This ratio was used by both homemade (HM) and commercial local (CLM) processors every time they need to make CBCF-F. Other rarely used ingredients included finger millet, millet, rice, wheat, soya and sesame. The maximum kg produced by HM and CLM per one bunch were 4.9 and 180 kg respectively (Table 4).

Table 4: Ingredients and their proportions used by HM and CLM processors for making Blended CBCF-F

Ingredients (cereals)	Product Type					
	HM			CLM		
	Minimum (kg)	Maximum (kg)	Average (kg)	Minimum (kg)	Maximum (kg)	Average (kg)
Maize	0.50	7.00	2.28	3.00	51.00	19.40
Finger millet	0.00	4.00	0.91	0.00	51.00	15.83
Rice	0.00	2.00	0.39	0.00	20.00	4.58
Millet	0.00	0.50	0.06	0.00	20.00	3.98
Groundnut	0.25	1.50	0.56	1.00	10.00	5.50
Wheat	0.00	2.00	0.39	0.00	40.00	7.03
Soya	0.00	2.00	0.28	0.00	42.90	7.01
Simsim	0.00	1.00	0.05	0.00	5.00	0.31
Total kg of CBCF-F produced per one batch by individual respondent	**2**	**14**	**4.9**	**17**	**180**	**63.18**

4.1.2.2 Sources of ingredients used by respondents in making blended CBCF-F

Most respondents (93.3% HM and 60% CLM processors) purchased ingredients for making blended CBCF-F from Morogoro markets. Few of the CLM processors (16.7%) added more cereals sourced from other districts and regions, and only 6.7% respondents of HM CBCF-F used their own produces from their farms apart from purchasing cereals from Morogoro markets (Table 5).

Table 5: Sources of ingredients used in making CBCF-F

	Types of blended CBCF-F			Overall Total
	Homemade	Commercial Locally Processed	Total number of CBCF-F-involved	Percent
Source of ingredient				
Purchase from Morogoro markets	28	18	46	76.7
Sourced from other districts	0	5	5	8.3
Purchased from Morogoro markets and sourced from other districts	0	5	5	8.3
Morogoro Markets and own produce	2	0	2	3.3
Morogoro Markets, own produce and sourced from other districts	0	2	2	3.3
Total	**30**	**30**	**60**	**100**

4.1.2.3 Storage of ingredients used by respondents in making blended CBCF-F

Majority of the respondents (86.7% and 50%) HM and CLM did not store ingredients for CBCF-F formulation. Ingredients stored by both respondents most was maize (63.2%), which was stored in steel-drums (42%). Majority of processors (52%) stored their materials for one year (Table 6).

Table 6: Storage of materials for CBCF-F formulation by HM and LCM processors

Descriptions	Type of product		Total
	HM	LCM	
	n(%)	n(%)	
Whether ingredients stored			
Yes	4 (13.3)	15 (50)	19 (31.7)
No	26 (86.7)	15 (50)	41 (68.3)
Total	**30 (100)**	**30 (100)**	**60 (100)**
Material stored			
Maize	4 (100)	8 (53.3)	12 (63.2)
Fingermillet	0 (0)	3 (20)	3 (15.8)
Groundnuts	0 (0)	1 (6.7)	1 (5.2)
All of the above	0 (0)	3 (20)	3 (15.8)
Total	**4 (100)**	**15 (100)**	**19 (100)**
Where store Materials			
Nylon bags	0 (0)	2 (13.3)	2 (10.5)
Drums (steel)	1 (25)	7 (46.7)	8 (42.1)
Drums (plastic)	2 (50)	4 (26.6)	6 (31.6)
Sacks	1 (25)	1 (6.7)	2 (10.5)
Drums and sacks	0 (0)	1 (6.7)	1 (5.3)
Total	**4 (100)**	**15 ()**	**19 (100)**
Duration of storage			
1< 2 weeks	0 (0)	2 (13.3)	2 (10.5)
1 Month	0 (0)	3 (20)	3 (15.8)
5- 6 Months	0 (0)	4 (26.7)	4 (21.1)
1 year	4 (100)	6 (40)	10 (52.6)
Total	**4 (100)**	**15 ()**	**19 (100)**

4.2 Status of Total Aflatoxin and Fumonisin in Homemade and Commercialized Blended Cereal Based Complementary Foods with Formula

4.2.1 Distribution of total aflatoxins among samples across the three types of blended CBCF-F

The data from laboratory analysis (Table 7) show that total aflatoxin distributions across all 3 samples (30-HM, 30-CLM and 10- CFM) ranged from 0 to 124 ppb (µg/kg) for HM which had 96.6% positive sample from which 43.3% samples were above the Tanzania

Bureau of standards (TBS) regulatory limit of (10ppb). For CLM the aflatoxins ranged from 0 to 174 ppb with 86.6% positive samples, from which 26.6% of the sample were above TBS regulatory limit. For CFM, the aflatoxins ranged from 0.9 to 257 ppb with all samples (100%) being positive and about 20% were above the recommended limit.

Table 7: Distribution of aflatoxins among samples across the 3 types of CBCF-F

No.	**Total aflatoxins (ppb) in three types of blended CBCF-F**		
	Homemade	**Commercial: Locally Made**	**Commercial: Factory Made**
1	0.0	0.0	0.9
2	1.4	0.3	2.0
3	1.6	0.3	3.7
4	1.7	0.4	3.7
5	2.7	0.7	3.7
6	2.7	0.8	3.9
7	2.8	1.6	4.5
8	3.1	1.7	6.6
9	3.6	1.8	11.1
10	4.0	1.9	257
11	4.6	2.1	
12	4.8	2.6	
13	4.8	3.0	
14	7.1	3.3	
15	8.1	3.3	
16	8.4	4.5	
17	9.4	4.8	
18	10.1	4.8	
19	10.6	4.9	
20	11.9	5.3	
21	19.5	5.4	
22	20.5	8.5	
23	23.5	12.6	
24	29.1	16.4	
25	40.7	40.1	
26	50.5	45.8	
27	68.1	50.0	
28	80.8	73.2	
29	89.4	87.9	
30	124.0	174.0	
Total sample	**30**	**30**	**10**
Mean	18.8	13.9	4.5
SD	24.7	23.3	2.9

N.B 5% of outliers were removed from each brand of CBCF-F

4.2.2 Distribution of total fumonisins among samples across the three types of CBCF-F

The data from laboratory analysis (Table 8) show that total fumonisins distributions across all 3 samples (HM, CLM and CFM) ranged from 0.1 to 1.4 ppm for HM, and 0 to 1.5 ppm for CLM and 0.1 to 1.7 ppm for CFM respectively. None of the three types of products had fumonisins above the TBS regulatory limit of 2ppm (TBS, 2014).

Table 8: Distribution of fumonisins among samples, across the three types of CBCF-F

		Total fumonisins (ppm) of three types of CBCF-F	
Sample No.	**HM**	**CLM**	**CFM**
1	0.1	0.0	0.1
2	0.1	0.1	0.1
3	0.1	0.1	0.1
4	0.1	0.1	0.2
5	0.1	0.1	0.2
6	0.1	0.1	0.2
7	0.1	0.1	0.3
8	0.1	0.1	0.6
9	0.1	0.2	1.1
10	0.2	0.2	1.7
11	0.2	0.2	
12	0.2	0.2	
13	0.2	0.2	
14	0.2	0.2	
15	0.2	0.2	
16	0.2	0.2	
17	0.2	0.2	
18	0.2	0.3	
19	0.2	0.3	
20	0.3	0.3	
21	0.3	0.4	
22	0.3	0.5	
23	0.3	0.5	
24	0.4	0.7	
25	0.4	0.8	
26	0.4	0.9	
27	0.5	1.0	
28	0.5	1.0	
29	0.5	1.3	
30	1.4	1.5	
Total	**30**	**30**	**10**

4.2.3 Comparisons of detected total aflatoxin and fumonisin with TBS-regulatory

4.2.3.1 Comparison of detected total aflatoxins and fumonisins with TBS-regulatory limit in all CBCF-F

The results in Table 9 show that the total number of the CBCF-F samples collected for analysis for total aflatoxins and fumonisins were 70 from which the majority (92.85 and 98.00%) of the samples were contaminated by aflatoxins and fumonisins respectively. For total aflatoxins, 32.85% of the samples exceeded the regulatory limit set by the Tanzanian Bureau of Standards (TBS, 2014) in cereal products. The TBS regulatory limit for human being and infants for total aflatoxins in cereal products is 10ppb and for total fumonisins is 2ppm. In general, total aflatoxins in the samples tested were statistical higher than the TBS regulatory limit ($p = 0.077$). Regarding fumonisin, there was no sample to all processors which exceeded TBS regulatory limit ($p < 0.000$).

Table 9: Overall total aflatoxins and fumonisins in CBCF-F detected across the three products

CBCF-F	Sample tested	Positive sample n (%)	Conc. (µg/kg) (Mean ± SD)	Range (µg/kg)	Exceeding legal limit, n (%)	Significance TBS standards
Overall mycotoxins						
Aflatoxins	70	65 (92.85)	14.67±22.56	0-257	23 (32.85)	0.077**ns**
Fumonisins	70	69(98)	0.35±0.37	0-1.7	0(0)	0.000*

ns indicates not significant and ***** indicates significant $p < 0.05$

4.2.3.2 Comparison of total aflatoxins and fumonisins contamination in CBCF-F across the three products

The Duncan's multiple range test for comparison of Least Significant Difference (LSD) across the 3 types showed no significant difference ($p > 0.05$) among the three products; HM, LCM and CFM implying that both types were related in terms of contamination of

total aflatoxins. Even though all types were not significantly different from each other, at least the CFM had low (4.5ppb) mean value of total aflatoxins which was within the recommended level. On other hand, the CFM had one sample with highest outlier for total aflatoxins (257ppb) which were excluded from the data analysis. Furthermore, there were no significant differences among HM, LCM and CFM in terms of total fumonisins implying that both types were related in terms of contamination for total fumonisin and also their mean value did not exceed the TBS regulatory limit (Table 10).

Table 10: Multiple Comparison of Least Significant Different (LSD) across the 3 types of CBCF-F for total aflatoxins

Type of CBCF-F	Total Aflatoxins (ppb) Mean ± SD	Total Fumonisins (ppm) Mean ± SD
Factory Made (CFM)	4.82 ± 2.48a	0.46 ± 0.54b
Local Commercial (LCM)	13.86±22.43a	0.40 ± 0.39b
Homemade (HM)	18.77±23.83a	0.27 ± 0.25b

Value in the same row and column sharing common superscript letter were statistically not significantly different at $p < 0.05$ by Duncan's multiple range test

4.3 Validation Test of ELISA Method

Validation test of the ELISA method was performed using High Performance Liquid Chromatography (HPLC) with fluorescence detection on 31 samples previously analyzed using Reveal AccuScan Pro reader III. The results indicated that both methods correlated in term of detection of total aflatoxins ($r = 0.87$). Also, there was no significant difference in terms of means for total aflatoxin contamination ($p = 0.07$) between the two methods and the allowable limit of TBS Standards (Table 11).

Table 11: Validation test of AccuScan data against HPLC data

Sample No.	AccuScan (µg/kg)	HPLC (µg/kg)
61	3.9	3.67
62	11.1	9.20
63	257	248.79
64	6.6	6.16
65	3.7	4.41
66	4.5	0.88
67	3.7	4.39
68	3.7	0.20
69	0.9	0.94
70	2	0.86
19	20.5	10.21
34	2.8	0.22
12	1.6	0.74
26	0	0.59
35	1.6	0.19
25	80.8	18.43
18	19.5	5.18
27	50	10.67
31	3.6	1.64
15	45.8	7.49
3	0.3	0.04
22	0.3	0.78
8	0	0.34
29	2.7	2.01
17	1.9	0.34
14	16.4	4.58
1	12.6	3.86
7	40.1	40.50
6	174	46.39
23	5.3	11.52
33	11.9	13.89
Average	**25.45**	**14.81**
Range	**0 to 257**	**0.04 to 248.79**

4.4 Factors Affecting Aflatoxin Contamination Levels in CBCF-F

4.4.1 Ingredients used in making CBCF-F and socio-economic factors of respondents

The results in Table 12 show a significant regression model with an R-square of 47.5% with several independent variables groundnuts, finger millet, rice, millet and status of house owned by respondent (rental / own) showing significant results with total aflatoxin levels in the respective CBCF-F. The findings suggest that use of groundnuts (0.152) as ingredients and house status (0.450) as socio-economic factors of respondent contribute

significantly toward explaining the occurrences of total aflatoxin levels (dependent variable) in CBCF-F. It should be noted that coefficients which were negative predicted (as seen in Table 9) implied a positive impact which means the variable decreased the level of total aflatoxin in CBCF-F products. However, coefficients which were positive predicted (as seen in Table 10) implied a negative impact which means the variable increased the level of total aflatoxin in CBCF-F products.

Table 12: Regression analysis of factors affecting aflatoxins level of contamination in CBCF-F

Independent variable	**Coefficients**			
	Unstandardized (B)	**Standardized (Beta weight)**	**T**	**P- value**
Constant)	3.033		3.697	0.001*
Groundnuts (kg)	0.152	0.807	3.510	0.001*
Maize (kg)	0.010	0.180	0.721	0.475**ns**
Fingermillet (kg)	-0.037	-0.697	-3.086	0.004*
Rice (kg)	-0.054	-0.374	-2.435	0.019*
Millet (kg)	-0.067	-0.488	-3.007	0.004*
Gender	-0.327	-0.178	-1.258	0.215**ns**
Age	-0.010	-0.183	-0.927	0.359**ns**
Experience	0.029	0.277	1.624	0.112**ns**
Education level	-0.038	-0.222	-1.590	0.119**ns**
House hold labor	-0.085	-0.216	-1.295	0.202**ns**
Status of house	-0.450	-0.338	-1.999	0.052*
Knowledge source	-0.214	-0.151	-0.942	0.352**ns**
Stored cereals	-0.188	-0.133	-0.965	0.340**ns**
Packaging materials used	0.359	0.270	1.433	0.159**ns**

Dependent Variable: log 10 of total aflatoxins in CBCF-Fs , R Square = 47.5%, adjusted R = 30.4 %, Annova P = 0.05, Darbin=2, ***** Statistically significant at 0.05 level, **ns** statistically non-significance at 0.05 level, B is unstandardized coefficient of dependent variable

4.4.2 Ingredient factor

According to the findings in Table 12, increase in one kilogram (kg) of groundnut and 1kg of maize in CBCF-F increased aflatoxin level by 0.152 ppb and 0.01 ppb respectively. On

the other hand as 1kg of fingermillet, rice and millet increased; 0.037, 0.054 and 0.067 ppb of aflatoxin level decreased in the CBCF-F.

4.4.3 Social demographic factors contributing to aflatoxin level in CBCF-F

4.4.3.1 Relationship between gender and processing CBCF-F

The results in Table 13 show that more females were involved than males in both HM and CLM CBCFs production. Unlike males, females also were predicted by the regression model (Table 12) to reduce total aflatoxins in their respective CBCF-F. Hence gender had significant association (p = 0.006) with processing of CBCF-F.

Table 13: Relationship between gender of respondent and production of CBCF=F cross tabulation

Gender of respondent	Product type		X^2- Value	P- value
	LCM n (%)	HM n (%)		
Male	9 (30)	1 (3.3)	**7.68**	**0.006**
Female	21 (70)	29 (96.7)		
Total	**30 (100)**	**30 (100)**		

X^2- indicates Chi-Square value, degree of freedom (df) = 1, n = number of respondents. Number of sample from each types of CBCF-F were 30; Total = 60

4.4.3.2 Relationship of age among production of CBCF-F types, respondents' level of awareness and level of total aflatoxins within their respective products

The results in Table 14 show that most respondents (86.7%) who were in reproductive age group (18 to 45years) were engaged in production of HM CBCF-F, whereas production of the CLM CBCF-F was mostly performed by aged group respondents of between 46 to 60 years (56.7%). This showed that there was significant association (p = 0.001) between age and a given set of activity (production of types of CBCF-F). Also, there was a significant association between age and levels of total aflatoxins in CBCF-F (p = 0.049). This was

justified by the level of aflatoxins found within their products; whereby aged (46 to 60) respondents had few samples (19.0%) which were above the recommended standard (10ppb) than their counterparts. Furthermore, regression analysis (Table 12) showed that the chances of decreasing level of total aflatoxins increased with respondent's age. However, there was no relationship (p = 0.338) between age of respondent and awareness on aflatoxin and fumonisin contamination in cereals.

Table 14: Relationship among age groups of respondents with respect to types of CBCF-F, aflatoxin awareness and aflatoxin regulatory limit

Age group of respondent	Production (%)		Level of awareness of aflatoxins contamination in cereals (%)		Level of aflatoxins (%)	
	HM (n=30)	CLM (n=30)	< 50% score	> 50% score	Within 10ppb	Above 10ppb
18-45	86.7	40.0	66.7	50.0	59.0	71.4
46-60	10.0	56.7	29.2	50.0	41.0	19.0
≥ 61	3.3	3.3	4.2	0.0	0.0	9.5
X^2-value		**14.96**		**2.17**		**6.03**
Significance		**0.001***		**0.338ns**		**0.049***

Note n= 60, > indicates greater than, <indicates less than, ≥ indicates greater than or equal to,
X^2- indicates Pearson Chi-Square, *****-indicates significant, **ns**- indicates not significance

4.4.3.3 Influence of education on total aflatoxins in CBCF-F and respondent's awareness on aflatoxins and fumonisins

The results in Table 15 indicate that, the high education of respondents has no influence (p = 0.325) on processing CBCF-F with recommended limit of the total aflatoxins. Moreover, it was observed from the regression analysis that increase in respondent education did not significantly decrease total aflatoxins, although its coefficient indicated decrease in total aflatoxins (Table 12). This suggests that, both respondents with low and high level of education produced products of similar level of contamination with total

aflatoxins. However, there was a significant association (P = 0.000) between education level of respondents and the level of awareness on aflatoxins and fumonisins.

Table 15: Association between education of respondent against level of awareness and total aflatoxin in their products

Education level	Total (%)	level of awareness (knowledge) (%)		Total aflatoxin level in CBCF-F (%)	
		Below 50% score	Above 50% score	Above recommended 10ppb	Within recommended 10ppb
Primary and adult education	48.3	60.4	0.0	57.1	43.6
Secondary, certificate, diploma and university	51.7	39.6	100.0	42.9	56.4
Significance			**0.000**		**0.232**
Spearman Correlation value			0.48		0.13

4.4.3.4 Relationship between household labour and processing groups on level of total aflatoxins in CBCF-F types

The quantity of assistants in processing of CBCF-F showed no influence in producing CBCF-F which are within recommended standards of total aflatoxins (p = 0.698) because both respondents who had one to three assistants and those with above 3 assistants produced products which were not significantly different (Table 16). Also, from the multiple linear regressions results (Table 12) it was observed that, as household labour increased also chances of decreasing total aflatoxin increased though the decrease was not significant (p = 0.202). Also, respondents who were involved in processing groups did not differ significantly (p = 0.439) with respondents not involved in groups in terms of total aflatoxin (Table 16).

Table 16: Relationship of household labor and processing groups on level of total aflatoxin in CBCF-F types

	Types according to level of aflatoxin (%)		(x^2) value	Significance
	Above recommended level	Within recommended level		
Family assistant				
1-3 people	34.1	65.9	0.72	0.698**ns**
4-6 people	40.0	60		
7 +	0.0	100		
Respondent involved into processing groups				
Individual	38.9	61.1	0.60	0.439 **ns**
Groups	29.2	70.8		

ns indicate not significant

4.4.4 Social economic factors

The findings suggest that respondents who were living and processing CBCF-F in a rental house had significant chances of increasing aflatoxin level by 0.45 ppb as opposed to their counterparts who processed foods in their own houses (Table 12).

Regarding respondents' knowledge source on processing CBCF-F, it was predicted that, respondents who got formal training had chances of decreasing aflatoxins level by 0.214 as opposed to those who did not (Table 12).

4.4.5 Other factors

Other factors influencing aflatoxins contamination in CBCF-F predicted from the regression model were storage and packaging materials used. Storage of cereals had a great chance of increasing aflatoxin contamination in CBCF-F as compared to cereals not stored. Also, the packaging materials used for packaging CBCF-F such as polythene bags were predicted to increase aflatoxin level by 0.359 ppb as opposed to product not packaged (Table 12).

4.5 Awareness and Perceptions of Respondents on Cereals Towards Mycotoxins

4.5.1 Level of awareness of processors about aflatoxin and fumonisin in cereal based complementary foods

The results in Table 17 show awareness of respondents on mycotoxins contamination. Most of the respondents (81.7%) had no knowledge of mycotoxins. A similar proportion of respondents (81.7%) had never heard about aflatoxin contamination in cereals. Regarding fumonisins, 95% of the respondents had never heard about them. Consequently, the majority of the respondents (83.3%) were not able to mention cereals which are susceptible to contamination with aflatoxins. Likewise, the majority of respondents (91.7%) were not able to mention cereals which are likely to be contaminated with fumonisins. Moreover about 76.7% of the respondents were aware of the conditions which influence growth of molds in cereals. The overall score of the respondents in assessing awareness of mycotoxins revealed that, the majority (80%) of the respondents scored below 50%, which indicated that most of them were not aware of aflatoxin and fumonisin contaminations in cereals.

Table 17: Awareness about aflatoxin and fumonisin in cereal based complementary foods

Description	Number of respondents	Percent
Aware of mycotoxin contamination in cereals		
Yes	11	18.3
No	49	81.7
Total	**60**	**100**
Aware of aflatoxins in cereals		
Yes	11	18.3
No	49	81.7
Total	**60**	**100**
Aware of fumonisins in cereals		
Yes	3	5.0
No	57	95.0
Total	**60**	**100**
Mentioned Maize and Groundnuts to be contaminated with aflatoxins		
Yes	10	16.7
No	50	83.3
Total	**60**	**100**
Mentioned Maize and Groundnuts to be contaminated with fumonisins		
Yes	5	8.3
No	55	91.7
Total	**60**	**100**
Conditions that favors growth of molds in cereals		
Moisture and high temperature	46	76.7
Dust and very low temperature -4 ºC	1	1.7
Don't know	13	21.7
Total	**60**	**100**
Overall knowledge		
Scored above 50 marks	12	20.0
Scored below 50 marks	48	80.0
Total	**60**	**100**

4.5.2 Awareness of respondents on issues related to health and aflatoxin and fumonisin

Table 18 present awareness of respondents on issues related to health and aflatoxin and fumonisin. The result shows that the majority of the respondents (76.7%) were not aware

that stunting and impairment of body immunity in children could be caused by consumption of cereal products contaminated by aflatoxin and fumonisin. Also about sixty percent of the respondents were not aware that consumption of cereal products contaminated by aflatoxin and fumonisin could cause abdominal pain.

Table 18: Awareness of respondents on issues related to health and aflatoxin or fumonisin

Description	Number of respondents	Percent
Aflatoxin and fumonisin contamination in cereals can cause stunting		
Yes	14	23.3
No	46	76.7
Total	**60**	**100**
Aflatoxin and fumonisin contamination in cereals can impair body immunity		
Yes	14	23.3
No	46	76.7
Total	**60**	**100**
Aflatoxin and fumonisin contamination in cereals can cause abdominal pain		
Yes	24	40
No	36	60
Total	**60**	**100**

4.5.3 Perceptions about aflatoxin and fumonisin in CBCF-F

Table 19 shows that the majority (90%) of the respondents had seen molds/fungi in cereals, but only 21.7% of them perceived those molds as possible causes of health complications. The rest (76.3%) perceived those molds as harmless to human beings. With regard to what respondents did with the molded portions or the whole lot of molded cereals, the majority (63.3%) said that they fed them to animals such as chickens, pigs and cattle.

Table 19: Perceptions about aflatoxin and fumonisin in CBCF-F

Description	Number of respondents	Percent
Respondents ever seen molds/fungus in cereals		
Yes	54	90
No	6	10
Total	**60**	**100**
Perceptions of respondents towards presence of molds in cereals		
Just dust present in food non harmful to human being	1	1.7
Just disease of crops not affecting human being	17	28.3
Just color / decayed food which are non-harmful to human being	29	48.3
Decayed food which are harmful to human being	13	21.7
Total	**60**	**100**
What do respondents do when find the whole cereal lot molded		
Leave it for adult use at home	6	10.0
Give it to animals such as pigs, cattle and chickens	38	63.3
Discard / put to garbage	13	21.7
Others-such as dehulling and use for adult	3	5.0
Total	**60**	**100**

4.5.4 Knowledge source of respondents about processing and handling CBCF-F

Figure 7 indicates that most processors (42 and 27%) acquired knowledge and experience of processing and handling blended CBCF-F from their own initiatives and their neighbours respectively. The rest (18%) received knowledge from Non-Governmental Organizations (NGOs) and only 5% from government health officers and 8% from the Small Scale Industries Organization (SIDO) of Tanzania.

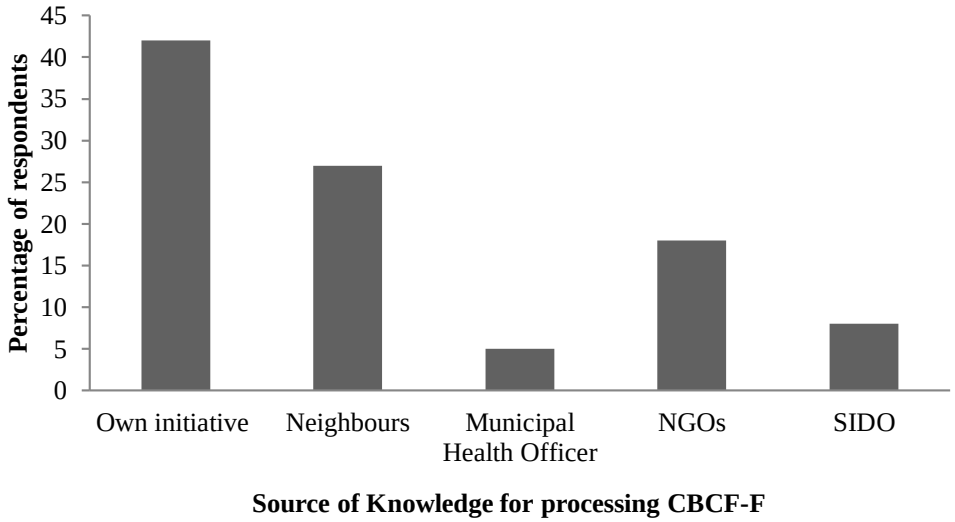


Figure 7: Knowledge sources of respondents on processing and handling of CBCF-F

4.5.5 Training on issue concerning processing and handling of CBCFs

Table 20. Shows about 57% of the processors had never attended any training concerning processing and handling of CBCF-F. Likewise, of the few respondents who had attended such training, only 19.23% had been trained on issues concerning contamination of cereals with aflatoxin and fumonisin.

Table 20: Percent of respondents attending training on issues related to processing of CBCFs and contamination of aflatoxin and fumonisin in cereals

Training	Number of respondents	Percent
Processing and handling of CBCFs		
Yes	26	43
No	34	57
Total	**60**	**100**
Aflatoxins and fumonisins being part of the training		
Yes	5	19.23
No	21	80.77
Total	**26**	**100**

CHAPTER FIVE

5.0 DISCUSSION

This chapter discusses results of this study according to the main objective which covers the status of aflatoxin and fumonisin in homemade and commercial CBCF-F and the specific objectives: factors affecting aflatoxins and fumonisins contamination in CBCF-F, the knowledge, perception and attitude of processors of CBCF-F towards aflatoxins and fumonisins.

5.1 Social Demographic Characteristics of Respondents

This study found that the majority of females (83.3%) were engaged in processing of CBCF-F for both HM and CLM. This study is in line with Rushunju (2012) who found that the majority (94.2%) of processors of CBCF-F were females. This implies that processing of CBCF-F is dominated by females for both homemade and commercial-made, although 90% of males were found engaged in CLM alone. This may be attributed to gender bias introduced from socialization. Age is an important factor in social analysis since different ages perform different sets of activities (Msokwa, 2008). The current study found that majority of respondents was aged between 18 to 45 years. According to African Child Policy Forum (2013), a person with ≥ 18 years old is considered to be mature enough to make their own decisions. This implies that the respondents were mature enough with enough energy to deal very well with processing of CBCF-F free of mycotoxins contamination. Furthermore, this study found that the majority of the respondents were literate with primary school education. This may be due to the government of Tanzania reinforcement on attaining the universal primary education to all people (TDHS, 2004-05). Moreover, according to TDHS-MIS (2015-2016), half of the population of men and women in Tanzania attained primary education. Household labour

is one of the factors which can influence minimization of contaminants in cereals. It was found that majority of the respondents had one to three people who were direct assisting in processing and handling of CPCF-F. This finding is in line with Tanzania's average household members as per house (4.9 people), although this study ignored children and old people who could not assist in the activities of CBCF-F. In addition, this study, found that 60% of the respondents were not engaged in groups for processing of CBCF-F. The majority of the respondents having 1 to 4 years' experience in processing CBCF may be contributed by the fact that most of them were youth (18-45 years old) who were actively involved in most developmental activities so we didn't expect them to have vast experience in product processing than the other category of age (≥ 46 years). So this low experience of the respondents may be the contributing factor for presence of AF above the allowable limit in the products (Magembe *et al*., 2016).

5.2 Ingredients Used in Processing CBCF-F and their Sources

It was found that all the respondents used maize and groundnuts as their main component ingredients in making CBCF-F with different ratios. Nevertheless, CBCF-F constituted other ingredients which were observed to be optional components added in CBCF-F. This study is in line with other studies which found that maize and groundnuts are the main components of food pattern of most developing countries (Kimanya *et al*., 2008a; Smith *et al*., 2017). Maize and groundnuts have been studied by many researchers to be good substrates for growth of fungus capable of causing aflatoxin, fumonisin and other mycotoxins.

Moreover, this study found that the majority of the respondents purchased raw materials for processing CBCF-F from Morogoro local market. Cereals found in local market in most developing countries are locally grown, and so are chronically exposed to multiple

mycotoxins (Williams *et al*., 2004; Lewis *et al*., 2005). This may be a reason as to why both types of CBCF-F were not significantly different from each other in terms of contamination of aflatoxin and fumonisin.

5.3 Status of Aflatoxins and Fumonisins in Homemade and Commercial CBCF-F

The current research is the first to conduct a study on distribution of aflatoxin and fumonisin among homemade, commercial locally processed and factory-made CBCF-F. Other studies investigated either commercial locally processed CBCF-F alone (Rushunju, 2012) or other cereal based complementary Foods (Kimanya, 2008, Kimanya *et al*., 2009, 2010 and 2014).

5.3.1 Status of aflatoxins in Homemade and Commercial CBCF-F

The study found that 96.6% of the HM samples had aflatoxin from which 43.3% had aflatoxins above the Tanzania regulatory limit. This implies that infants depending on home processed CBCF-F and who are the primary user of CBCF-F can be chronically exposed to aflatoxins thus are likely to suffer from aflatoxicosis such as abdominal pain, vomiting, diarrhea, underweight, growth retardation and for a long run use of this product may pose them to risk of getting cancer. These findings agree with those of a study by Lewis *et al*. (2005) who stated that the majority of home grown and stored maize was contaminated by aflatoxins.

Furthermore, this study found that the commercial CBCF-F both from locally processed and Factory-made had many samples confirmed to be with aflatoxins (86.6 and 100%) and others with higher amount than the recommended regulatory limit (26.6 and 20%) respectively. The results are in line with findings by Rushunju (2012) and Rushunju *et al*. (2013) who conducted studies in other parts of Tanzania and found that majority of

CBCF-F were contaminated by aflatoxins, and some samples had aflatoxins above the regulatory limit.

In addition, the status of aflatoxin in the current study of CBCF-F regardless of being HM, CLM or CFM showed that the majority (92.85%) of samples were contaminated by aflatoxin, from which 32.85% of the positive were above the allowable limit implying that consumers, especially children, can be chronically exposed to aflatoxins though levels may differ from one place to another. Therefore, they are likely to suffer from Aflatoxicosis or related infections as observed by Gong *et al*. (2003) and Shirima *et al*. (2016). Results of the current study agreed with other reports as cited elsewhere.

5.3.2 Status of fumonisins in homemade and commercialized CBCF-F

This study found that all samples regardless of HM, CLM and CFM contained detectable level of fumonisin although in smaller amounts and below the regulatory limit (2 ppm). The low level of fumonisin found in this study of CBCF-F might be contributed by the fact that fumonisins are water soluble toxins, thus they may be reduced from the ingredients through washing. All the respondents interviewed revealed that washing of all ingredients was part and parcel of their daily routine before they processed CBCF-F into flour. The finding of the study concurred with other studies which revealed that fumonisins can be reduced significantly from corn products by various processing procedures such as washing and dehulling (Codex Alimentarius Commission (2000). In additional, Yau *et al*. (2016) found that people in Hong Kong were exposed to cereal foods with lower limit of fumonisin. However, this study different from previous studies conducted in other parts of Tanzania, which found that 131 infants depending on maize (CBCF) had fumonisins in their blood sample, ranging from 21 to 3201 ppm of whom 26 infants had fumonisins above the Tanzanian regulatory limit of 2 ppm (Kimanya *et al*.,

2009, 2010 and 2012). Williams *et al*. (2004), Fung and Clark (2004) argued that chronic exposure to low level of fumonisins and other mycotoxins may also expose the human body into risk of getting mycotoxin related complications. Infants' exposure to fumonisins is also linked with growth retardation (Kimanya *et al*., 2010; Shirima *et al*., 2016). Furthermore, according to Etzel (2015) consuming foods contaminated with fumonisins increases the risk of having a child with neural tube defect and the risk of developing esophageal cancer during adulthood.

5.4 Factors Affecting Aflatoxins Level of Contamination in CBCF-F

5.4.1 Ingredients used in making CBCF-F

This study found out that groundnuts and maize, as the ingredients used, increased the level of aflatoxins in CBCF-F, having groundnuts showing high significant levels in aflatoxins than maize. This finding is in line with finding of some other studies (Grace *et al*., 2015; Lindahl *et al*., 2016; Magembe *et al*., 2016) which found that groundnuts contain higher amount of aflatoxins and Nyangi (2014) who found that some maize samples had aflatoxin above the allowable limit. So complementary foods based on maize and groundnuts cereals are likely to be contaminated by aflatoxin and fumonisin. Further studies are required to investigate whether maize contributes significantly to increasing level of total aflatoxin in CBCF-F, not just CBCF.

Other ingredients such as fingermillet, rice and millet statistically seemed to have significantly reduced levels of total aflatoxins in CBCF-F. This finding is similar to finding by Bandyopadhyay *et al*. (2007); Chala *et al*. (2014) Ezekiel *et al*. (2014) who found that fingermillet and millet may not favour growth of aflatoxin. Furthermore, the fact that rice did not increase aflatoxin was due to the fact that all processors of this study used dehulled rice. Dehulling of cereals reduce some level of aflatoxins (Kimanya, 2008).

This suggests that complementary foods based on fingermillet, dehulled rice and millet will be in low risk to be contaminated by aflatoxin.

5.4.2 Social demographic characteristic of respondents

5.4.2.1 Gender

The findings revealed that female participants had higher chances of processing CBCF-F with low level of aflatoxins. This may be due to the fact that Tanzanian women are trained from the grassroots on issues concerning child rearing which include cooking and food preparation as identified by Waithanji and Grace (2014) and also it was observed from this study that more women (97 and 70%) were engaged in processing of HM and LCM respectively than men. Similarly, Magembe *et al*. (2016) found that female respondents in Kilosa District who stored maize and groundnuts showed greater awareness than males on issue pertaining to molds contamination and how to mitigate them in cereals. Also Kiama *et al*. (2016) found that women were sensitive to food safety especially molded food in feeding cattle.

Furthermore, it was observed through this research that most of male respondents were involved in LCM but not in HM CBCF-F. This was due to gender bias introduced from socialization, that kitchen activities should be implemented by females (Kiama *et al*., 2016).

5.4.2.2 Age

This study found that age was significantly associated with processing of types of CBCF-F. The results were similar to those of some other findings that age is a key factor in social analysis since different age groups perform different sets of activities as reported by Msokwa (2008). Likewise, it was found that as age of respondents increased, chances of

total aflatoxin in CBCF-F decreased. This may be due to long experience of the aged respondents (46 to 60 years) who had more experience than those in the reproductive age (18 to 45 years). Similarly, Magembe *et al*. (2016) found that farmers with age greater than 35 years who had been farming maize and groundnuts in the previous 20 years were more likely to perceive that mold infections had been a problem than people with age less than 35 years.

However, lack of significant relationship between age of respondents and awareness on issues concerning cereals and mycotoxin contamination may be due to lack of training either from formal or informal education concerning mycotoxins contamination in cereals. This finding is in line with Shirima (2016) who reported that the majority of people in Tanzania and also in Kenya (Kiama *et al*., 2016) are not aware of mycotoxin contamination in cereals which are the main component of processing CBCF-F.

5.4.2.3 Production period in processing CBCF-F

The regression analysis indicated that, as number of years involved in production and processing CBCF was one of the factors affecting aflatoxins in CBCF-F, as number of years of respondents in processing increased, levels of aflatoxins contamination increased. This might be due to the fact that respondents with many years of experience in processing CBCF-F got those skills from their own initiatives and friends (43 and 27%) respectively. Moreover, this study found that the majority (57%) of the respondents had not attended any proper training on processing and handling of CBCF-F. So this can be the main contributing factor to AF contamination in CBCF-F. From this findings, it is argued that the current situation in Tanzania of processing and handling of CBCF-F is based on local know how skills that were confirmed with other researchers to increase aflatoxin and fumonisin in cereals (Williams *et al*., 2004; Lewis *et al*., 2005).

5.4.2.4 Education level and household labour/assistants

The findings in the current study did not indicate significant relationship between education level of respondents and decrease in total aflatoxin in CBCF-F. This means that both respondents of low and high level of education produced products of the same total aflatoxin and fumonisin. This study indicates that there is lack of training and emphasis on mycotoxin contamination in cereals used in processing of CBCF-F. This was revealed from this study that only few (19%) processors had received training concerning aflatoxin and fumonisin contamination in cereals. However, this study found that there was good relationship between education level of respondents and level of awareness concerning aflatoxin and fumonisin. This finding is related to those reported by Magembe *et al*. (2016) who found that level of awareness on issues pertaining to mycotoxins contamination was significantly higher among people with higher education as opposed to people with low level of education.

Moreover, this study found that as labour increased in processing of CBCF-F the chances of decreasing aflatoxin levels in CBCF-F decreased by 0.085 bbp as compared to respondents without or with little assistance. This research agrees with Covey (2004) who said that one plus one is more than two. This means that when two or more efforts are synergized together to tackle a certain problem, the chances of doing best is more than one person alone.

5.4.3 Social economic characteristic of the respondents

5.4.3.1 Status of house of processors of CBCF-F

The status of having own houses among processors of CBCF-F contributed significant on reducing aflatoxin levels by -0.450 ppb times among respondents who lived in rented houses. This may be due to the fact that respondents who live in rented houses may be

occupying few rooms for living and at the same time for food processing and storage. This may result on poor ventilation which increases moisture contents and temperature for fungi and other microbial multiplication. Moreover, most of rented houses in Morogoro are characterized by dampness around the home with poor or frequent cleanness or fumigation a condition known as sick building syndrome (personal observations). Sick-building syndrome is characterized by poor ventilation, poor cleaning supply chemicals and different forms of microbial contamination that Bennett and Klich (2003) associates with the presence of toxic molds. Similarly, Thrasher *et al*. (2011) found that a family of 5 people and pet dogs with no history of health related problems found themselves in complicated mycotoxin related problems after they moved into a rental house in Hawaii.

5.4.3.2 Source of knowledge for processing CBCF-F

Source of knowledge of processors for processing CBCF-F was observed to be an influencing factor of aflatoxins which means that respondents who had formal knowledge for processing of CBCF-F were predicted to produce products with low level of total AF as compared to processors who obtained that knowledge from informal source such as indigenous knowledge. The results concurred with other studies which found that knowledge is power in reducing aflatoxins in cereals (Magembe *et al*., 2016). This may be due to the fact that most respondents obtained informal knowledge for processing CBCF-F which was based on traditional norms.

5.4.4 Other factors

5.4.4.1 Stored cereals

Stored cereals from this study were observed to increase total aflatoxin levels in CBCF-F as compared to cereals not stored. This finding agree with those reported by Williams *et al*. (2004), Nyangi (2014) and Magembe *et al*. (2016) who found that stored cereals

increase total aflatoxin levels and other mycotoxins. This might have been contributed by steady subsistence farming methods based on homegrown and local storage of produce that have been proved by other researchers (Williams *et al*., 2004) to increase mycotoxin contents in developing countries. However, duration of storage and storage facilities used may have further contributed to increase in aflatoxin concentration in stored cereals. This could be due to microbial and rodents' activities which destroy the seed coat of the stored cereals and accelerate fungal penetration to interior parts of the grain (Achaglinkame *et al*., 2017). Moreover, pests activities accumulate moisture which provide conducive environment for fungal growth (Chulze, 2010). In this study, it was observed that majority of the processors (52%) who stored cereals, stored for one year and most of them stored in steel drums. If the storage conditions were not conducive, the materials stored were likely to be contaminated with mycotoxins. It was also confirmed by other researcher that, length of time for example in five (5) months period of time regardless of storage types, there was severe increase in pests' development and aflatoxins (Hell *et al*., 2000).

5.4.4.2 Packaging materials

Polythene bags as packaging materials used for CBCF-F were observed to increase the level of aflatoxin in the respective products. This finding is in line with other studies by Nyangi (2014) and Azziz-Baumgartner *et al*. (2005) who found that polythene bags increase level of aflatoxins when used to pack cereals. Hence, this finding argues that polythene bags should be avoided in packaging of CBCF-F. Also, further studies should be employed in order to come out with suitable materials for packaging of this important complementary foods product for Tanzanian's infants.

5.5 Awareness and Perception of Processors of CBCF-F on AF and FM

5.5.1 Awareness

The majority of processors of CBCF-F were not aware of occurrence of mycotoxins in cereals particularly aflatoxins and fumonisins despite some of them having many years of experience in processing of CBCF-F. This might be contributed due to the fact that most of processors lack training concerning contaminants of CBCF-F specifically aflatoxin and fumonisin. This fact was observed from this research that most of processors had not attended training concerning processing and handling of CBCF-F. Also it was observed that even those who had attended such training only a few of them (19%) had been trained on aflatoxin and fumonisin.

Furthermore, lack of knowledge concerning aflatoxin and fumonisin may be a contributing factor to the processors being not conscious of the health implications of AF and FM to human beings like abdominal pain, immunity impairment, stunting and risk of getting cancer. This finding is related to other study by Kisai's (2015) who conducted a research in some parts of Tanzania and found that farmers and handlers of cereals (Magembe *et al*., 2016) were not aware of presence of mycotoxins in cereals, also the health implications to human being (Shirima, 2016) and similar observation was done in Kenya (Kiama *et al*., 2016). This finding argues that, lack of awareness on issues related to AF and FM and other mycotoxins may pose most of processors to risk of producing CBCF-F with aflatoxins and fumonisins and possibly with other mycotoxins.

5.5.2 Perceptions of processors of CBCF-F towards AF and FM

The majority of producers of CBCF-F had seen molds with green or dark blue colors in cereals and perceived them as harmless to human beings although this study find that, small amounts of the molded portion of cereals were sorted out from the ingredients of

making CBCF-F, because they said ‘‘the molds carry bitter taste and also impart bad aroma to CBCF-F.” Processors also added that, ‘‘the molded portion if found in flour for preparation of *Ugali* (stiff porridge), also the *Ugali* would taste bitterly with bad aroma rendering it unpalatable.’’ This finding is similar to findings by Hell and Mutegi (2011), Magembe *et al*. (2016) and Kiama *et al*. (2016) who found that the majority of farmers had seen molds but did not associate them with health implications to human beings.

Nevertheless, the processors once found that most of the portions or whole lots of cereals were molded; few of them said that they could process it for adult use and not for infants because infants don’t like unpleasant foods. Further, the majority of processors reported that infected cereals were used to make feeds for pigs, chickens and cattle. The results obtained from this study are similar with results of some other studies which found that molded cereals that should be discarded for human consumption and as feeds were consumed by poorest producers and labourers and some sold for animal feeds (Williams *et al*., 2004; Kiama *et al*., 2016 and Magembe *et al*., 2016). Meat, eggs and milk contaminated with aflatoxin and the side effects of aflatoxicosis can have hazardous side effects to human beings and animals as observed in 2016 in Tanzania (Kondoa district) during the aflatoxin outbreak when eight (8) puppies were reportedly dead due to suckling milk contaminated with aflatoxin from their mother that had consumed contaminated foods.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

i. It is concluded that CBCF-F from all producers are contaminated with AF and fumonisin hence consumers depending on any type of CBCF-F can be chronically exposed to AF and FM that can pose them into risk of getting mycotoxins health related problems.

ii. Groundnuts use and living in rental houses contribute significantly to increasing total AF in CBCF-F.

iii. The majority of processors of CBCF-F are not aware of multiple occurrences of mycotoxins in cereal products and its related health implications to human beings, this may expose processors to the risk of producing CBCF-F contaminated by multiple mycotoxins which in return pose health risks to consumers.

6.2 Recommendations

6.2.1 Status of AF and FM in HM and marketed CBCF-F

i. Mycotoxin should be checked in the raw materials (cereals or nuts) to be used in CBCF-F formulation.

ii. Cereal-based foods, especially CBCF-F, should be checked or tested for aflatoxin and other important mycotoxins before sale and marked for easy monitoring of the problem and proper measures be instituted to non-compliance because this is a health risk to general public.

iii. TFDA and TBS should ensure all CBCF-F producers are knowledgeable enough on issue of mycotoxins and on the importance of observing GMP. Also to encourage dealers of CBCF-F be it CLM or CFM to be keen on the importance of

checking the safety limit for AF & FM before marketing. TFDA, TBS together with municipalities, should set by-laws and enforce them to ensure that CBCF-F marketed are labeled with safety limit of AF and FM and other important mycotoxins in recognition with nutritive value labeled.

iv. The government should provide or initiate mini-laboratories at each district headquarters through its department of health under each district with AccuScan or M-reader and reagents easy for use by farmers, dealers of CBCF-F and anyone who wishes to check for AF or FM safety-limit at low cost which will not be expensive for a citizen to pay but also which will enable the health department to run that laboratory for sustainable development, because it is difficult for someone who wishes to check the safety limit of mycotoxins in his/her product.

v. Furthermore, mycotoxins contaminations in babies' foods should be viewed as Malaria and HIV in Tanzania and all over the world. Therefore, for those min-laboratories suggested from this study that they should be initiated to all over the districts of Tanzania and Africa as we have lab for malaria and HIV where people go and check their healthy status these laboratories can be useful. Because people will be sensitized on the impact of mycotoxins but yet they will not be sure as to what safety level they are consuming or producing product of cereals.

6.2.2 Factors that influence AF and FM contamination in CBCF-F

i. It is time now for researchers to get out from their offices and train and demonstrate to Tanzanian mothers on the benefits of replacing cereal based complementary foods with other discovered complementary foods (such as *Uji bora* which comprise of orange sweet potatoes flour, millet and soya) which suit both rural and urban Tanzanian which also are easy to care and handle throughout the time, moreover mothers should be advised on the use or replace of maize and

groundnuts with other least contaminated cereals such as rice, and sesame, millet and sorghum and also on the importance of pre-preparatory methods such as dehulling maize prior to preparation of CBCF-F. Vitamins or minerals removed during dehulling can be replaced with other ingredients such as carrots and vegetables during cooking.

ii. Municipalities should provide dealers of CBCF-F be it the CLM or the CFM with loans/credit to enable them employ high quality facilities such as solar delayers, quality storage facilities that reduces accuracy of mycotoxins together with this, the municipalities should allocate specific areas and buildings for processing of CBCF-F because the CBCF-F is consumed by vulnerable groups that require safe foods.

iii. Appropriate interdisciplinary technologies for minimizing mycotoxins in maize and groundnuts along the value chain should be adopted and used during farming, transportation, storage, and proper handling during marketing of cereals.

iv. Mothers and care givers as well as dealers of CBCF-F should be trained by health workers on good manufacturing practices of cereals that reduce mycotoxins; moreover, they should be advised to be keen in selecting good quality raw material for complementary foods. Together with these ingredients for CBCF-F prior to storage should be well dried and sorted out with infected grain. Furthermore, the store and storage facilities should be in good environment that hinders growth of AF and FM and other mycotoxins. Therefore, these findings suggest that cereals for processing CBCF-F should be treated as foods for special vulnerable groups of people and be put separate from other cereals during storage time and also proper storage practices such as proper drying of cereals (< 13% moisture content), sorting of infected and defective grain before storage should be followed. Finally, moisture free environment accompanied by low temperature of storage facilities

that hinders growth of mycotoxins and respiration activities of other vector insects should be followed.

v. Another research should be employed to check on the accuracy of nutritive-values and ingredients used in making CBCF-F subject to mycotoxins occurrence among the homemade and commercialized CBCF-F.

6.2.3 Awareness and perception on issue related to AF and FM in cereals

i. Agricultural extension officers are the ones who advise farmers on good agricultural practices (GAP); so the government should train them on AF and FM and other mycotoxins contamination in cereals and how to mitigate them. Furthermore, they should be given package of GAP that they should be promoting to rural & urban farmers for implementation in Tanzania. If adopted by farmers, GAP would minimize mycotoxins in food chain and reduce food losses related to fungi and mycotoxins contamination.

ii. The government and stake holders of mycotoxins should provide awareness raising, creation and sensitization to the whole public on the occurrence of AF and FM and other mycotoxins in cereals, and their negative impacts to human health and economy should be implemented. Furthermore, the public should be sensitized on the circle chain of mycotoxins from animal and animal products to human being such as meat, eggs, and milk.

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APPENDICES

Questionnaire No…….

Appendix 1: Processors' questionnaire for the study on aflatoxin and fumonisin contamination in homemade and commercial blended cereal based complementary food with formula in Morogoro Municipality- Tanzania

Name of product …….....Tell phone No……. GPS……..

Name of Enumerator……
Interview Date …./…./2016…

SECTION A: BACKGROUND INFORMATION

1. Sex of respondent …….
2. Age of respondent …….
3. Ward-product purchased……live-processor ……..
4. Processing experience (in years)……
5. Education level of Processer / caregiver (indicate by putting tick)
    1. = No formal education( )
    2. = Adult education ( )
    3. = Primary education
    4. = Secondary education
    5. = certificate education ( )
    6. =diploma ( ) 7= Other (Specify) ( ) …….
6. (a) Are you involved in a processing group? Yes..... No .....
   (b) How many people or family members are involved in processing of CBCF-F ? .....
7. Do you have a tap water at home which provides water every day? Yes/No
8. (a) Do you live in a rental house? Yes/No
    1. (b)If yes to (1) part of rooms (2)whole house

PART B: Perception about aflatoxin and fumonisins contamination in materials used for process of CBCF-F

9. Have you ever heard about mycotoxins contamination in food staff? Yes / No
10. Specifically Aflatoxin……..Yes / No and Fumonisin…….. Yes / No
11. If Yes, mention 2 food staffs which are likely to be contaminated by
    a) Aflatoxin ……, ……
    b) Fumonisin ……, ……
12. Can you identify presence of mycotoxin in cereals by your naked eyes?
    Yes ( ) No ( ) Don't know ( )
13. If Yes, which features will you ascertain about the presence of mycotoxin contamination in the food materials. ……, ……., …….
14. Which food groups among the following ones do you think favours most the growth of aflatoxin and fumonisin?
    i) Maize, groundnuts and cash-nuts, ii) Millet, finger millet and sorghum, iii) Soya-beans, green pees and simsim, vi) None of the above v) Don't know
15. Which conditions among the following ones do you think favours growth of aflatoxin and fumonisin in food-stuffs?
    i) Moisture and high temperature, ii) Dust and very cold condition (-21^{o}C), iii) all of the above, iv) Don't know.
16. Can aflatoxin and fumonisin contamination in food-stuffs contribute to
    a) Growth failure/stunting?…Yes/ No/ Don't know
    b) Impairs immunity?…Yes/ No/ Don't know
    c) Abdominal pain and diarrhea? …Yes/ No/ don't know

Part C: practice

17. Where did you get knowledge about processing of this *unga wa lishe*?
    (a)Through experience, (b) Neighbors, (c) Health officer-Munispal, d) NGOs, (e) SIDO, (f) Others (specify) ……
18. Have you ever attended a seminar / work shop concerning processing and handling of *unga wa lishe*? (a) Yes (b) No
19. If Yes, where (Mention)……
20. Was aflatoxin and fumonisin contamination part of that training you attended? Yes/No

21. Where do you get materials for processing of this composite flour/*unga wa lishe*?
    (a) Purchase from Morogoro markets, (b) Import from other district, (c) Own produces, (d) in shops.
22. Do you store your cereals? Yes / No. Which one? ……. ……,…….
23. If Yes, Please specify for how long you store cereals before using them for processing them into composite flour? . …….
24. Where do you store your cereals/ ingredients ……
25. In order to make your product safe and of good quality, what do you normally do? Please mention all procedures involved in the process of your *Unga wa lishe* e.g. winnowing, sorting, ……, ……, ……..…….. ……., ……..,……..,…….
26. Where do you dry your materials? .....
27. Do you use dehulled maize? (1) Yes, (2) No
28. Do municipal council health officers come to check your products if they are within the recommended standards? (1) Yes, (2) No
29. If yes How many times per a year? .....
30. (a) What materials /ingredients do you use in making of this product?
    ……
    ……
    ……
    (b) How many kilograms per each of the ingredients mentioned above do you use in blending your products
    ……
    ……
    ……
    ……
    ……
    (c) Why do you think it is important to blend those materials before milling them?
    ……
31. How many bags (1kg capacity) do you make per one batch? …….
32. What materials do you use to pack / store this product for use? .....
33. How long does it take to finish selling the banch produced? …….
    Or (**For homemade**) how long does it take to finish up this product?.....

34. Who are the primary user of this product? ……
    a) Children under five years of age, b) Children under five years of age, sick people like those infected of HIV and pregnant mothers, c) Others (*Specify*)……..
35. Why do you think those are the groups of people that should use this product? …….
36. Have you ever seen molds/ harz like materials in food-stuffs like those seen in these pictures?
37. What assumptions did you get about the presence of those materials in food-stuffs?
    i) Just dust present on food non harmful to human being ii) Just disease of crops not affecting human beings iii) Just colour / decayed food which are non-harmful to human being iv) Others (*specify*) ……
38. What do you normally do when you find the whole materials are contaminated of those mold?
    i) Continue with usual processing because it is non harmfully to human being ii) you leave it for other use at home, iii) you give it to animals such as Pigs, Cow and chickens, iv) You sell to someone else, v) other, (If other Please specify) ……
39. (**For commercial**) Are you registered under Morogoro Municipal to run this business? Yes / No / other (Specify) ……..

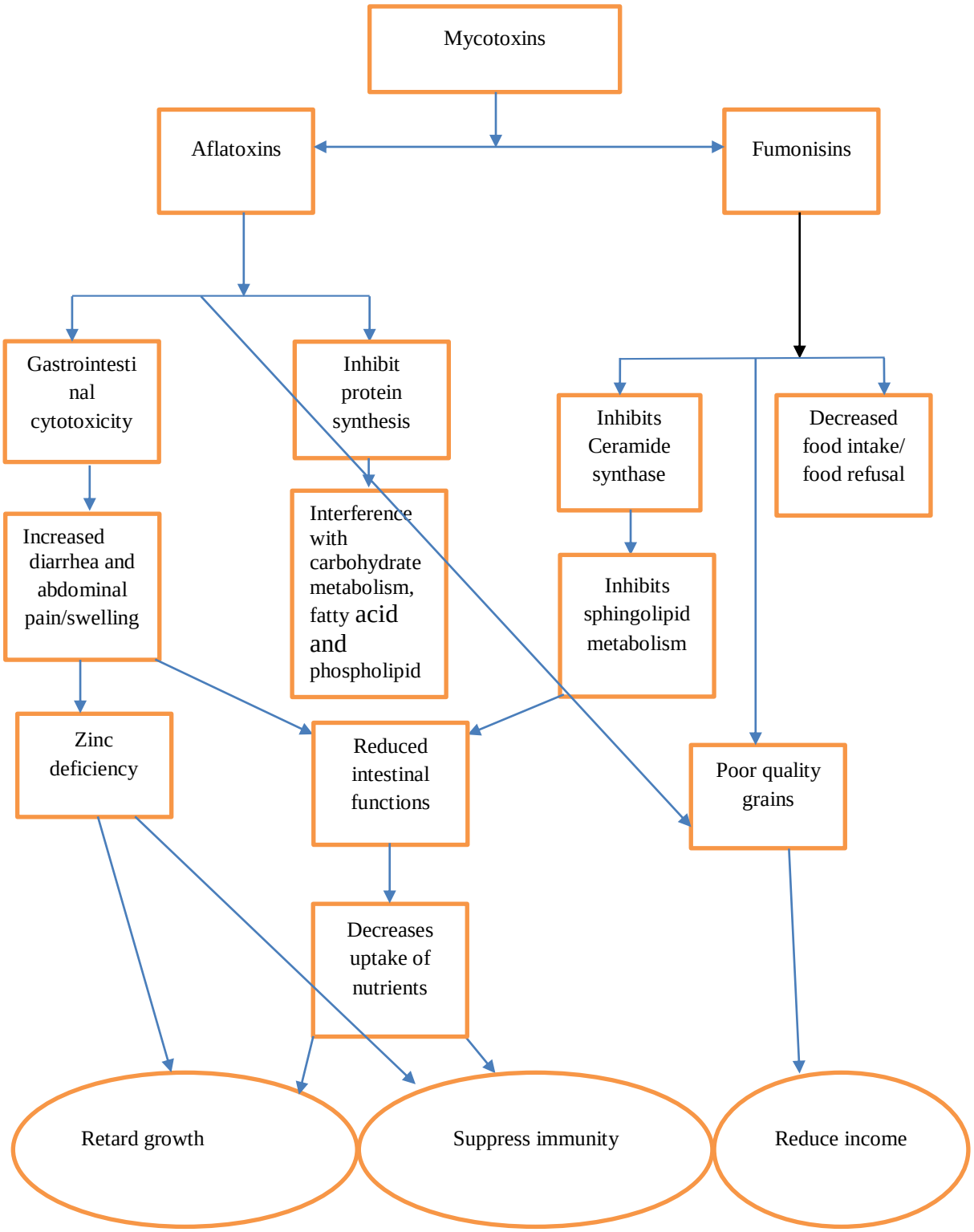
    N.B

    Mark where the product were found; eg. On shelf in supermarket /on the open place under the sun? .....

THANK YOU VERY MUCH FOR BEING COOPERATIVE!!!!!!!

Appendix 2: Conceptual frame work

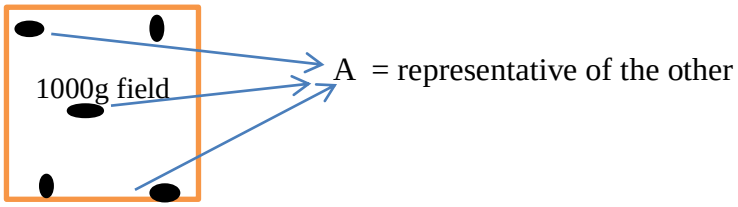
The conceptual frame work is built on the hypothesis that chronic exposure to mycotoxins specifically aflatoxins and fumonisins contribute to growth failure/stunting and immunity impairment in human beings.



Source: Modified from Smith *et al*. (2012)

Appendix 3: Sampling procedures

Procedure i) sample for each respondent of HM was taken from different part of the container at the milling machines as shown below;



Container containing CBCF-F

Appendix 4: Sampling protocol according to IITA (2009)

1. Briefly and clearly explain the interviewee your intention and reason of collecting from him/her the sample of the crop/flour in question and also request her/his willingness to participate in the research.
2. Fill in the questionnaire provided with all information needed as indicated.
3. Show the colored-printed photographs of crops infected with mycotoxins to the interviewee and ask if he/she has seen such symptoms in his/her crops. Fill in his/her answer on the appropriate space in the questionnaire.
4. See the package(s) of the sampling space and draw sub-samples from each if there are more than one packages of the same lot as will be explained by the interviewee, then mix the sub-samples to have the required quantity of sample.
5. Put the sample in the paper bag (envelope) provided.
6. Correctly, using a pencil write a label by copying the sample code already filled in on the questionnaire on a piece of paper and put this label inside the envelope containing the sample.
7. Roll the envelope containing the sample and the label from the bottom upwards, and when reaching the flap remove the paper tissue from the flap to expose its sticky side, then press the flap on the side of the envelope to hold on and prevent unrolling.
8. Correctly write the same sample code on the roll and wrap on a rubber band.

NOTE: Sample code writing on the questionnaire is very important and labels must be written onto outside of envelope and also onto a piece of paper placed inside the envelope. The label should be made up of the following sequence:

day and month / crop / district / farm code.

The crop names will have to be abbreviated (CA. for cassava, GR. for groundnuts and MA. for maize) but the district names written in full. In the sample code everything will be written in capital letters, for example, 1405/CA/HANDENI/01 where 14 stands for the 14th day and 05 for the month of May, CA for cassava, HANDENI is the name of a district and 01 is the farm code meaning that it was the first farm visited in Handeni District. The next sample collected on the same day in this example will then be 1405/CA/HANDENI/02.

Based on this information Samples of CBCF-F were labeled as follows; for HM as 1405/HM/MAZIMBU/KIHONDA/01 for CLM as 1405/CLM-R/MAZIMBU-MARKET/KIHONDA/02 or 1405/CLM-NR/MAZIMBU-MARKET/ KIHONDA /02 for CFM as 1405/CFM/MAZIMBU-MARKET/KIHONDA/03

Slight modification done means Mazimbu market- where exactly the sample were collected eg Mazimbu milling machine or Mazimbu market, Kihonda- where the processors live, 01 or 02 or 03 corresponding sample number

9. Place the rolled envelope of the sample in the polrique sack and proceed for the next sampling station (household/market).
10. When the polrique sack has accommodated samples of approximately 50kg, tie it up at the 'neck' using sisal rope and pack it in the car. Start another empty sack in the next sampling station.
11. Keep all samples dry in the vehicle and avoid any moisture risk. For this reason a vehicles used in these surveys are preferably station wagons to avoid spoilage by rain if the vehicle is a pick-up.
12. Temporary storage of the samples waiting for dispatch to IITA Dar-es-Salaam should also be done in a moisture-free environment.

13. Dispatch the samples to IITA Dar-es-Salaam, ensuring moisture-free environment.
14. In case the collected sample (especially cassava) is not dry (i.e. feels moist and cool to the touch) the sample must be air-dried. This can be done in either the village or ward or district office room or in the hotel room. This is to avoid keeping moist samples that may develop unwanted microbes and contaminants. This should be done carefully with clear labeling in order to not mix up samples and in a way to prevent spoilage, theft, wind disruption or eaten by animals.

Appendix 5: Images of affected crop products of Aflatoxin in whole-grain/ groundnuts and fumonisins in maize-crop

Source: UNIDO (n.d) for aflatoxin contaminated groundnuts, and sampling protocol according to IITA (2009) for Fumonisins contaminated maize

Appendix 6: Source of knowledge for processing CBCF-F

Knowledge Source	Number of Respondents	Percent
Own initiative	25	42
Neighbours	16	27
Municipal Health Officer	3	5
NGOs	11	18
SIDO	5	8
Total	**60**	**100**