

**THE INFLUENCE OF AWARENESS, KNOWLEDGE AND PRACTICES OF
COMMUNITIES ON CHILDHOOD DIETARY EXPOSURE TO AFLATOXINS IN
CENTRAL REGIONS OF TANZANIA**

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

Complementary foods in Tanzania are heavily contaminated with aflatoxin (AFs), a group of highly toxic metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Many people and especially children largely depend on consumption of cereal-based diets as complementary foods. Unfortunately, cereals and nuts have been associated with aflatoxin contamination where conditions favour growth and proliferation of the causative moulds. This study was conducted with an overall objective to examine the influence of awareness, knowledge and actions of communities on childhood dietary exposure to aflatoxins in Dodoma and Singida regions. The specific objectives were: (i) to assess communities' level of awareness, knowledge, attitude and perceptions of aflatoxins, (ii) to identify practices that contribute to levels of contamination of aflatoxins in these areas (iii) to identify local barriers and practices associated with reducing aflatoxins contamination in complementary foods in the community (iv) to determine complementary feeding practices among infants aged between 6-23 months and (v) to determine the level of aflatoxins in ready-to-cook foods used in complementary feeding. A descriptive study of the awareness, attitude, perception and actions of communities towards aflatoxin contamination in complementary foods and its health risks was conducted in Bahi and Chamwino districts in Dodoma Region; and Manyoni and Ikungi districts in Singida Region, central Tanzania. The districts were chosen as they represented the semi-arid condition which is characterized by high temperature during the day up to 35°C and cool to 10°C during the night. Both temperature and humidity favour growth of fungi which indicate possibility of aflatoxin production. The respondent's level of awareness of aflatoxin contamination in crops used in preparation of complementary foods, and health risks associated with its ingestion was assessed. A suitable scale was developed used to measure awareness of aflatoxins contamination in complementary foods

and its health effects in the community of the children aged 6-23 months, and Likert-type scale was used to rate respondent's attitude and perceptions towards aflatoxin contamination in complementary foods and its managements. Health Belief Model (HBM) was used to determine parents' perception and attitude towards aflatoxins contamination and control. Exploratory Factor Analysis identified the underlying constructs of the Health Belief Model (HBM). Data were collected using an interview schedule which was administered to 364 randomly selected respondents and 228 ready-to-cook complementary foods samples collected with parents/caregivers of children aged between 6-23 months from households, and focus group discussions (FGDs) with 121 (105 females and 16 males) participants to obtain personal experiences about aflatoxin contaminations in complementary foods and its health effects on the community. The mean age of respondents was of 30 ± 8.3 years and the majority (73.1%) was married. Among 364 respondents, 87.6% were farmers and 70.3% earned less than or equal to US\$ 22.8 per month. The majority of respondents (56%) had primary school education while 0.8% were university graduates. About 81.9% respondents were not aware of aflatoxin contamination and associated health effects, and half of parents who were responsible for preparation of complementary foods had low perception and attitude of harmful effects of aflatoxins contamination to human and animals in general. Fifty seven samples were collected from each of the four studied districts of Dodoma and Singida regions and used to determine aflatoxin (AF) contamination of ready-to-cook complementary foods of the children aged between 6-23 months. Contamination was correlated with levels of awareness, perception and actions of parents towards contamination and control. The Romar's all-purpose method was used for the extraction of total aflatoxins for analysis by HPLC. The total aflatoxins contamination in 228 the ready-to-cook complementary food samples had level up to $60.3\mu\text{g}/\text{kg}$. About 53% samples were contaminated by aflatoxins and 24.1% of the samples exceeding the maximum permissible levels of $10\mu\text{g}/\text{kg}$. Samples with aflatoxin B₁

had level up to 38.2 $\mu\text{g}/\text{kg}$ and 15.4% of all samples were contaminated. Samples that were contaminated, (45.7%) had levels above 5 $\mu\text{g}/\text{kg}$ which is the recommended limit. Manyoni and Chamwino had equal proportions of complementary food samples with aflatoxins B₁ contamination of 19.3%. Ikungi district had the least prevalence of aflatoxins B₁ (7%). The results of the Univariate analysis showed that, awareness, knowledge, perception, attitude and dehulling were all significantly associated with aflatoxins (AFB₁) contamination levels ($p < 0.05$). The multiple logistic regression model of awareness of aflatoxins B₁ and the chance of having food with aflatoxins B₁ was significantly higher among respondents not aware of aflatoxins contamination (OR=2.929, $p=0.015$). People with no knowledge of aflatoxins (OR =2.739, $p=0.019$) had significantly greater odds of having food contaminated with aflatoxins B₁ in comparison to people with knowledge of aflatoxins. The odds of having food with aflatoxins B₁ contamination for individual with less than 22.23 perception score was almost 3 times that of people with at least 22.23 perception score (OR =3.101, $p=0.022$). Those respondents with less than 22.23 perception score toward aflatoxins contamination were significantly more likely to have food with aflatoxins B₁ contamination. The risk of having ready-to-cook complementary foods contaminated with aflatoxins B₁ were also found to be significantly higher among respondents not dehulling the crops used to make children's food than those who dehulled grains (OR=2.763, $p=0.028$). It might be concluded that considerable differences in contamination level were observed among the four districts. Parents in the four districts practiced similar post harvest actions though with varying degrees. Most processing activities like dehulling, milling, drying, and storage showed significant association with aflatoxins contamination of complementary foods. Due to food shortage and low knowledge or awareness on fungal and aflatoxins contamination in the study areas, the respondents sometimes ate undehulled, unsorted and mouldy crops without washing or winnowing them, hence exposing themselves to high health risks of aflatoxin

contamination in their diet. Thus, it is recommended that an aggressive campaign on the use of best agricultural practices in pre- and post-harvest activities at the household level should be emphasized. There is an urgent need to raise awareness and educate parents/caregivers on aflatoxin health risks associated with complementary foods and the appropriate strategies to minimize contamination. In addition, research on climatic change and mycotoxin production during pre and post harvest practices such as tillage, breeding varieties resistance to mycotoxin, harvesting time, drying, storage, and dehulling practices of crops in Tanzania is needed in order to improve food safety.

LIST OF PUBLICATIONS

- i. Ngoma, S., Tiisekwa, B., Mwaseba, D. and Kimanya, M. (2016). Awareness of Aflatoxin Health Risks among Parents with Children Aged Between 6-23 Months in Central Tanzania. *International Journal of Nutrition and Food Sciences*. Vol. 5, No. 6, 2016, pp. 429-436. doi: 10.11648/j.ijnfs.20160506.19.
- ii. Ngoma, S., Tiisekwa, B., Mwaseba, D. and Kimanya, M. (2016). Parents' Practices Associated with Aflatoxin Contamination and Control of Complementary Foods in Central Tanzania. *Journal of Food and Nutrition Sciences* Vol. 4, No. 6, 2016, pp. 152-161. doi: 10.11648/j.jfns.20160406.13.
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DECLARATION

I, Selestin Joseph Ngoma, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is the result of my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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DEDICATION

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LIST OF ABBREVIATIONS AND ACRONYMS

AF	Aflatoxin
AF-alb	Aflatoxin albumin
AFB1	Aflatoxin B ₁
AFB2	Aflatoxin B ₂
AFG1	Aflatoxin G ₁
AFG2	Aflatoxin G ₂
AFs	Aflatoxins
AIDS	Acquired Immunodeficiency Syndrome
BLM	Baseline Logit model
CAST	Council for Agricultural Science and Technology
CD4	Cluster of Differentiation 4
CDC	Center for Diseases Control
DED	District Executive Director
EAC	East Africa Community
EU	European Union
FAO	Food and Agriculture Organization of United Nations
FB ₁	Fumonisin B1
FBs	Fumonisin
FDA	Food and Drugs Authority
FGD	Focus Group Discussion
FLD	Fluorescence Detection
HBM	Health Belief Model
HBV	Hepatitis B virus
HCC	Hepatocellular Carcinoma

HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
Ibid	In the same quotation/place mention above
IITA	International Institute of Tropical Agriculture
ISO	International Standard Organisation
JECFA	Joint Expert Committee on Food and Additives
LOD	Limit of Detection
LOQ	Limit of Quantification
Mg/Kg	Milligram per Kilogram (ppm)
MTL	Maximum Tolerable Limits
N	Sample size
NBS	National Bureau of Statistics
NM-AIST	Nelson Mandela African Institution of Science and Technology
OAS	Organization of American States
Op cit	In the Citation above
PI	Principal Investigator
SE	Standard Error
SPSS	Statistical Package of Social Sciences
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standard
UK	United Kingdom
UNAIDS	United Nation of HIV/AIDS
UNDP	United Nations Development Programme
UNICEF	United Nations International Children's Emergency Fund

URT	United Republic of Tanzania
US\$	United State Dollar
USA	United States of America
USAID	United States Agency for International Development
USDA-ARS	Southern Regional Research Center of the United States
UV	Ultra Violet
WHO	World Health Organisation
µg/Kg	Microgram per Kilogram (ppb)

THESIS ORGANISATION

The thesis is composed of seven chapters; chapter one contains introduction, problem statement and justification, objectives and study areas. Discussion and details of the main objectives are organized in the manuscripts in the form of chapters starting from chapter two.

Chapter two addresses the levels of awareness of parents or caregivers on aflatoxin contamination in complementary foods and its health effects. Specifically this chapter was designed to assess the levels and factors of awareness of aflatoxin health risks among parents or caregivers with children aged between 6-23 months in Central Tanzania (Manuscript I published).

Chapter three determines parents' perception and attitude of aflatoxins. The objective being to assess perception and attitude towards aflatoxins contamination in child foods and its management among 364 parents with children aged between 6-23 months in central Tanzania (Manuscript II published).

Chapter four investigates the parents' practices that contribute to aflatoxin contamination and control in complementary foods in Central Tanzania (Manuscript III published).

Chapter five assesses parents' local barriers and actions associated with reducing aflatoxins contamination in complementary foods among parents with children aged between 6-23 months in Central Tanzania (Manuscript IV).

Chapter six provides an analysis of aflatoxin levels in household ready-to-cook complementary foods and to correlate with parents' awareness, perception and actions of aflatoxin contamination and control in Bahi and Chamwino Districts in Dodoma Region, and Manyoni and Ikungi Districts in Singida region (Manuscript V).

CHAPTER ONE

1.0 INTRODUCTION

Aflatoxins are a major source of disease outbreaks due to a lack of awareness, knowledge and consumption of contaminated food and feed worldwide (Kumar *et al.*, 2017). The level of aflatoxin awareness in many developing countries is extremely low or non-existent altogether (Ephrem, 2015). Narrod *et al.* (2011) associated the lack of awareness, inadequate knowledge about aflatoxins contamination with the high rate of exposure to aflatoxins. Jolly *et al.* (2009) demonstrated that education on risk factors of aflatoxins among people had an influence on aflatoxin reduction in the communities. In Ghana, women had high levels of awareness and knowledge on aflatoxins than men and that education levels played a great role in addressing the problem (Ibid). Aflatoxins are metabolites produced by moulds *Aspergillus flavus* and *Aspergillus parasiticus* (Wild and Gong, 2010) and, are among the most potent carcinogens found in human and animal foods (IARC, 1993; 2002).

In developing countries, many people depend on consumption of largely cereal-based diets (Wu *et al.*, 2011) that are deficient in other essential nutrients. This practice may contribute to nutritional deficiency especially growth failure in children who are exposed to high level of mycotoxins in their complementary foods (Gong *et al.*, 2008). More importantly, consumption of foods containing high levels of aflatoxins has been associated with liver failure, weakened immune systems and rapid death (Williams *et al.*, 2004; Turner *et al.*, 2007). In the year 2004, 317 people including children became ill and 125 of them died in the central provinces of Kenya as a result of consuming contaminated food (CDC, 2004; Azziz-Baumgartner *et al.*, 2005; Strosnider *et al.*, 2006). Contamination is often unavoidable because many African countries do not test crops for aflatoxins, have

low level of awareness and knowledge of aflatoxins which as a result, leads to consumption of contaminated foods (Azziz-Baumgartner *et al.*, 2005; Kimanya *et al.*, 2008). Infection of crops with aflatoxins producing fungi is most common in the tropical regions in which humidity and temperature are high (IARC, 1993; Schmale *et al.*, 2012; Kamala *et al.*, 2016).

Over 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Shephard 2003; Williams *et al.*, 2004). Aflatoxin contamination occurs in crops during plant growth, maturation, harvesting and processing of grains (Cotty and Ramon, 2007; Cotty *et al.*, 2008). Fungi infection can be induced when maturing corn is under drought conditions and during prolonged periods of hot weather (Cotty and Ramon, 2007; Ncube *et al.*, 2010). Contamination during storage of the crop can occur if moisture and relative humidity, oxygen availability, damaged or broken grain kernels are allowed to go beyond critical values (Lanyasunya *et al.*, 2005; Ncube *et al.*, 2010). Crops grown in warm climates conditions have greater chances of infection by toxin moulds and in some areas, infection occurs only when temperatures rise in connection with drought (Cotty and Ramon, 2007). A climatic change influences not only the amount of aflatoxins, but also the types of aflatoxin producers present in the area (Op.cit). Thus, drying, proper storage and suitable transportation are of prime importance in prevention of aflatoxins (Williams *et al.*, 2004).

The wide range of food products which are contaminated with aflatoxins include cereals like maize, sorghum, pearl millet, rice and wheat, oilseeds such as groundnuts, soybean, sunflowers and cotton, spices like chillies, black pepper, coriander, turmeric and zinger; tree nuts such as almonds, pistachio, walnuts and coconuts; and milk and milk products (CAST, 2003; Jolly *et al.*, 2009). The highest concentration of aflatoxins is often found in

nutritive seeds such as maize, nuts and some cereals grains in Africa and, rice in China and Southeast Asia because millions of people use these commodities as the primary source of carbohydrates (Bennett and Klich, 2003; WHO, 2000; Otsuki *et al.*, 2002; Kitya *et al.*, 2010). Maize is probably the commodity of greatest worldwide concern, because it is grown in climates that are likely to have perennial contamination with aflatoxins, and is the staple food of many countries (Kimanya *et al.*, 2010; Marechera and Ndwiga, 2014). Most of the people living in rural areas use local products in preparation of complementary foods, mainly cereals like maize, and groundnuts. These come with an added risk of exposure to aflatoxins (WHO, 2006).

Aflatoxins exposure through food poses challenges in pre- and post-harvest handling of crops such as lack of data on prevalence of aflatoxins in foods, absence of surveillance programmes, co-occurrence of aflatoxins with other mycotoxins, lack of regulations and inadequate enforcement, lack of consumer education programmes, lack of qualified personnel and increased susceptibility in the community due to other infections like HIV/AIDS (Ibid).

Awareness of the potential danger posed by aflatoxins contamination of foodstuffs and the basic knowledge of aflatoxin were extremely low among communities in Kenya and Mali (Narrood *et al.*, 2011). Dietary exposure to aflatoxin B₁ has been identified as a major etiological risk factor for the development of hepatocellular carcinoma (Bbosa *et al.*, 2013). Therefore, from the foregoing, aflatoxins risks can be reduced through awareness campaigns (CDC, 2004; Strosnider *et al.*, 2006).

The study done by Kamala *et al.* (2015) has looked at multiple mycotoxin contamination in stored maize in rural Tanzania which also used as an ingredient in complementary foods. Results from the mentioned study indicated high levels of AFs and FBs among

other mycotoxins with the co-occurrence in 45% of samples. AFs and FBs were detected in 50% at levels up to 1081 µg/kg and 73% at levels up to 38 217 µg/kg, respectively. Other studies in the country have reported occurrence of these toxins at important levels (Kimanya *et al.* 2008, 2009). In addition, studies in the country have reported high exposure of infants and young children to AFs and FBs through maize based diet (Shirima *et al.* 2014; Magoha *et al.* 2014c) and AFM₁ (Magoha *et al.* 2014b) and FB₁ (Magoha *et al.* 2014a) through breast milk from mothers whose predominant diet is maize.

The study examined existing aflatoxin levels in household ready-to-cook complementary foods, level of community's awareness, attitude and perception of aflatoxins contamination in food and, the actual knowledge gap of the communities with regard to reduction of aflatoxins in complementary foods contamination. Also, the relationship between awareness, knowledge, perception and attitude of communities on aflatoxins contamination were analyzed. Factors affecting aflatoxins reduction by the communities were analysed in detail and were found to be obstacles in improving aflatoxins reduction. Therefore, necessary information to formulate strategies for increasing level of awareness, knowledge, perception and attitude about aflatoxin contamination in the complementary food is compulsory in central regions of Tanzania.

1.1 Description of Key Concepts

1.1.1 Aflatoxins

Aflatoxins are poisonous carcinogens that are produced by certain moulds (*Aspergillus flavus* and *Aspergillus parasiticus*) which grow in soil, decaying vegetation, hay, and grains. These are a group of structurally related polyketide mycotoxins that contaminate field crops such as groundnuts, maize, cottonseed, cassava, cashew nuts, sorghum, millet and rice (Williams *et al.*, 2004; Strosnider *et al.*, 2006; Liu *et al.*, 2012). Contamination

starts in the field and is exacerbated when crops are damaged by drought or insect infestation, or when the produce comes into contact with soil and is not properly dried (Wild and Gong, 2010; Kew, 2013). The most toxic of the aflatoxins is B₁, which is poorly degraded in the rumen and it is quickly excreted in milk as the metabolite aflatoxin M₁ (Strosnider *et al.*, 2006). The aflatoxins produced by *Aspergillus spp.* are proven carcinogens, immunotoxins which cause growth retardation in animals (IARC, 1993; Raisuddin *et al.*, 1993 and Hall and Wild, 1994).

1.1.2 Aflatoxicosis

The aflatoxicosis is the disease resulting from exposure of humans or animals to aflatoxins. The response of humans and animals to aflatoxins exposure is related to the rate of the metabolism and type of metabolites being produced which can result in acute, chronic or sub-chronic forms (Riley *et al.*, 2011).

1.1.3 Awareness

Awareness is the state or ability to perceive, to feel, or to be conscious of events, objects, or sensory patterns. In this level of consciousness, sense data can be confirmed by an observer without necessarily implying understanding. More broadly, it is the state or quality of being aware of something. In biological psychology, awareness is defined as a human's or an animal's perception and cognitive reaction to a condition or event (Barroso and Llobet, 2012).

1.1.4 Knowledge

According to Webster's Dictionary, knowledge is "the fact or condition of knowing something with familiarity gained through experience or association". However, in practice, there are many possible, equally plausible definitions of knowledge. A frequently

used definition of knowledge is "the ideas or understandings which an entity possesses that are used to take effective action to achieve the entity's goal(s). This knowledge is specific to the entity which created it." However, recognition of the difficulties inherent in transferring knowledge from one person to another has tended to highlight the importance of implied knowledge (Polanyi, 1975; Nonaka and Takeuchi, 1995).

1.1.5 Perception

This is a process by which people translate sensory impressions into a coherent and unified view of the world around them. Though necessarily based on incomplete and unverified (or unreliable) information, perception is equated with reality for most practical purposes and guides human behaviour in general (Reber and Perrig, 2001). Perception is our sensory experience of the world around us and it involves both the recognition of environmental stimuli and actions in response to these stimuli. Through the perceptual process, we gain information about properties and elements of the environment that are critical to our survival. Perception not only creates our experience of the world around us; it also allows us to act within our environment. Perception includes the five senses; touch, sight, taste, smell and taste. It also includes what is known as proprioception, a set of senses involving the ability to detect changes in body positions and movements. It also involves the cognitive processes required to process information, such as recognizing the face of a friend or detecting a familiar scent (Pentz and Gerber, 2013). Perception is mental organization and interpretation of sensory information. Costanzo *et al.* (1969) concluded that people base their perceptions on experience and knowledge.

1.1.6 Attitude

Attitude is a predisposition or a tendency to respond positively or negatively towards a certain idea, object, person, or situation. Attitude influences an individual's choice of

action and responses to challenges, incentives and rewards (together called stimuli) (Chaiklin, 2011). There are four major components of attitude which are: (1) Affective: this has to do with emotions or feelings. This involves a person's feelings and/or emotions about the attitude object. For example: "I am scared of spiders". (2) Cognitive: belief or opinions held consciously. This involves a person's belief and/or knowledge about an attitude object. For example: "I believe spiders are dangerous". (3) Conative/behavioural: This has to do with inclination for action. That is, the way the attitude we have influences how we act or behave. For example: "I will avoid spiders and scream if I see one". (4) Evaluative: This has to do with positive or negative response to stimuli (Dahlberg *et al.*, 2005; Chaiklin, 2011). An attitude is a relatively enduring organization of beliefs, feelings, and behavioural tendencies towards socially significant objects, groups, events or symbols (Hogg and Atkinson, 2005). It is a psychological tendency that attitude is expressed by evaluating a particular entity with some degree of favour or disfavour (Eagly and Chaiken, 1993).

1.1.7 Complementary feeding practices

Complementary feeding is defined as the process starting when breast milk is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed along with breast milk. The target range for complementary feeding is generally taken to be 6 to 23 months of age, (WHO, 2004) even though breastfeeding may continue beyond two years (WHO, 2009).

1.1.8 Complementary feeding

Complementary feeding is the process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants and therefore other foods and liquids are needed along with breast milk (WHO, 1999).

1.1.9 Complementary feeding period

This is the period when an older infant (6 to 12 months) and young child (12 to 36 months) transitions from exclusive breastfeeding and/or breast milk substitutes to feeding on the family diet (FAO, 2011).

1.1.10 Ready-to-cook food

These are foods prepared ready to be cooked for the consumption of the family.

1.1.11 Food frequently consumed

These are foods which are often consumed in the family.

1.1.12 Exclusive breastfeeding

Exclusive breastfeeding means that an infant receives only breast milk from his or her mother or a wet nurse or expressed breast milk and no other liquids or solids, not even water with the exception of oral rehydration solution, drops or syrups consisting of vitamins, minerals supplements or medicines (WHO/UNICEF/USAID, 2008).

1.1.13 Local barriers to aflatoxins reduction

These are the norms or and obstacles which are in the society which may hinder minimization of aflatoxins in food or crops.

1.1.14 Practices to aflatoxins reduction

These are local practices like hygiene which are in the community that promote aflatoxins reductions in crops or food.

1.1.15 Quantitative research

Quantitative research is an inquiry into an identified problem based on testing a theory and measured with numbers and analyzed using statistical techniques. The goal of quantitative method is to determine whether the predictive generalizations of a theory hold true (Creswell, 1994).

1.1.16 Qualitative research

This is study based upon a qualitative process of inquiry which has the goal of understanding a social or human problem from multiple perspectives (Mason, 1996). Qualitative research is conducted in a natural setting and involves a process of building a complex and holistic picture of the phenomenon of interest (Creswell, 1994).

1.1.17 Health belief model (HBM)

The Health belief model (HBM) is a psychological model that attempts to explain and predict health behaviours. The model is most commonly used in health education and health promotion and it focuses on the attitudes and beliefs of individuals (Glanz *et al.*, 2002). The HBM was first developed in the 1950s by social psychologists working in the United States Public Health Services in response to the failure of free tuberculosis (TB) health screening programme (Hochbaum, 1958). The Health Belief Model is a Conceptual Framework used to understand health behaviour and possible reasons for non-compliance with recommended health action (Becker and Rosenstock, 1984). It provides guidelines for programme development allowing planners to understand and address reasons for non-compliance. It is used to guide the design of interventions to enhance compliance with preventive procedures (Janz *et al.*, 2002).

1.2 Aflatoxins

Aflatoxins are among the most potent and dangerous groups of worldwide mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Strosnider *et al.*, 2006; Wu *et al.*, 2011; Michele, 2011). It is estimated that more than five billion people in developing countries are at risk of chronic exposure to aflatoxins through contaminated foods (Williams *et al.*, 2004; Strosnider *et al.*, 2006). The *A. flavus* and *A. parasiticus* are soil-borne moulds that grow on living and decaying plant matter. *Aspergillus* is a mould that essentially belongs to grains storage flora. It grows optimally at 25 °C with a minimum necessary water activity of 0.75. It starts to produce secondary metabolites at 10-12 °C, but the most toxic ones are produced at 25°C with a water activity of 0.92 (Liu *et al.*, 2017). Those toxic secondary metabolites named aflatoxins (AF) are a group of mycotoxins produced by a large number of *Aspergillus* species, basically by three phylogenetically distinct sections. The main producers are *A. flavus* and *A. parasiticus*, but it has been verified that *A. nomius*, *A. pseudotamarii*, *A. parvisclerotigenus*, and *A. bombycis* of section *Flavi*, *A. ochraceoroseus* and *A. rambellii* from section *Ochraceorosei* and *Emericella astellata* and *E. venezuelensis* from *Nidulatan*s section also generate aflatoxins (IARC, 2002; Frisvad *et al.*, 2005). All of them contaminate a large fraction of the world's food including maize, rice, sorghum, barley, rye, wheat, peanut, groundnut, soya, cottonseed and other derivative products made from these primary foodstuffs in low-income countries (Rizzi *et al.*, 2003; Saleemullah *et al.*, 2006; Strosnider *et al.*, 2006; Masoero *et al.*, 2007; Caloni and Cortinovis, 2010).

1.2.1 Type of aflatoxins

There are four main types of aflatoxins: B₁, B₂, G₁, and G₂ because of their blue colour (B) and green (G) fluorescence under ultraviolet light respectively, based on structure, chromatographic and fluorescent characteristics. Aflatoxin B₁ is considered the most toxic

and is produced by both *A. flavus* and *A. parasiticus* which are the most common in the tropical regions (NTP, 2011). Aflatoxin G₁ and G₂ are produced exclusively by *A. parasiticus*. Aflatoxin M₁ is a metabolite of aflatoxin B₁ in humans and animals. Aflatoxin M₂ is a metabolite of aflatoxin B₁ in milk of cattle fed on contaminated foods (NTP, 2011; Kumar, 2018).

Table 1.1: Chemical and physical properties of aflatoxins

Aflatoxin	Molecular formula	Molecular weight
B1	C ₁₇ H ₁₂ O ₆	312
B2	C ₁₇ H ₁₄ O ₆	314
G1	C ₁₇ H ₁₂ O ₇	328
G2	C ₁₇ H ₁₄ O ₇	330
M1	C ₁₇ H ₁₂ O ₇	328
M2	C ₁₇ H ₁₄ O ₇	330

Source: Syed *et al.*, 2013.

1.2.2 Chemical structure of the different aflatoxins

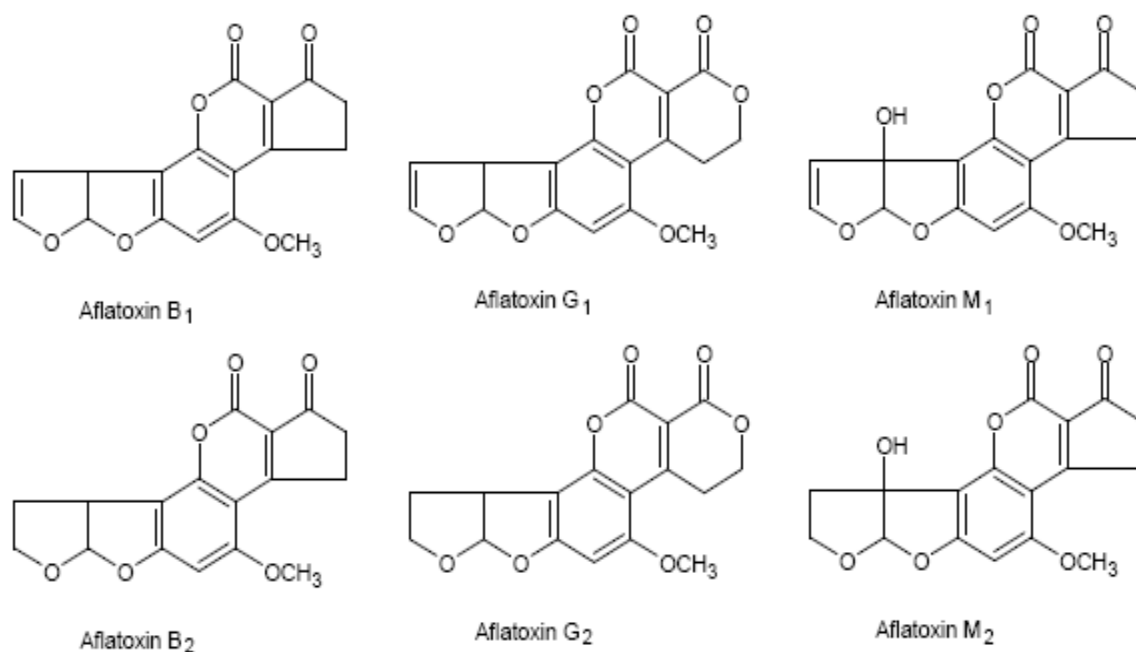


Figure 1.1: Chemical structures of major dietary aflatoxins namely aflatoxin B₁, G₁ and M₁ with the double bonds in 89 positions and aflatoxins B₂, G₂ and M₂ without the double bond.

Source: Syed *et al.*, 2013

1.2.3 *Aspergillus*

The *A. flavus* is a mould. In nature, *A. flavus* is capable of growing on many nutrient sources. It is a saprophyte and grows on dead plant and animal tissue in the soil (Yu *et al.*, 2005). *A. flavus* can also be pathogenic on several plant and animal species including humans and domestic animals (Payne, 1998). The fungus can infect seeds of corn, peanuts, and cotton and nut trees. The fungus can often be seen sporulating on injured seeds such as maize kernels, groundnuts and sorghum (Bennett, 2009). Growth of the fungus on a food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound (Payne and Brown, 1998). *A. flavus* is also the second leading cause of aspergillosis in

humans (Amaike and Keller, 2011). Patients infected with *A. flavus* have reduced or compromised immune systems (Richard and Payne, 2003 and Amaike and Keller, 2011).

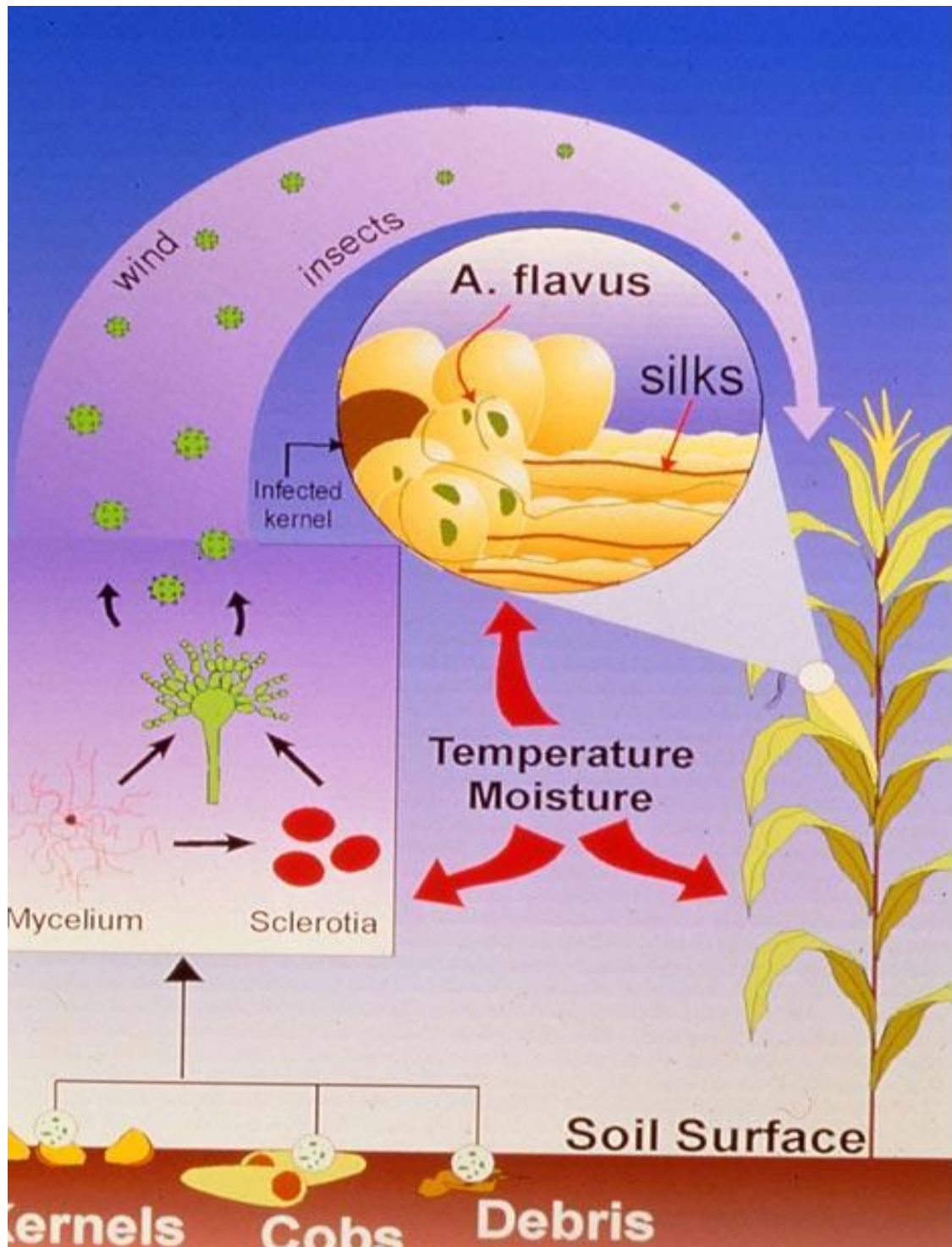


Figure 1.2: Life cycle of *Aspergillus flavus* on maize

Source: Scheidegger and Payne, 2003

Life cycle of *Aspergillus flavus*

The epidemiology of *A. flavus* differs depending on the host species. Fig 1.2 above shows the life cycle of the *A. flavus* on maize. The fungus grows in the soil either as mycelium or as resistant structures known as sclerotia. The sclerotia either germinate to produce additional hyphae or they produce conidia (asexual spores), which can be dispersed in the soil and air. These spores are carried to the maize ears by insects or wind where they germinate and infect maize kernels (Diener *et al.*, 1987; Hedayati *et al.*, 2007). Unlike most fungi, *A. flavus* is favoured by hot dry conditions. The optimum temperature for growth is 37°C, but the fungus readily grows between the temperatures of 25 and 42°C and will grow at temperatures from 12 to 48°C. Such a high temperature optimum contributes to its pathogenicity on humans (Scheidegger and Payne, 2003).

1.2.4 Mycotoxins in Africa

The food-borne mycotoxins are of great importance in Africa and other parts of the world. The impact of such toxins on human health, animal production and economy has attracted worldwide attention (WHO, 2006). Many African countries had set up prevention, control and surveillance strategies to reduce the incidence of mycotoxins in foods. The available information on the incidence, public health importance, prevention and control of mycotoxins in many African countries is still lacking (Darwish *et al.*, 2014). This may be due to limited monitoring systems and failure to adopt preventive and control measures in these countries. The updated and available information on the occurrence, public health importance, prevention and control of mycotoxins in Africa were listed in table 1.2.

Table 1.2: Mycotoxins in the agricultural crops and foodstuffs in different African countries

Country	Mycotoxin	Foodstuffs	Concentr. (ppb)	Reference	
Egypt	Aflatoxins	Cereal grains	36	Motawee <i>et al.</i> , 2009	
		Nuts and seeds	24	El-Tras <i>et al.</i> , 2011	
		Medicinal plants	49		
		Milk	50-270		
		Infant milk formula	9.796		
Tunisia and Morocco	NIV	Cereals and cereals products	135-961	Serrano <i>et al.</i> , 2012	
	Beauvericid	„	2.1-844		
	a	„	5.5-66.7		
	Aflatoxins	„	75-112		
	OTA	„	121-176		
Sudan	Aflatoxins	Sesame oil	0.2-0.8	Idris <i>et al.</i> , 2010	
		Groundnut oil	0.6	El shafie <i>et al.</i> , 2011	
		Peanuts butter	121-170		
Tanzania	FUMs	Maize	11,048	Kimanya <i>et al.</i> , 2008	
	Aflatoxins	Maize	158		
	Af-B ₁	Maize	3-1081		Kamala <i>et al.</i> , 2015
	FUMs B ₁	Maize	16-18,184		
	FUMs-B ₂	Maize	178-38,217		
Zambia	FUMs	Maize	20,000	Mukanya <i>et al.</i> , 2010	
Uganda	Aflatoxins	Maize	0-435	Probst <i>et al.</i> , 2014	
		Groundnuts, cassava, millet, sorghum flour and eshabwe sauce	0-55	Kitya <i>et al.</i> , 2010	
Kenya	Aflatoxins	Animal feed and milk	>5	Kang'ethe and Lang'a 2009	
Kenya(Rift valley)		Maize	>20	Daniel <i>et al.</i> , 2011	
Somalia	Aflatoxins	Maize	0-87	Probst <i>et al.</i> , 2014	
	Aflatoxins	Maize	1-1407	Probst <i>et al.</i> , 2014	
Malawi	Aflatoxins	Maize	0-185	Matumba <i>et al.</i> , 2014	
	FUMs	Maize	493-3303		
Cameroon	Aflatoxins	Maize	0-122	Probst <i>et al.</i> , 2014	
Sierra Leone	Aflatoxins	Maize	2-162	Probst <i>et al.</i> , 2014	

Source: Darwish *et al.*, 2014 and Udomkun *et al.*, 2017.

Table 1.2: Mycotoxins in the agricultural crops and foodstuffs in different African countries continues

Country	Mycotoxin	Foodstuffs	Concentr. (ppb)	Reference
Ethiopia	Aflatoxins	Sorghum, barley, teff and wheat	0-26	Ayalew <i>et al.</i> , 2006
	OTA		54.1-2,108	
	DON	Sorghum, barley and wheat	40-2,340	
	FUM	wheat	2,117	
	ZEA	Sorghum Sorghum Sorghum	32	
Nigeria	Aflatoxins	Rice	28-372	Makun <i>et al.</i> , 2011
	OTA	Rice	134-341	
	Aflatoxins	Weaning food	46-530	Ibeh, 2011
	Aflatoxins	Maize	4-1400	Perrone <i>et al.</i> , 2014
Ghana	Aflatoxins	Maize	4-1400	Perrone <i>et al.</i> , 2014
Benin	Aflatoxins	Maize	5	Hell <i>et al.</i> , 2000
		Chips	2.2-220	Bassa <i>et al.</i> , 2001
Benin, Mali and Togo	Aflatoxins	Dried vegetable (Baobab leaves, hot chill and okra etc)	3.2-6.0	Hell <i>et al.</i> , 2009
South Africa	FUMs	Maize	222-1,142	Burger <i>et al.</i> , 2010
	FUMs	Compound feeds	104-2,999	Njobeh <i>et al.</i> , 2012
	DON	Compound feeds	124-2352	
	ZEA	Compound feeds	30-610	

Source: Darwish *et al.*, 2014 and Udomkun *et al.*, 2017.

The wide range of food products which are contaminated with aflatoxins include cereals like maize, sorghum, pearl millet, rice and wheat, oilseeds such as groundnuts, soybean, sunflowers and cotton, spices like chillies, black pepper, coriander, turmeric and zinger; tree nuts such as almonds, pistachio, walnuts and coconuts; and milk and milk products (CAST, 2003; Jolly *et al.*, 2009). The major commodities affected by aflatoxins are listed in table 1.3.

Table 1.3: Major commodities affected by aflatoxins

Type of aflatoxin	Producer fungal species	Affected commodities
B (B ₁ ,B ₂)	<i>A. flavus</i> , <i>A. parasiticus</i> , <i>A. tamarii</i> , <i>A. pseudotamarii</i> , <i>A. bombycis</i> , <i>A. parvisclerotigenus</i> , <i>A. nomius</i> , <i>A. minisclerotigenes</i> , <i>A. oryzae</i> , <i>A. toxicarius</i> , <i>A. versicolor</i> , <i>A. rambellii</i> , <i>A. arachidicola</i> , <i>A.</i> <i>ochraceoroseus</i> , <i>Emericella</i> <i>astellata</i> , <i>E. venezuelensis</i> .	Cotton seed, peanuts, peanut butter, pea, sorghum, rice, pistachio, maize, oilseed rape, maize flour, sunflower seed, figs, spices, meats, dairy products, fruit juices (apple, guava)
G (G ₁ ,G ₂)	<i>A. parasiticus</i> , <i>A. nomius</i> , <i>A. bombycis</i> , <i>A. pseudotamarii</i> , <i>A. terreus</i> , <i>A. versicolor</i> , <i>A.</i> <i>arachidicola</i> , <i>A. toxicarius</i> , <i>A. minisclerotigenes</i> .	Peanuts, cotton seed, sunflower seed, tree nuts, pistachio, peanut butter, maize flour, pea, cereals, corn, figs, meats, spices, dairy products, fruit juices (apple, guava)

Source: Abdin *et al.*, 2010.

1.2.5 Exposure and absorption of aflatoxins into the organism

Aflatoxins commonly occur in feedstuffs, feed and animal products. This type of mycotoxins poses a serious health threat to humans and animal species (Strosnider *et al.*, 2006; Lizárraga-Paulín *et al.*, 2011; Probst *et al.*, 2013). The oral route is the main contamination means of aflatoxins, although inhalation may also occur as a result of people or animals being exposed to the grains' dust (Bhat and Vasanthi, 2003). After respiratory exposure, aflatoxin B₁ appears in the blood more quickly than after oral exposure. After four hours, the plasmatic concentration does not differ between the two

routes of contamination (Gong *et al.*, 2004; Williams *et al.*, 2004). Aflatoxin B₁ is efficiently absorbed in the intestinal tract of which the duodenum appears to be the major site of absorption (Riley, 1996). Due to the particle's low molecular weight, the main mechanism of absorption of mycotoxin as suggested by several authors is passive diffusion, in which no efflux pumps or transporters are involved (Kumagai, 1989; Hsieh and Atkinson, 1995; Fernandez *et al.*, 1997; Mata *et al.*, 2004). Daily human aflatoxin exposure varies between countries and was estimated between 4184 ng/kg body weight in various African countries, 122027 ng/kg body weight in Southern China and 753 ng/kg body weight in Thailand, compared to <3 ng/kg body weight in the USA (Hall and Wild, 1994; Williams *et al.*, 2004).

In addition, among processed infant and adult foods all including nuts, grains and powdered milk, Mushtaq *et al.* (2012) found that the magnitude of AFB₁ contamination varied widely. However, the levels of aflatoxin in the processed foods intended for infant consumption were found to be higher than the maximum allowable amounts set by the European Union, which can be more hazardous for infants since they are more sensitive and prone to exposure and toxic effects of such highly carcinogenic food contaminants (Ibid). The systems that are mainly affected by aflatoxins were indicated in Table 1.4.

Table 1.4: The systems mainly affected by aflatoxins

Affected system	Effects/Signs/Symptoms
Genes/Gene expression	Teratogenic effects - Birth defects of the offspring
Genes/Gene expression	Carcinogenic Effects - Higher incidence of cancer in exposed animals
Pathological changes	Weight variation of the internal organs (liver, spleen, kidneys enlargement, fatty liver syndrome), Bursa of Fabricius and thymus reduction, change in the texture and colouration of the organs (liver, gizzard)
Circulatory system	Hematopoietic effects (haemorrhages, anaemia)
Immune system	Immuno suppression (decreased resistance to environmental and microbial stressors; increased susceptibility to diseases)
Nervous system	Nervous syndrome (for example abnormal behaviour).
Skin	Dermatotoxic Effects (impaired feathering)
Urinary system	Kidney inflammation
Digestive system	Impaired rumen function, with decreased cellulose digestion, decreased volatile fatty acid formation, decreased proteolysis, decreased rumen motility, diarrhoea.
Reproductive system	Decreased breeding efficiency (birth of smaller and unhealthy offspring)

Source: Bbosa *et al.*, 2013

1.2.6 The acceptable limits of aflatoxins

The European Union (EU) has the maximum tolerable limits of aflatoxins allowed in cereals according to the European Commission (2007) (Regulation No. 1881/2006) and the Commission regulation (EU) no 165/2010 of 26 (2010). The most stringent regulations of 2 and 4 µg/kg AFB₁ and total aflatoxins in food, and 0.05 µg/kg of AFM₁ in milk. Most of the other countries are somewhere in the middle with 5 and 10 µg/kg AFB₁ and total aflatoxins in food, respectively. The US Food and Drug Administration (US-FDA) is among the most permissive, allowing 20 µg/kg total aflatoxins in food and up to

100 µg/kg aflatoxins in feed (Li *et al.*, 2013). . The East African Commission has set the regulatory limit of 5µg/kg and 10µg/kg for AFB₁ and total aflatoxins, respectively (EAC, 2015). The United States food and drug authority limit for aflatoxins in human food, animal feed and animal ingredients were listed in Table 1.5.

Table 1.5: United States Food and Drug Authority action levels for aflatoxin in human food, animal feed and animal feed ingredients

Intended use	Grain, grain by-product, feed or other products	Aflatoxin level [parts per billion]
Human consumption	Milk	0.5 (aflatoxin M ₁)
Human consumption	Foods, peanuts and peanut products, brazil and pistachio nuts	20
Immature animals	Corn, peanut products, and other animal feeds and ingredients, excluding cottonseed meal	20
Dairy animals, animals not listed above, or unknown use	Corn, peanut products, cottonseed, and other animal feeds and ingredients	20
Breeding cattle, breeding swine and mature poultry	Corn and peanut products	100
Finishing swine 100 pounds or greater in weight	Corn and peanut products	200
Finishing (i.e., feedlot) beef Cattle	Corn and peanut products	300
Beef, cattle, swine or poultry, regardless of age or breeding status	Cottonseed meal	300

Source: United States Food and Drugs Administration

1.2.6 Regulations for aflatoxin M₁ worldwide

All European Union (EU) Member States other than Germany do not have any additional national limits laid down for aflatoxins M₁. The German national legislation specifies an additional maximum limit for aflatoxin M₁ for dietetic foodstuffs for infants, and young children at 0.01 µg/kg, (EMAN, 2012). Limits for aflatoxin M₁ worldwide are generally only laid down for milk and milk products and in some cases for infant and products for infants, as illustrated in Table 1.6.

Table 1.6: Limits for aflatoxin M₁ by countries, mycotoxins legislation worldwide

Country	Foodstuffs	Aflatoxin M₁ (µg/kg)
EU	Raw milk, heat-treated milk	0.050
Bosnia and Herzegovina	and milk for the	
Turkey	manufacture of milk-based products	
	Infant formulae and follow-on formulae, including infant milk and follow-on milk	0.025 (products ready to use)
	Dietary foods for special medical purposes intended specifically for infants	0.025 (products ready to use)
China	Milk and milk products (for milk powder, calculated on a fresh milk basis)	0.5
	Formulated foods for infants (milk or milk protein based)	0.5 (calculated on a dry powder basis)
	Formulated foods for older infants and young children (milk or milk protein based)	0.5 (calculated on a dry powder basis)
	Formulated foods for special medical purposes intended for infants	0.5 (calculated on a dry powder basis)
Codex, GCC, India, Kenya, USA	Milk	0.5
Argentina	Milk, liquid including milk used in the manufacture of milk and milk products and reconstituted milk	0.5 ⁽¹⁾
	Milk, powder	5.0
	Milk formula	ND
Mexico	Pasteurized, ultra pasteurized, sterilized and dehydrated milk, milk formula and combined milk products	0.5 ⁽¹⁾
South Africa	Milk	0.05

ND: Not Detectable, (1) Given in µg/l

Source: European Mycotoxins Awareness Network (EMAN), 2012.

1.2.7 Evaluation of chronic exposure to aflatoxin in human food samples

Food samples collected either from prepared meals and ingredients or from markets provide the most commonly available data (Udomkun *et al.*, 2018). The most reliable sample source for a measure of exposure is through analysis of prepared meals because people may sort grain and remove those kernels that are considered unfit to eat. However, market and trade samples provide information on the risk of exposure from various foods in the diet particularly when local food processors undertake operations such as milling without any quality control (Williams *et al.*, 2004).

1.2.8 Biological markers of exposure

Blood, milk or urine samples are obtained from humans and analyzed for the presence of aflatoxins, each of which has a characteristic half-life in the body (Makarananda *et al.*, 1998). Exposure to aflatoxin is reflected in the urine as directly excreted AFM₁ and other detoxification products, but only a small fraction of the dose is excreted in this way. Measurements of aflatoxin and its by-products in urine have been found to be highly variable from day to day, which reflects the wide variability in the contamination of food samples, and, for this reason, the measurement of AFM₁ on a single day may not be a reliable indicator of a person's chronic exposure (Makarananda *et al.*, 1998; Groopman, 1993). The aflatoxin-albumin adduct is measured in peripheral blood and has a half-life in the body of 30-60 days. Therefore, it is a measure that integrates the exposure over a longer period and hence is a more reliable indicator of a person's chronic exposure.

1.3 Detection of Aflatoxin

Aflatoxin not only has adverse effects on human health but also causes serious economic losses when tons of foods have to be discarded or destroyed as a result of aflatoxin contamination. To ensure food safety, maximum levels for aflatoxins in food and feed have

been set by national and international organizations and various approaches have been developed for the determination of aflatoxin concentrations in food and feed commodities (Bacaloni *et al.*, 2008; Anfossi *et al.*, 2011; Darwish *et al.*, 2014)..

1.3.1 Chromatography

Chromatography is one of the most popular methods to analyze mycotoxins such as aflatoxins. In the beginning of aflatoxin analysis and research, gas chromatography (GC) was frequently used for detection and quantification of compounds. However, later on, new chromatography-based techniques were developed for aflatoxins. Examples of these improvements are liquid chromatography (LC), thin layer chromatography (TLC) (Stroka *et al.*, 2000), and high-performance liquid chromatography (HPLC) (Bacaloni *et al.*, 2008) which nowadays is the most commonly used chromatographic technique for detection of a wide diversity of mycotoxins, especially for aflatoxin derivatives (Cavaliere *et al.*, 2006; Sapsford *et al.*, 2006; Vosough *et al.*, 2010; De Rijk *et al.*, 2011).

Frisvad and Thrane (1987) described HPLC method to identify 182 mycotoxins and other fungal metabolites based on their alkylphenone retention indices and diode array spectra. Coupling of HPLC with mass spectroscopy or tandem mass spectroscopy allows for highly accurate determination of toxin concentrations and compound identification in one analysis (Sobolev, 2007). Alternatively, fluorescence detection of the unmodified aflatoxins is widely used in HPLC applications as well as in thin layer chromatography. Furthermore, there are combinations of the methods above with pre-process techniques, which can detect the concentration of aflatoxin in a solution in a better way. For example, immunoaffinity column sample clean-up followed by a normal or reverse phase of HPLC separation with fluorometric detection is mostly used for quantitative determination of

AFM1 due to the characteristics of specificity, high sensitivity and simplicity of operation (Muscarella *et al.*, 2007).

1.3.2 Immunoassay

Immunochemical detection for aflatoxins is based on antibody-antigen reactions (Ab-Ag) (Lee *et al.*, 2004). Since different kinds of aflatoxin molecules can be considered as antigens, it is possible to detect them by developing antibodies against the compounds. Most of the immunological methods are based on enzyme-linked immunosorbent assays (ELISA), which have good sensitivity, speed and simplicity. In addition, some lateral flow immunoassays (LFIA) also are applied for the qualitative and semi-quantitative detection of aflatoxin in food, feed and milk (Ho and Wauchope, 2002; Anfossi *et al.*, 2011; Salter *et al.*, 2006). Even though several reports have been published on the immunochemical determination of aflatoxin in food, only a few validation studies are available to show that the results comply with certain regulations because of the requirement for expensive instrumentation.

1.3.3 Biosensors and other methods

The term biosensors refers generally to a small, portable and analytical device based on the combination of recognition bio-molecules with an appropriate transducer, and able of detecting chemical or biological materials selectively and with a high sensitivity (Paddle, 1996). Biosensors, an alternative to improve the disadvantages of the previous methods, are multidisciplinary tools with an enormous potential in detection and quantification of aflatoxin. There are all kinds of biosensors that base their performance on different physical or biochemical principles, such as optical, optoelectronic, electrochemical, piezoelectric, DNA and combined. Thus, such devices have a huge impact on healthcare, food management, agronomical economy and bio-defense (Nayak *et al.*, 2009).

Many kinds of biosensors are applied to detect aflatoxin. However, they mainly work in conjunction with immunochemical methods. Such junctions are based on the high affinity of antigen-antibody interaction and have the aim of increasing the sensitivity and shortening the detection time of the toxic element (Dinçkaya *et al.*, 2011). More methods exist which are less common than the previously described methods but have a wide utility as well. The most important are those ones that base their principle on electrochemistry, spectroscopy and fluorescence. Compared with traditional methods for aflatoxin determination, electrochemical techniques offer some advantages such as reliability, low cost, *in-situ* measurements, fast processes, and easier methodology than common chromatography techniques through a similar performance. Especially for measurement of AFM₁, the disposable immuno-sensors have been applied directly in milk following a simple centrifugation step without dilution or other pre-treatment steps. Exhibition of a good working range with linearity between 30 and 240 ng/ml makes this method useful for AFM₁ monitoring in milk (maximum acceptable level of AFM₁ in milk is 0.05 ppb) (Micheli *et al.*, 2005). Spectroscopy techniques have been popularized due to the characteristics that fast, low-cost and non-destructive analytical methods suitable to work with solid and liquid samples. Among them, near infrared spectroscopy (NIRS) is an excellent method for a rapid and low cost detection of aflatoxin in cereals (Fernández-Ibáñez *et al.*, 2009). When incorporated with a bundle reflectance fiber-optic probe, NIRS was successfully applied to quantify aflatoxin B₁, ochratoxin A and total aflatoxins in paprika (Hernández-Hierro *et al.*, 2008). Aflatoxins have a native fluorescence due to their oxygenated pentaheterocyclic structure, which is the basis of most analytical and microbiological methods for detection and quantification of aflatoxins (Rojas-Durán *et al.*, 2007; Rasch *et al.*, 2010).

1.4 Effects of Aflatoxin on Human and Animal Health

Human and animals are exposed to aflatoxins primarily by eating contaminated foods and feeds. Occupational exposure for instance, is usually through inhalation of dust contaminated with aflatoxins and farmers and agricultural workers have the greatest risk of getting exposure to aflatoxins (NTP, 2011). Infants are exposed to aflatoxin through breast feeding and during introduction of complementary foods (Zarba *et al.*, 1992). The adverse effects of aflatoxins on animal can be categorized into two general forms; acute toxicity and chronic toxicity (Wu *et al.*, 2011).

1.4.1 Acute toxicity in animals

Acute toxicity is caused when moderate to large doses of aflatoxin are ingested. This is common in livestock. The principal target organ for aflatoxins is the liver (Wu *et al.*, 2011). After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes and lead to necrosis or liver cell death. This is mainly because aflatoxin metabolites react negatively with different cell proteins which lead to inhibition of carbohydrate and lipid metabolism and protein synthesis (Wild and Gong, 2010; Wu and Khlangwiset, 2010). The specific acute episodes of disease may include haemorrhage, acute liver damage, oedema, alteration in digestion, absorption and/or metabolism of nutrients and possibly death (NLM, 2002; Otsuki *et al.*, 2002; Liu *et al.*, 2012; Thrasher, 2012).

1.4.2 Chronic toxicity in animals

This is due to long term exposure of moderate to low aflatoxin concentration. The symptoms include decrease in growth rate, lowered milk or egg production and immuno suppression (Khlangwiset *et al.*, 2010; Wu *et al.*, 2011; Wu, 2010). Immuno-suppression is due to the reactivity of aflatoxins with T-cells, decrease in Vitamin K activities and a decrease in phagocytic activity in macrophages.

In animals, the effects of aflatoxins are variable depending on sex, age, species and even animal breed. The main target organ for aflatoxins is the liver. Due to the toxin's interference and reactions with nucleic acids, RNA and DNA, proteins and enzymes, their effects on domestic animals are not only hepatotoxic and expressed by toxic hepatitis and jaundice, but involve a broad range of organs, tissues and systems. No animal species has been found to be immune to the effects of aflatoxins (Murphy *et al.*, 2006).

1.4.3 Acute toxicity in humans

Acute exposure is experienced when individuals ingest high levels of aflatoxins which as a result lead to aflatoxicosis, which can cause rapid death from liver failure. Besides carcinogenic effects, immunomodulatory effects are also observed in the humans along with infectious disease and growth problems in children (Lewis *et al.*, 2005; CDC, 2004).

Acute dietary exposure to aflatoxin B₁ has been implicated in epidemics of acute hepatic injury (Sudakin, 2003; Farombi, 2006). Evidence of acute aflatoxicosis in humans has been reported worldwide especially in the third world countries like Taiwan, Uganda, India, Kenya, Tanzania and many others (Levin, 2012).

Outbreaks of acute aflatoxicosis from highly contaminated food have been documented in Kenya, India and Thailand (CAST, 2003). In rural Kenya, the outbreak resulted into 317 cases and 125 deaths (CDC, 2004). The cause for this 39% fatality rate was contaminated maize with aflatoxin levels up to 8mg/kg (Lewis *et al.*, 2005). In addition, preliminary results from the cereals (maize, sorghum and millet) tests by the CDC showed aflatoxins contamination about 200 ppb which was remarkably above the tolerant limit to human being (Buguzi, 2016). In Dodoma region, (Kondoa, Chemba, Dodoma, Chamwino districts) and Manyara (Kiteto District), the outbreak of aflatoxin contamination in cereals

was revealed where 54 people were reported to have been exposed to the poison. Out of those exposed people, 14 deaths were reported due to liver failure with the majority of the affected being children aged below 12 years old (Ibid).

Aflatoxin-contaminated home-grown maize was the source of the outbreak as it was the case in north-western India in year 1974 where 25% of the exposed population died from consumption of molded maize with levels from 6 250 mg to 15 600 mg/kg (Krishnamachari *et al.* , 1975).

1.4.4 Chronic toxicity in humans

The chronic aflatoxicosis results from ingestion of low to moderate levels of aflatoxins (Williams *et al.*, 2004; Levin, 2012; Laurian and Delia, 2013). The effects of aflatoxins are usually subclinical and complicated to recognize. Some of the common symptoms are impaired food conversion and slower rates of growth with or without the production of aflatoxin disease (WHO, 2000). Hepatocellular carcinoma (HCC) is a major health problem in China where each year approximately 110,000 patients are diagnosed with it. The HCC cases in China account for almost 45% of HCC incidences worldwide (Op.cit). The mortality rate for HCC is more than 95%. Excluding other risk factors, the consumption of aflatoxin contaminated food such as corn, soya-based products and peanut oil was correlated to the HCC fatality rates in people living in ten Chinese villages (Yu, 1995).

The immunosuppressive effects of aflatoxin have also been shown to be transferred across the placenta and usually affect the unborn foetus in porcines, suggesting that unborn babies could equally be affected (Waliyar *et al.*, 2009). Poor nutrition is usually attributed to food insecurity and is clearly exacerbated by exposure to aflatoxins thus leading to

increased disease prevalence and further reduction in the ability of individuals to cope with mycotoxin exposure (Gong *et al.*, 2002; Waliyar *et al.*, 2009).

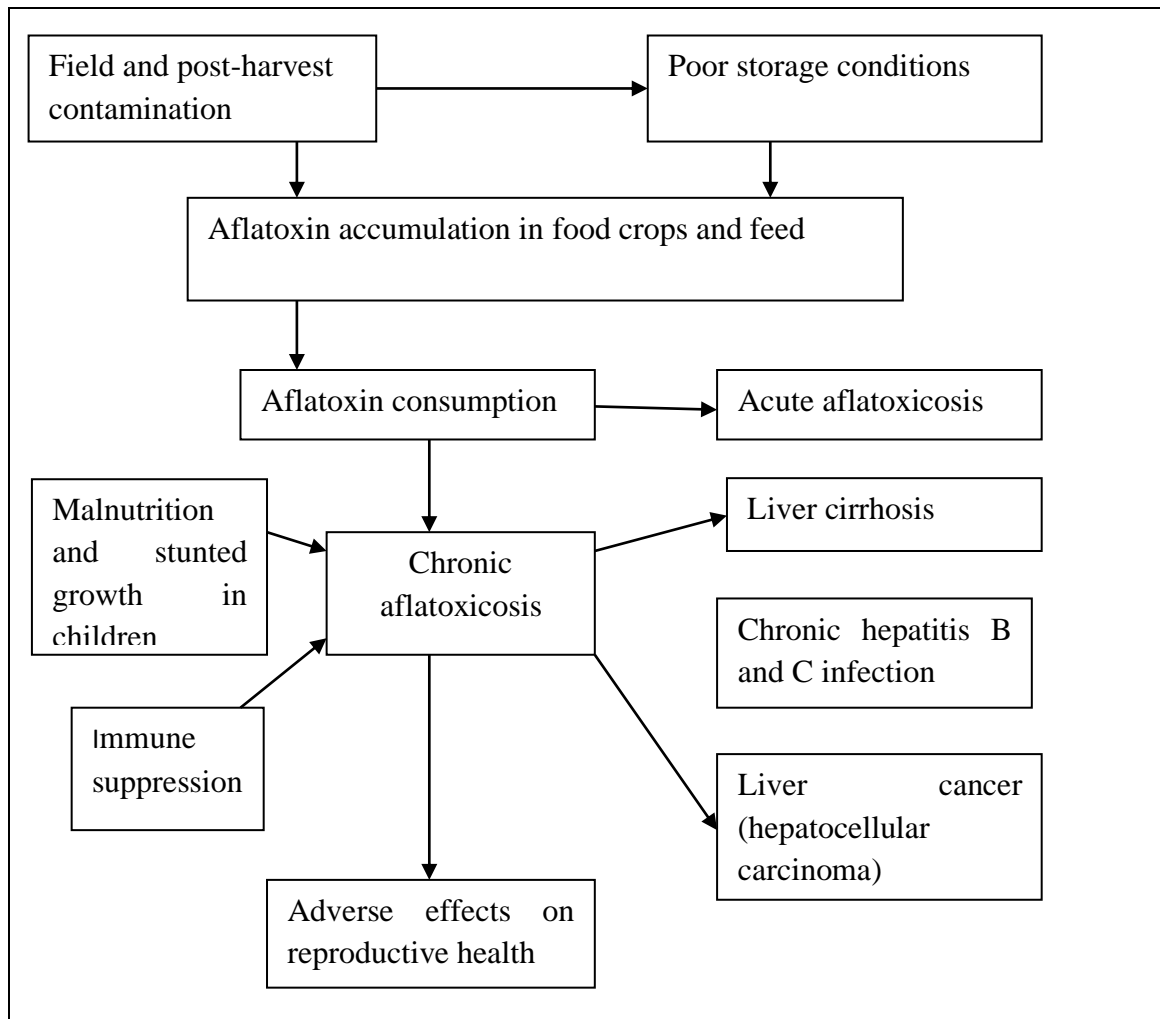


Figure 1.3: Aflatoxin disease pathways in humans

Source: Wu, 2010; Levin, 2012; WHO, 2011; Wu and Tritscher, 2011

1.5 Liver Cirrhosis, Cancer and Aflatoxins

Cancer diseases is among the leading causes of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million cancer related deaths in 2012 (World Cancer Report, 2014). The most common causes of cancer death are cancers of lung (1.59 million deaths), liver (745 000 deaths), stomach (723 000 deaths), colorectal (694 000 deaths), breast (521 000 deaths) and oesophageal cancer (400 000 deaths). Worldwide, it

is estimated that over 600 000 people die each year due to liver cancer with a majority of cases in China, South East Asia and Sub-Saharan Africa (Parkin, 2006). Aflatoxin exposure and chronic hepatitis B infection contains either separately or in synergy the agents responsible for most liver cirrhosis (Kuniholm *et al.*, 2008). International Agency for Research on Cancer (IARC, 1993) classified that those naturally occurring mixtures of aflatoxins are as a Group 1 human carcinogens based on the evidence from animal studies like rats, mice, hamsters, trout, salmon, ducks, tree shrews and monkeys.

The co-exposure of aflatoxins and the hepatitis B virus (HBV) in developing countries is common and greatly increases hepatocellular carcinoma risk (Wu *et al.*, 2013). Persons with both exposures have multiplicatively greater risk of developing HCC than those exposed to aflatoxins or HBV alone (Groopman *et al.*, 2008). Co-occurrence of aflatoxins and hepatitis B virus (HBV) produces a synergistic effect which increases the risk of liver cancer twelve times that of the people infected with the virus which already increases the relative risk five-fold (WHO, 2006).

A systematic review and meta-analysis determined that the risk of developing liver cancer was over 6 times higher in persons with detectable aflatoxin biomarkers than in those without, over 11 times higher in individuals with chronic HBV infection than in those without and over 73 times higher in individuals with both detectable aflatoxin biomarkers and HBV positivity compared with those with neither risk factor that is a nearly perfectly multiplicative relationship (Liu *et al.*, 2012). The two separate analyses were conducted to estimate the global burden of liver cancer attributable to aflatoxins. Liu and Wu (2010), used a quantitative cancer risk assessment approach, using dose-response data for the relationship between aflatoxins and liver cancer risk in populations of HBV-negative and HBV-positive individuals (JECFA, 1998; Henry *et al.*, 1999) and multiplying the related

cancer potency factors by aflatoxin exposure data for multiple nations worldwide. The analysis included about 5 billion individuals around the world where aflatoxin data were available in different nations and it was estimated that 25 200-155 000 liver cancer cases annually can be attributed to aflatoxin exposure (Liu and Wu, 2010).

Cancer is a generic term for a large group of diseases that can affect any part of the body. In the study by Liu *et al.* (2012) different approach to estimate global burden of cancer caused by aflatoxins was used. The estimating population-attributable risk from a systematic review and meta-analysis of 17 epidemiological studies on aflatoxins, HBV and liver cancer in Africa and Asia was used. It was estimated that about 23% (21-24%) of all HCC cases annually may be attributable to aflatoxins for a total of up to 172,000 cases per year. Since liver cancer is the third-leading cause of cancer deaths worldwide and mortality rapidly follows diagnosis, the contribution of aflatoxins to this deadly cancer is significant (WHO, 2008).

1.6 Aflatoxin and Growth Impairment

Aflatoxin exposure has been associated with stunting growth of the children, a condition in which the child's height for his or her age is two standard deviations or more below a World Health Organization (WHO) growth reference. Stunting is important from a public health perspective because it is associated with effects such as increased vulnerability to infectious diseases and cognitive impairments that last well beyond childhood (Ricci *et al.*, 2006). Khlangwiset *et al.* (2011) reviewed the epidemiological studies that show an association between child growth impairment and aflatoxin exposure. They noted that studies in Togo and Benin in West Africa (Gong *et al.*, 2002, 2004) showed that height and weight for children's ages were lower in a dose-dependent fashion for higher aflatoxin exposures and children's growth over eight months was also compromised. Studies of

infants and children in The Gambia and Tanzania (Turner *et al.*, 2003, 2007; Shirima *et al.*, 2014) showed that aflatoxin-albumin adduct (AF-alb) levels in maternal blood, cord blood, infant blood and children's blood were associated with poorer growth indicators. AF-alb is a biomarker of aflatoxin exposure and biological activation in humans. Aflatoxin levels in household flour in Kenya were associated with wasting in children (Okoth and Ohingo, 2004). A Ghanaian study (Shuaib *et al.*, 2010) linked mothers' AF-alb levels with low-weight babies at birth. In Iran and Tanzania, studies (Sadeghi *et al.*, 2009; Mahdavi *et al.*, 2010; Magoha *et al.*, 2014; Shirima *et al.*, 2013) explain that aflatoxin M₁ in mothers' breast milk was associated with reduced length and weight of infants at birth. Khlangwiset *et al.* (2011) also provide discussions of animal studies linking aflatoxin exposure with impaired growth outcomes and of the importance of aflatoxin-free weaning foods. However, since there were a relatively small number of epidemiological studies undertaken and the limited nature of dose-response relationships, it is not possible to conduct a quantitative risk assessment linking an aflatoxin dose with a particular risk of stunting in a population. However, while causality has not yet been confirmed, the body of evidence consistently shows an association between aflatoxin exposure and growth impairment in children (Gong *et al.*, 2003; Egal *et al.*, 2005).

Chronic exposure to aflatoxin has been shown to stunt growth and even contribute to infant mortality when it coincides with kwashiorkor (Tchana *et al.*, 2010), a form of malnutrition caused by dietary deficiency of protein and other nutrients. The insidious combination of impaired development and undernourishment accounts for about half of the 4.5 million deaths of children under the age of 5 occurring annually in sub-Saharan Africa (Science of Africa, 2005).

Aflatoxin is fat soluble and can be measured in blood as an Aflatoxin-albumin adduct, in urine (AF M₁) as an Aflatoxin-guanine adduct and in breast milk as AF M₁. There are few human data, but animal studies provide evidence that chronic exposure to aflatoxin retards growth and interferes with micronutrient absorption and utilization (Smith *et al.*, 2012; Rubert *et al.*, 2014). Turner *et al.* (2007) suggested that intestinal mal-absorption leads to zinc deficiency, which then causes growth faltering and immune deficiency. Abdulrazzaq *et al.* (2004) suggested that aflatoxin exposure inhibits the synthesis of proteins, enzymes and clotting factors and impairs glucose metabolism, fatty acid synthesis, and phospholipid synthesis. Aflatoxin exposure can cause enterocyte damage and increased intestinal permeability (Turner *et al.*, 2007).

1.7 Breast Feeding and Complementary Feeding

It is estimated that sub-optimal breastfeeding, especially non-exclusive breastfeeding in the first 6 months of life, results in 1.4 million deaths and 10% of the disease burden in children younger than 5 years (WHO, 2009). Complementary feeding means giving foods in addition to breast milk. Malnutrition can result from suboptimal breastfeeding practices, poor quality complementary foods, detrimental feeding practices and contamination of complementary food and feeding utensils (WHO/UNICEF, 1998). The second half of an infant's first year is an especially vulnerable time because infants are learning to eat and must be fed soft foods frequently and patiently. If nutritional intake is inadequate, the consequences persist throughout life (WHO/UNICEF, 1997, 2000).

Malnutrition is responsible, directly or indirectly, for over half of all childhood deaths (WHO, 2002). Infants and young children are at increased risk of malnutrition from six months of age onwards, when breast milk alone is no longer sufficient to meet all nutritional requirements and complementary feeding needs to be started (Ibid). Global

recommendations for appropriate feeding of infants and young children are: Breastfeeding should start early within one hour after birth, breastfeeding should be exclusive for six months and appropriate complementary feeding should start from the age of six months with continued breastfeeding up to two years or beyond (WHO, 2002).

Poor breastfeeding and complementary feeding practices are widespread (WHO, 2009). Worldwide, it is estimated that only 34.8% of infants are exclusively breastfed for the first six months of life, meaning that the majority receive some other food or fluid in the early months (WHO, 2005). Complementary foods are often introduced too early or too late and are often nutritionally inadequate and unsafe (WHO, 2009).

1.8 Breast Feeding and Aflatoxins

Aflatoxin M (AFM) is a hydrolyzed metabolite of AFB formed in liver by means of cytochrome P450 associated enzymes (WHO/FAO, 2002; Cavaliere *et al.*, 2006). When feed contaminated with AFB is ingested by dairy cattle, up to 0.3-6.2%, it will appear in the milk as AFM (Barbieri *et al.*, 1994). It is excreted in milk following exposure to AFB₁ contaminated food and transferred to dairy products such as cheese which represents an important risk factor for consumers (Creppy, 2002). AFM₁ can be transmitted to a newborn offspring by the human's milk (Moore-Landecker, 1996; Abdulrazzaq *et al.*, 2004; Smith *et al.*, 2012). Infants and children living in developing countries have many other problems compromising health, such as general food shortages, malaria, diarrhoea, measles and protein energy malnutrition that may make them more susceptible to AFM₁ detrimental effects (Ghiasian *et al.*, 2012; Magoha *et al.*, 2014).

1.9 Aflatoxins and Complementary Foods

Exposure to aflatoxins occurs primarily through ingestion of contaminated food. Cereals are probably the most contaminated food by fungi and that contamination can occur at different steps of the food chain from field to processing into final products (Bankole and Mabekoje, 2004; Wu and Khlangwiset, 2010), and are the most important ingredients used in preparation of complementary foods (Kimanya *et al.*, 2008; Azziz-Baumgartner *et al.*, 2005).

In the developing world, many people live largely on cereal-based diets. Therefore, nutritional deficiencies are very common in populations consuming high levels of cereal crops (Bankole and Adebajo, 2003; Kamala *et al.*, 2016), particularly children. In addition, many children in the developing countries are also exposed to high levels of mycotoxins in their diets (Cardwell *et al.*, 2001; Kimanya *et al.*, 2010).

1.10 Aflatoxin and Immune System Disorders

Aflatoxin exposure is associated with immune system disorders and reduces weight and height in children. In several studies of animal over three decades, it was found that aflatoxin may have immunosuppressive impacts (Jolly *et al.*, 2008). Aflatoxin and immuno-suppression in humans has been relatively less well-characterized in several studies but could have a great significance from a global health perspective (Williams *et al.*, 2004). Several human studies have shown evidence of immuno-modulation (Turner *et al.*, 2003, Jiang *et al.*, 2005; 2008), though the actual outcomes of such immuno-modulation have yet to be characterized in humans. But in reality, aflatoxins' immuno-toxicity may be one -enlightenment for the stunted growth in children that appears to follow a dose-response relationship with aflatoxin exposure (Gong *et al.*, 2002, Turner *et al.*, 2003). Another explanation may be altered intestinal integrity (Gong *et al.*, 2008).

Aflatoxin impairs growth and contributes to immune suppression in animals; however, immune suppression in humans has been currently investigated mostly in children. In Benin and Togo, stunted and/or underweight children had an average of 30 to 40 percent higher levels of aflatoxin-albumen levels in the blood than children with a normal body weight (Gong *et al.*, 2008). In addition to the above mentioned negative effects of aflatoxin,, it also suppresses immune systems in susceptible populations such as young children and HIV and AIDS patients (Williams *et al.*, 2004).

When aflatoxin is consumed either directly or indirectly through contaminated foods or feeds, it can exert toxicity in numerous ways. It may alter intestinal integrity (Gong *et al.*, 2008) or modulate the expression of cytokines, such as proteins that signal to each other and to the immune system components hence causing disorders of immune system. Both of these effects may result in stunted growth in children and/or immune suppression due to difficulty in absorption of nutrients in the body (Ibid).

1.11 Aflatoxins and HIV Progression

The Sub-Saharan Africa has the largest number of HIV/AIDS epidemic worldwide according to UNAIDS, (2012) and the millions of HIV infected people in this part of the world are likely to be chronically exposed to aflatoxins in their diets (CAST, 2003; IARC, 2002; Jiang *et al.*, 2008). Both aflatoxins and the human immunodeficiency virus (HIV) are immunosuppressive agents that may adversely influence the HIV infection and lead to faster progression to acquired immune deficiency syndrome (AIDS) in the infected individual. In the study done by Hendrickse *et al.* (1989) in Netherlands and Scotland among the heroin addicts, it was revealed that there was a rapid progression of human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS). They also found that the people who use heroin were often contaminated with aflatoxins and the

aflatoxins derivatives were commonly found in the body fluids of the addicts (Jaffar *et al.*, 2004, and Morgan and Whitworth, 2001). A strong correlation between aflatoxin exposure and the perceived faster rate of HIV progression in developing countries in developed countries (Europe or the USA). Turner *et al.* (2003) in Gambian children reported that immunoglobulin A in saliva may be reduced by dietary aflatoxins.

Different species of animals exposed to low level of aflatoxins and revealed that there was an increase of susceptibility to infectious diseases like dysentery in swine and, *salmonella* and fowl adenovirus serotype-4 infectious in chickens (Boonchuvit and Hamilton, 1975; Joens *et al.*, 1981). These also show that reactivated chronic *Toxoplasma gondii* infection in mice (Venturini *et al.*, 1996) reduces the antibody response to vaccines in animals (Gabal and Dimitri, 1998) and decreases the cell-mediated immune response to a vaccine antigen in pigs (Meissonier *et al.*, 2008). Jolly *et al.* (2013) showed that the higher viral loads in HIV positive people also revealed higher aflatoxin albumin levels among Ghanaian adults. Jolly *et al.* (2011) that there is a strong association between high aflatoxin albumin levels with high viral load. Jiang *et al.* (2008) found that in HIV+ and HIV- in Ghanaians, higher levels of AF-albumin were associated with lower levels of CD4+ T regulatory cells and naïve CD4+ T-cells as well as lower B-cells. All these cells are associated with immune responses. In Ghana it was revealed that other types of cells which were involved in immune response were found to be lower in individuals with higher AF-alb (Jiang *et al.*, 2005). Another study showed that Gambian children with higher levels of AF-alb had lower levels of secretory IgA in their saliva, which is another immune parameter (Turner *et al.*, 2003).

1.12 Factors Influencing the Occurrence of Aflatoxin

The occurrence of aflatoxins (or other mycotoxins) in agricultural commodities is a major health concern for livestock and humans. Crops frequently contaminated include cereals,

peanuts, tree nuts, spices, corn, rice, cottonseed, dry fruits and copra (FAO/WHO, 2007). Many African countries, including East African countries do not regularly test their crops for aflatoxins, thus leading to consumption of contaminated and suspect grain (Azziz-Baumgartner *et al.*, 2005; Kimanya *et al.*, 2008). Stressful environments in maize and other cereals and groundnut crops, in particular, favour aflatoxin production (Cotty and Ramon, 2007). Drought and high temperatures, insect and physical plant damage, Crop residues left on the field and soil type and tillage practices provide these stresses (Schmale *et al.*, 2012).

1.12.1 Pre- and post-harvest handling

The occurrence of aflatoxin on crops is strongly influenced by weather during and after the growing season (Cotty and Ramon, 2007). Cool, wet growing seasons may delay grain maturity, especially for corn, and therefore result in mould and mycotoxin formation in the field. Climate change is likely to lead to an increase in hot and dry spells; this implies an expectation of increased risk of aflatoxin contamination (Ongoma, 2013; Cotty and Ramon, 2007).

Factors influencing fungal growth and toxin development include growth cracks, mechanical injury and damage by pests which all lead to infestation by fungi. Toxins produced under high temperatures, drought, high insect activity prior to harvest and wet conditions at harvest lead to higher contamination and innovative breeding is needed to be explored to produce cereals that are more resistant to fungal infection. Mature maize that remains in the field (as dry heaps) or maize that is stored without proper drying is susceptible to *Aspergillus* fungi growth and aflatoxin production (Lunyasunya *et al.*, 2005). Poorly stored feeds and grains can indeed become contaminated with aflatoxin (Lunyasunya *et al.*, 2005; Hell *et al.*, 2008).

A. flavus comes in contact with crops before harvest, it however remains associated with the crop through harvest and storage (Lillehoj, 1987; Hell *et al.*, 2003, 2008). Thus, seed kernels become contaminated with aflatoxin both before and after harvest (Diener *et al.*, 1987; Cotty and Lee, 1989, 1990). The contamination is however, more likely to occur in the post-harvest stage if the produce is not handled properly to minimize the thriving of the fungal species (Hell *et al.*, 2003, 2008; Kaaya *et al.*, 2005). Poor harvesting practices, inappropriate storage and less than optimal situation during transport and marketing can also contribute to fungal development and increase the risk of aflatoxins production (Bhat and Vasanthi, 2003; Ngoma *et al.*, 2016).

1.12.2 Drought and High Temperatures

Drought occurs when plant water demands cannot be met due to soil water deficiency resulting from dryness brought on by meteorological or hydrological drought (USAID and OAS, 2001). In such cases, plant water stress may be evidenced from reduced biomass and plant yield. Meteorological drought is defined mainly by deficiencies in precipitation. Along with deficient rainfall, conditions during drought may be accompanied or aggravated by high temperatures, strong winds, low relative humidity, greater sunshine and less cloud cover. These conditions can be expected to bring increased evaporation and transpiration, reduced water infiltration into soils and a reduction in deep percolation and ground water recharge (op.cit).

Drought stress and high temperature are key factors that raise concentrations of aflatoxins. The two climatic factors directly have an impact on cereals and *A. flavus*. Dry conditions and high temperatures facilitate the growth, conidiation and dispersal of *A. flavus* that weakens growth and maturity of maize (Cotty and Ramon, 2007). Drought and semi-arid to arid conditions are linked to contamination, and it is most dependent on frequently

contaminated staples especially in tropical countries. This shift in weather patterns may lead to acute aflatoxicosis and thus death (Lewis *et al.*, 2005).

Aflatoxins contamination has been associated with prolonged high day and night temperatures especially during the growing season and severe drought conditions during grain fill (Cotty and Ramon, 2007; Schmale *et al.*, 2012). Risk factors for aflatoxin contamination include end of season drought, high moisture contents and/ or relative humidity and high temperature with optimum temperatures between 25-35 °C (Cotty and Antilla, 2003). *Aspergillus flavus* colonization in maize and oil seeds are encouraged by high humidity (80-89 %) and heat (10-40 °C) (Lunyasunya *et al.*, 2005). Drought stress has been found to increase the number of *Aspergillus* spores in the air (Sorenson *et al.*, 1984). Nitrogen stress (low soil fertility) and other stress that affect the plant growth during pollination can increase the level of aflatoxins production by *Aspergillus* fungi. During high humidity, initially dry seed develops water content conducive to contamination. The combination of moisture content and temperature dictate the extent of contamination. According to Jaime-Garcia and Cotty (2003), influences of delayed harvest on contamination are most severe when crops are caught by rain just prior to or during harvest. When temperatures are below 18.3 °C and the moisture of the corn is below 12 to 13 percent, development of the fungus usually stops.

1.12.3 Insect and physical plant damage

Aspergillus flavus is considered mainly as a storage fungus although it can produce aflatoxin in the field under conditions which favour their growth (Cotty and Ramon, 2007; Hell *et al.*, 2011). Parts of maize or groundnuts are more likely to be substrate for aflatoxin production than whole seeds in certain conditions. Whatever the thing that damages the seed coat and allows the fungus access into the carbohydrate-rich endosperm, increases the

risk of fungal proliferation and aflatoxin production (Hell *et al.*, 2008; 2011). Insects increase *A. flavus* infection and aflatoxin contamination by feeding on and damaging developing kernels and by transporting *A. flavus* conidia into the ear (Ni *et al.*, 2011). The relationship between insect damage and aflatoxin levels has at times been difficult to document. This relationship has been shown to vary among genotypes and locations (Abbas *et al.*, 2006; Bowen *et al.*, 2014).

1.12.4 Soil type and tillage practices and aflatoxins

Tillage is defined as mechanical manipulation of soil to provide a favourable environment for good germination of seeds and crop growth to control the weeds to maintain infiltration capacity and soil aeration (FAO, 2002). A well planned tillage practice provides a favourable environment, suitable for better seed germination and effective plant growth. In addition, it also protects and maintains a strong soil structure to fight against erosion. Fields that vary in cropping history, tillage practices, planting date, soil type, or hybrid may differ greatly in aflatoxin vulnerability (Kress, 2014).

1.12.5 Small-scale subsistence farming systems

Tanzanian agriculture is dominated by small-scale subsistence farming. The Tanzanian agricultural sector is pivotal to the country's economy and social structure. The rural population accounts for almost 80 percent of the total, and more than 90 percent of female rural employment and 78 percent of male rural employment are in the agricultural sector (World Bank, 2000). Most of the 4.4 million farm families in Tanzania are engaged in subsistence cultivation of food crops and in cash cropping. The main subsistence crops, which account for 55 percent of total agricultural output, are maize, sorghum, millet, cassava, rice, plantains, and vegetables.

Subsistence farmers are “people who grow what they eat, build their own houses, and live without regularly making purchases in the marketplace” (Waters, 2007). The typical Tanzanian family consists of a single mother raising four or five children, and thus many of the Tanzanian subsistence farmers are women striving to grow enough food to feed their children (UNDP, 2012).

The number of poor, particularly in rural areas, is still high; about 12 million people, among them 10 million in the rural sector, continue to live in poverty. Heads of households with less education and a large number of children and who are engaged in subsistence agriculture and living in communities lacking infrastructure are likely to be the most poor and many of them will pass on their poverty to their offspring (World Bank Report, 2015).

The fungi that produce aflatoxins grow best under warm conditions and therefore, aflatoxins are of greatest concern in warm agricultural production areas especially during dry periods (Cotty and Bhatnagar, 1994). Such areas of high vulnerability are common in parts of Africa where subsistence farmers frequently rely on contaminated maize and groundnuts as life-sustaining staples (Egal *et al.*, 2005; Hell *et al.*, 2003; CDC, 2004). Extensive subsistence farming systems, lack of irrigation and inadequate drying and storage facilities hinder prevention and detection of aflatoxin in crops.

1.13 Strategies for Reducing Aflatoxins Risks

The majority of people in developing countries are not aware of risks associated with contaminated foods (Jolly *et al.*, 2009). Mycotoxins production depends on many factors such as biological and environmental factors. Moisture contents and temperature are the crucial factors in fungal growth and toxins elaborations (CAST, 2003; Bryden, 2007).

1.13.1 Pre-harvest strategies

In the pre-harvest period, crops can be affected by stress either by drought or insects which can lead to fungal invasion therefore preventive measures are needed to reduce the problems by using good agricultural practices, use of insecticides and fungicides to reduce fungal and insects infestation and irrigation to avoid moisture stress, also harvesting at maturity and breeding programme to improve genetic resistance to fungal growth (Bryden, 2007; Russell *et al.*, 2009).

1.13.2 Post-harvest strategies

During post harvest period, crops can be affected through moisture contents of commodities and temperature during storage therefore control of moisture through proper drying of crops prior to storage will minimize fungal attack (Wu *et al.*, 2009; Kamala *et al.*, 2016). The methods like dehulling and washing grains before processing will minimize contamination. Harvesting must be done in such a way that it will prevent damage to the seed coat and to assure maximum cleaning of grain, since damaged seed and foreign material contribute to the development of aflatoxin. Following these practices will reduce the likelihood of this problem (Sumner and Lee, 2012).

Reducing post harvest aflatoxin accumulation can begin with simple physical methods. Mechanical sorting can separate aflatoxin-contaminated seeds from relatively cleaner ones and proper drying can further reduce risks. To prevent the growth of *Aspergillus* in food storage, it is essential to control moisture, temperature and pests (Kabak *et al.*, 2006). Post-harvest methods can be the least resource intensive for developing countries to put into practice (Turner *et al.*, 2005).

1.13.3 Biological control of aflatoxins

A biological control method involves the use of biological agents to control pests or toxin production in pre-harvest period and has been used in maize, groundnuts, and cottonseed in different parts in the world (Pitt and Hocking, 2006; Cotty and Ramon, 2007). This is the use of organisms to reduce the occurrence of toxigenic *Aspergillus* in susceptible crops and in that way reduce aflatoxin contamination (Wu and Khlangwiset, 2010). The most commonly biological control method for aflatoxin is by using non-toxigenic strains of *Aspergillus* that can eliminate toxigenic strains from colonizing crops (Russell *et al.*, 2009). Grain seeds (of wheat, barley, sorghum, or other small grains) are either briefly colonized by or coated with conidia of a non-toxigenic strain and these seeds are applied to agricultural fields during a period favourable for competitive exclusion of toxigenic strains (Pitt and Hocking, 2006; Cotty and Ramon, 2007).

Biological control is highly cost-effective and reduces aflatoxin at its earliest stages but professional staff and training requirements may be high (Wu *et al.*, 2009). However, individual farmers may not have enough money to pay even this relatively small amount, if they do not understand the risks of aflatoxin contamination.

1.13.4 Chemical control of aflatoxins

A chemical control method is used in the post harvest period and especially in the diet and it is consumed with meals; hence dietary interventions can reduce aflatoxin-related health risks (Russell *et al.*, 2009). One simple dietary intervention method is to consume less maize and groundnuts, in favour of other food crops that usually have lower aflatoxin levels such as sorghum and pearl millet (Bandyopadhyay *et al.*, 2007). Dietary additives to reduce aflatoxin-induced risk include enterosorbents that “trap” aflatoxin in the gastrointestinal tract thus facilitating elimination (Phillips *et al.*, 2008). NovaSil clay is an

enterosorbent of aflatoxin therefore it can be blended into food or feed, or taken separately in capsule form during the meal to bind aflatoxin in the gastrointestinal tract, hence reduce aflatoxin bioavailability in the body (Wu and Khlangwiset, 2010).

The following are some of the materials that have the ability to bind aflatoxin. These include: bentonites, zeolites, diatomaceous earth, activated charcoal, yeast cell walls, and fibres from plant sources (Wu and Khlangwiset, 2010). Calcium montmorillonite, marketed as NovaSil clay (NS) has proven effective in animal feed and shown promise in human trials. NS has been shown to prevent aflatoxicosis in many animal species when introduced in their diet, by binding aflatoxin with high affinity and high capacity in the gastrointestinal tract (Phillips *et al.*, 2008).

Some strains of *Lactobacillus* shows effectiveness in binding dietary mycotoxins, and also selenium supplementation change the negative effects of aflatoxin B₁ in Japan, while butylated hydroxytoluene gives some protection in turkeys. Oltipraz is a drug that in the beginning was used to treat schistosomiasis, and then it was tested in human populations in China where it has shown some success (Bennett *et al.*, 2007).

1.13.5 Awareness, knowledge and perception of aflatoxins

In many areas, farmers have never heard of aflatoxin. Therefore, radio broadcasters can do a great service to farmers by passing on reliable information about aflatoxin, including the kinds of management and storage practices necessary to prevent aflatoxin contamination (Azziz-Baumgartner *et al.*, 2005; Wu *et al.*, 2011; Ngoma *et al.*, 2016). In India, farmers are not aware of aflatoxins, and they do not perceive aflatoxin contamination as a problem in their crop production systems. They do not perceive any economic risks in producing crop that may carry aflatoxin contamination since, neither are

crops prices influenced due to aflatoxin contamination nor are there any market restrictions on its sale. They also do not have information on the health risks involved in consumption of aflatoxin contaminated products (Waliyar, 2003). Rajendra *et al.* (2014) indicated that majority of consumers in India were not aware about aflatoxins contamination. In Malaysia, Sabran *et al.* (2012) indicated that having low personal income was the determinant of adults' knowledge about aflatoxins and fungal contamination in the diets and also their study shows that women had higher knowledge level of fungal and aflatoxins contamination than men. This author and others indicate that possessors of high level of education status had high level of knowledge on the occurrence of fungal infections in diets compared with those with low level of education status. On the other hand, education is positively related to awareness, knowledge and perceived benefits. Aflatoxin exposure in Ghana has been shown to be significantly correlated with farmer's knowledge of aflatoxin risk (Jolly *et al.*, 2006) while farmer's knowledge of aflatoxin risk in Benin has been correlated with the motivation to implement aflatoxin-reduction education (Jolly *et al.*, 2009).

Jolly *et al.* (2012) indicated that in Ghana health professionals and agricultural officers have had heard mycotoxins and aflatoxins but they were less aware about their effects in humans and animals. Ilesanmi *et al.* (2011) indicate that in Nigeria, health workers had awareness and good knowledge of aflatoxin contamination and the risks of its ingestion but picking up mouldy nuts to reduce aflatoxins infection were not done. In Kenya and Mali, consumers lacked knowledge about what aflatoxins were their consequences, origin and health effects. Although some farmers were aware of the consequences of contamination and exposure, knowledge about reducing risk has not been translated to action (Narrood *et al.*, 2011). According to Hell and Mutegi (2011), in Kenya, people were not aware of the risks at all levels of contamination and had insufficient knowledge on

options to reduce aflatoxin contamination from farm to fork. Public education on the health effects caused by aflatoxins and the method to manage aflatoxins at the field level must be provided regularly in order to encourage people to adopt any new technology, arise and change their behaviour to protect themselves from aflatoxins exposure.

1.13.6 Local practices to reduce aflatoxins

Rural populations generally have higher levels of aflatoxin exposure than urban dwellers in developing countries (Wild *et al.*, 2000). This is because, urban populations typically consume more diversified diets than do rural dwellers and may have food that is better controlled for contaminants. Traditional methods of cooking food with alkaline compounds (i.e. nixtamalization) are used to reduce aflatoxin exposure in the food by soaking, though the chemical reaction may involve temporary inactivation of aflatoxins, but this process may reverse the gastric acid of the stomach (Mendez-Albores *et al.*, 2004). Despite their usefulness, these methods do not all the time sound well to other communities because these communities do not accept them (Fardohan and Zoumenou, 2005).

1.14 Problem Statement and Justification

Aflatoxins are known to be highly carcinogenic and have been classified as group one carcinogens by International Agency for Research on Cancer (IARC, 1993). Thus, foods contaminated by aflatoxins represent one of the biggest global public health problems (Wu *et al.*, 2011; Strosnider *et al.*, 2006). Their effects are evidenced by an increase of extreme health conditions such as malnutrition (as indicated by stunting in children), hepatitis B and tuberculosis which suppress immune system as well as economic issues (Jolly *et al.*, 2009; Turner *et al.*, 2007; Khlangwiset *et al.*, 2010). People suffer from diseases associated with consumption of maize and groundnuts infected with aflatoxins (Wu *et al.*,

2011), and these crops are used as an ingredient in formulation of complementary foods consumed in Tanzania (Kimanya *et al.*, 2008; Mamiro *et al.*, 2005).

Aflatoxin may enter the food chain directly, through pre and post harvest contamination of susceptible crops, or indirectly, for example through contamination of milk from animals as carryover from contaminated feed (WHO, 2006). Studies in West Africa and elsewhere have shown strong links between aflatoxin levels and health in humans, especially children. Lack of awareness of aflatoxin, lack of aflatoxin resistant varieties, non-availability of cheap diagnostic kits and inadequate monitoring skills all exacerbate the problem (Jolly *et al.*, 2009).

Studies conducted in Tanzania have focused on incidences and levels of contamination (Shirima *et al.*, 2013; Kimanya *et al.*, 2014; Kamala *et al.*, 2016; Magoha *et al.*, 2014; Kimanya *et al.*, 2008). Narrod *et al.* (2011) associated lack of awareness, inadequate knowledge about aflatoxins contamination and high rate of exposure to aflatoxins, however, no study in Tanzania has addressed relationship of consumer awareness, knowledge, perception and levels of aflatoxins exposure. Therefore, it is not known whether consumers in Tanzania are aware of aflatoxin problem and whether knowledge of the problem has any association with level of exposure. Understanding the relationship between communities' awareness, knowledge and practices with aflatoxins exposure provide an opportunity for intervention to minimize exposure.

In Tanzania, there has been an inadequate understanding of the relationship between existing levels of aflatoxin contamination in complementary foods, level of community's awareness and knowledge, attitude and perception of aflatoxins contamination. There is a need to determine aflatoxin levels in household ready-to-cook complementary foods in

Dodoma and Singida regions, assess levels of awareness, knowledge and perception on aflatoxins, and practices of aflatoxin contamination and its reduction on one hand and contamination levels, on the other. Factors affecting aflatoxins reduction by the communities in central Tanzania required a detailed analysis in order to improve aflatoxins reduction and to provide important and necessary information to create strategies aimed at increasing level of awareness and knowledge about aflatoxin contamination in the complementary food.

Results from this study will be useful for understanding the levels of aflatoxins contamination in complementary foods, levels of awareness, knowledge, attitude and perception associated with levels of aflatoxins contamination in complementary foods. Information that was generated from the study also provides an insight on people's perspective of effectiveness of aflatoxins reduction from their food and identifies gaps that will form a basis for intervention. Furthermore, information from this study will give insight to policy makers on policy review, enforcement of laws and regulations and formulation of guidelines on aflatoxins management in Tanzania.

1.14.1 Objectives

1.14.1.1 Overall objective

The overall objective of the study was to examine the influence of awareness, knowledge and actions of communities on childhood dietary exposure of aflatoxins in Dodoma and Singida regions.

1.14.1.2 Specific objectives

The specific objectives of the study were to:

- (i) Measure communities' level of awareness, knowledge, attitude and perceptions of aflatoxins.

- (ii) Identify practices used that can contribute to high levels of contamination of aflatoxins in these areas.
- (iii) Identify local barriers and practices associated with reducing aflatoxins contamination in complementary foods in the community.
- (iv) Determine the level of aflatoxins in ready-to-cook foods used in complementary feeding.
- (v) Determine complementary feeding practices among infants aged between 6-24 months.

1.14.1.3 List of manuscripts

- i. Awareness of Aflatoxin Health Risks Among Parents with Children Aged Between 6-23 Months in Central Tanzania.
- ii. Parents' Practices Associated with Aflatoxin Contamination and Control of Complementary Foods in Central Tanzania.
- iii. Perception and Attitude of Parents towards Aflatoxins Contamination in Complementary Foods and their Management in Central Tanzania.
- iv. Parents' Barriers and Actions Associated with Reducing Aflatoxins Contamination in Complementary Foods in Central, Tanzania.
- v. The Influence of Awareness, Knowledge, Perception, and Actions of Community on Childhood Dietary Exposure to Aflatoxins in Central, Tanzania.

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CHAPTER TWO

2.0 Awareness of Aflatoxin Health Risks among Parents with Children Aged Between 6-23 Months in Central Tanzania

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2.1 Abstract:

In Tanzania, aflatoxin contamination and exposure in complementary foods was very high. However, it remains unknown whether the extent of the risks of exposure is linked to the levels of awareness among parents especially mothers who prepare and feed infants with cereal based complementary foods. This study was a cross-sectional study designed to assess the levels and factors of awareness of aflatoxin health risks among parents or caregivers with children in Central Tanzania. Data for the study were collected using an interview schedule which was administered to 364 households with parents/caregivers of children aged between 6-23 months, and focus group discussions (FGDs) with 121 (105 females and 16 males) participants. The results show that 82% of the parents/caregivers were not aware of aflatoxin contamination in complementary foods and their health effects. The odds [odds ratio (OR)=0.3, 95% Confidence Interval (CI): 0.1-0.6] of a parent

with low (less than US\$ 22.8) monthly income to be aware of aflatoxin contamination and its effects, was significantly ($p < 0.05$) less compared with that of a parent whose monthly income was high (more than US\$ 22.8). An employed participant (OR=13.5, 95% CI: 1.7-105.2) is significantly ($p < 0.05$) more likely to be aware of aflatoxin contamination than a farmer. The findings were complemented by results of the FGDs which showed that people were not aware of aflatoxin contamination in complementary foods. The FGD showed that participants were only aware of the presence of fungi in cereal type of foods, which leads to changes of taste and imparts unpleasant smell in foods. It is concluded that the level of awareness about aflatoxin contamination and health risks is very low in the study community. As such, there is an urgent need to raise awareness and educate parents/caregivers on aflatoxin risks associated with complementary foods in Central Tanzania.

Keywords: *aflatoxin, awareness, complementary foods, parents*

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2.2 Introduction

Aflatoxins are toxic secondary metabolites produced by molds; mainly those of the species *Aspergillus flavus* and *Aspergillus parasiticus* (Wild and Gong, 2010). These toxins are common contaminants in cereals and nuts such as maize and groundnuts where they exist in four main forms: aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁) and G₂ (AFG₂). AFB₁ is among the most potent carcinogenic compounds found in human and animal foods (IARC, 1993) Consumption of foods containing aflatoxins has been associated with liver cancer, weakened immune systems, impaired child growth and death (Williams *et al.*, 2004; Turner *et al.*, 2007). The health outcomes such as liver cancer and immune suppression can be exacerbated when such foods are deficient in essential nutrients as is the case in

developing countries where the majority of people depend on cereal-based diets (Wu *et al.*, 2011).

Acute toxicity of aflatoxin is exemplified by the aflatoxicosis which occurred in Kenya in 2004. In that year, 317 people including children became ill and 125 of them died after consuming aflatoxin contaminated cereals (CDC, 2004; Azziz-Baumgartner *et al.*, 2005; Strosnider *et al.*, 2006). Aflatoxin contamination in crops is possible along the food chain: during plant growth, maturation, harvesting, storage and processing (Cotty and Bhatnagar, 1994; Cotty and Jaime-Garcia, 2007; Cotty *et al.*, 2008). Fungi infection can be induced when maturing crop is under drought conditions and during prolonged periods of hot weather (Cotty and Jaime-Garcia, 2007; Ncube *et al.*, 2010).

Contamination during storage of the crop can occur if moisture and relative humidity, oxygen availability, temperature, time, and damaged or broken grain kernels are allowed to go to critical levels (Lanyasunya *et al.*, 2005; FAO, 1998, 2004). Crops grown in warm climate conditions have high chances of infection by aflatoxin producers and in some areas, infection occurs only when temperature rises in connection with drought (Cotty and Jaime-Garcia, 2007). A climatic change influences not only the amount of aflatoxins, but also the types of aflatoxin producers present in the area (Op.cit). Thus, drying, proper storage, and appropriate transportation are of prime importance in prevention (Williams *et al.*, 2004). Factors promoting aflatoxin contamination in developing countries include poor pre- and post-harvest handling of crops, low consumer education programmes, limited numbers of qualified personnel, inadequate surveillance programmes and enforcement of regulations (WHO, 2006).

The wide range of food products which are contaminated with aflatoxins include cereals like maize, sorghum, pearl millet, rice and wheat; oilseeds such as groundnuts, soybean, sunflowers and cotton; spices like chillies, black pepper, coriander, turmeric and zinger; tree nuts such as almonds, pistachio, walnuts and coconuts; milk and milk products (Lopez-Garcia and Park., 1998; CAST, 2003; Jolly *et al.*, 2008). Maize is probably the commodity of greatest worldwide concern because it is grown in climates that are likely to influence perennial contamination with aflatoxins, and it is the staple food in many countries (Marechera and Ndwiga, 2014). Besides, in the preparation of complementary foods, most of the people living in rural areas use local products, mainly cereals like maize, and groundnuts, which come with an added risk of exposure to aflatoxins (WHO, 2006).

Contamination and exposure of aflatoxins are often unavoidable because the levels of awareness and knowledge of aflatoxins are low among the majority of people in rural areas who rely on own grown food that cannot be subjected to regulatory controls (Azziz-Baumgartner *et al.*, 2005; Cotty and Jaime-Garcia, 2007). This explains why over 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods (Wu *et al.*, 2011). Aflatoxins are not visible, neither do they have a particular flavour hence it is not easy to convince consumers about their existence in food. A review of literature shows an association between lack of awareness and inadequate knowledge about aflatoxins contamination with high rate of exposure to aflatoxins (Jolly *et al.*, 2008; ICRISAT and NASFAM, 2009; Monyo *et al.*, 2010). In Ghana, it has been reported that women had high levels of awareness on aflatoxins than men and such education levels played a great role in addressing the problem and how to overcome the situation (Jolly *et al.*, 2008). The information gap of aflatoxin risks can be reduced through awareness campaigns (Ibid).

In Tanzania, aflatoxin contamination and exposure in complementary foods has been reported to be very high (Shirima *et al.*, 2013; Kimanya *et al.*, 2014; Kamala *et al.*, 2016). In addition, studies in the country have reported high exposure of infants and young children to aflatoxins through direct consumption of maize based diet as well as through breast milk of mothers whose main diet is maize (Shirima *et al.*, 2014; Magoha *et al.*, 2014a; 2014b). However, it remains unknown whether the parents especially mothers who prepare and feed infants with cereal based complementary foods in Tanzania are aware of the aflatoxin problem or not. Such understanding is important to develop workable measures to mitigate the problem in the country. This study, therefore, assessed the level and factors of awareness of aflatoxin health risks among parents with children in Central Tanzania.

2.3 Materials and Methods

2.3.1. Study areas

This study was carried out in four randomly selected districts of Chamwino and Bahi in Dodoma Region and Manyoni and Ikungi districts in Singida Region, in Central Tanzania. These regions experience low rainfall and short rainy seasons which are often erratic with long periods of drought. The regions were selected because of the semi-arid condition which is characterized by high temperature during the day (up to 35°C) and cool (to 10°C) during the night. Both temperature and humidity favour the growth of fungi which signaling possibility of aflatoxins production in improperly stored crops (Cotty and Jaime-Garcia, 2007).

2.3.2. Study population

The study population consisted of males and females who were parents/caregivers of children aged between 6-23 months found in the household and who reside in the randomly selected villages, streets, or hamlets in the study areas.

2.3.3 Study design

The study design is cross-sectional study which involves the data collection at one point (households). The cross-sectional design also allows one to either use the entire population or a subset. Therefore, data were collected from a few individuals to help answer research questions. Hence, this design was considered to be capable of providing some base-line information that could be used for future studies in the country. Both quantitative and qualitative approaches to data collection with pre-tested questionnaires and structured interviews were used. The pre-tests were carried out in an area not selected for the study. A multistage sampling technique was used to select 364 households. The households with children aged 6-23 months were identified after which the sampling was done. Awareness variables were derived by computing the awareness test index (ATI). A suitable scale was developed to measure parents' or caregivers' awareness of aflatoxins. The respondents' responses were recorded as correct/incorrect or yes/no against each statement of awareness. A unit score was given to correct/yes answer and zero to incorrect/no answer. The total scores obtained by a respondent for all the statements were summed up to obtain the individual respondent's score. The awareness test index (ATI) was created by summing up the number of correct responses, with a possible score ranging from 0 to 9. The generated ATI was then recorded to generate a binary variable with two levels (aware or not aware). Respondents having an ATI of 1 and above were considered to be aware of aflatoxins contamination and health risks while those with ATI equal to zero (0) were classified as not aware. All components of the study received University Research and Publication approval Ref: SUA/CB/26.

2.3.4 Data collection procedure

Six research assistants were selected by the principal researcher (PR) to aid in data collection. Research assistants were selected based on previous experience in research

work in communities and ability to understand and write Kiswahili and English languages. They were trained by the PR to understand the objectives of the study, the purpose and the procedure of the interview process; in order to have a common understanding of the questions in the interview schedule, and also to ask the questions to ensure that participants understanding them. The questions were translated from English to Kiswahili language by the PR and were explained to the participants for data collection. Quantitative data were collected by administering an interview to a random sample of 364 households with parents/caregivers of children aged between 6 to 23 months. The interview contained both open and close-ended questions developed and used to gather basic information on respondents' demographics and awareness levels of aflatoxin contamination in complementary foods as well as associated health effects after consumption.

Qualitative data were collected by using the Krueger methodology for conducting focus group discussions (FGDs) (Krueger and Casey, 2015). The questionnaire was developed after pre-tested through conducting a focused group discussion and interview involving parents or caregivers. The Focus Group Discussions were carried out after setting appointments with stakeholders (parents or caregivers) to be interviewed. The FGDs were conducted by using a checklist of the semi-structured, open-ended questions to allow the researcher guide the sessions and obtain the participants views. Participants (including parents or caregivers with children aged between 6 to 23 months) in Focus Group Discussion (FGD) were purposefully selected from the four randomly selected districts. These were the criterion that was used in inclusion and exclusion of participants. The research assistants had been trained on how to probe for specific issues when running FGDs. One pair of assistants (male and female) facilitated the FGDs by using a discussion guide and the principal researcher (PR) served as the assistant moderator. Another pair took notes during the FGDs. Information was collected by research assistants through 17

Focus Group Discussions (FGDs) with 121 participants (105 females and 16 males). The composition of all 17 FGDs was six (6) participants except six groups which had nine participants each and one group which had seven participants. The discussions were held nearby primary school class rooms, office of village leaders or office of ward executive officer. The information collected was on awareness of the crops mostly contaminated by aflatoxin or fungi, its causes, and health effects to the adults or children and also personal experiences about aflatoxin contaminations in complementary foods. The interview lasted for approximately 40 minutes for each session. All the interviews were audio recorded, after obtaining the consent from participants and then the tapes were transcribed and translated into English by principal researcher.

2.3. 5 Data analysis

The Statistical Package for Social Sciences (SPSS) program 21.0 version was used to analyze the data after cleaning. A 5% level of significance was used throughout the study and an independent variable with a p-value less than 0.05 was considered as statistically (significant) associated with outcome variable. For the qualitative part, coding was done using NVivo 7 software. The NVivo package has the ability to code and sort narrative data, interface with SPSS, and has good modelling facility and is user friendly (Hancock, 1998). It also combines best the NUD*IST computer software package with much more flexibility friendly (Hancock, 1998). The FGDs were analysed by using Thematic Content Analysis method.

2.4 Results

2.4.1 Socio-demographic characteristics of the respondents

Table 2.1 shows the distribution of parents/caregivers by socio-demographic characteristics. Generally, majority of the respondents were aged below 34 years, earned

monthly income less than US\$ 22.8, had primary education, were farmers, and were in marital union.

Table 2.1: Distribution of parents/caregivers by socio-demographic characteristics

Characteristics	Number	(%)
Age Group (Years)		
≤ 34	270	74.2
> 34	94	25.8
Monthly income (US\$)		
≤ 22.8	256	70.3
> 22.8	108	29.7
Level of education		
Never been to school	64	17.6
Partial primary	49	13.5
Primary	204	56.0
Partial secondary	18	4.9
Secondary	29	7.9
Respondent's occupation status		
Farmer	287	78.8
House wife	32	8.8
Employee	10	2.7
Petty trader	35	9.6
Marital status		
In Union	272	74.7
Not in Union	92	25.3

2.4.2 Awareness of aflatoxin contamination

The majority of respondents (82%) were not aware of aflatoxin contamination and their health effects. They scored 0 out of 9 statements while those who were aware scored the mean of 0.67 out of a scale of 1 to 9.

Table 2.2: Distribution of parents/caregivers according to their level of awareness of aflatoxins contamination (n=364)

Attribute	Number (n)	Affirmative Yes (%)
Ever heard of a mould toxin that may be present in crops	55	15.1
Ever heard of a mould toxin that may be present in food	56	15.4
Ever heard of the word “aflatoxin”	13	3.6
Aware that aflatoxins can contaminate crops on farm	21	5.8
Aware that aflatoxins can contaminate crops in storage	28	7.7
Aware that aflatoxins can contaminate food	23	6.3
Aware that aflatoxins can contaminate complementary food	26	7.1
Aware that aflatoxins can affect human health	19	5.2
Aware of one or more health effects of aflatoxins	5	1.4

Number “n” is the number of respondents who gave an affirmation to the asked question.

Multiple logistic regression models were employed to find out the demographic characteristics associated with awareness on aflatoxin contamination. The parameter estimates and associated odds ratios (OR) of the fitted models for awareness are presented in Table 3. Respondents with less or equal to US\$22.8 monthly income (OR=0.3, 95% CI: 0.1-0.6) were 0.3 times, significantly ($p<0.05$) less likely to be aware of aflatoxin contamination compared to respondents whose monthly income was higher than US\$22.8. An employed participant (OR=13.5, 95% CI: 1.7-105.2) was significantly ($p<0.05$) 13.5 times more likely to be aware of aflatoxin contamination than a farmer. Age, education level, and marital status were not statistically significantly associated with low awareness of aflatoxin contamination or health risks.

Table 2.3: Multiple logistic regression models for awareness of aflatoxin contamination

Variable	Parameter Estimate (se)	OR	95% CI	P-Value
Age (Years)				
≤ 34	Reference	Reference	Reference	Reference
> 34	-0.9 (0.3)	0.9	(0.5, 1.8)	0.8
Monthly Income (US\$)				
> 22.8	Reference	Reference	Reference	Reference
≤ 22.8	-1.4 (0.4)	0.3	(0.1, 0.6)	0.001
Education Level				
Never been to School	Reference	Reference	Reference	Reference
Partial Primary	-0.5 (0.5)	0.6	(0.2, 1.6)	0.3
Primary	-0.3 (0.4)	0.8	(0.4, 1.5)	0.4
Partial Secondary	-0.1 (0.7)	0.9	(0.2, 3.9)	0.9
Secondary	-0.6 (0.8)	0.6	(0.1, 2.9)	0.5
Occupation Status				
Farmer	Reference	Reference	Reference	Reference
House wife	0.5 (0.5)	1.6	(0.6, 4.1)	0.3
Petty trader	-0.4 (0.7)	0.7	(0.2, 2.6)	0.6
Employed	2.6 (1.1)	13.5	(1.7, 105.2)	0.01
Marital Status				
Not in Union	Reference	Reference	Reference	Reference
In Union	0.01 (0.3)	1.01	(0.5, 1.9)	0.97

Reference= The first category acts as a baseline, and can interpret the other coefficients as an increase or decrease in the log odds ratio over the baseline category

2.4.3 Themes and sub themes of awareness of aflatoxin contamination

Coded texts were categorized into three sub themes as shown in Table 4.

Table 2.4: Sub-themes: Describing awareness of aflatoxin contamination from focus group discussion participants

Sub theme	Message/finding
Crops which are mostly contaminated by aflatoxins (fungi/mouldy)	Cereal type of crops
Causes of aflatoxins (fungi) found in crops or grains that are used as food for adults or infants	Fungi (<i>ukungu</i>) when stored in moist conditions
Effects of aflatoxins (fungi) found in crops or grains that are used in preparation of food for adults or infants	Change of taste and unpleasant smell of the food, get stomach ache or flatulence

2.4.5 Awareness of the crops mostly contaminated with fungi

During FGDs, various crops were mentioned as being vulnerable to fungal contamination. The mostly mentioned as vulnerable crops were cereals mainly maize. Participants were not aware about aflatoxin. This was testified by participants who stated that, regarding these mould/fungus (aflatoxins);for them who cultivate sorghum, maize and millet/finger-millet, these crops are the most vulnerable to the “*uvundouvundo*” moist-like condition and most of the time fungi come when crops are growing, but they are not aware if it is aflatoxin“(*sumu kuvu*)”.

2.4.4 Awareness of the causes of fungifound in crops or grains that are used as food for adults orinfants

In FGDs, most of the participants mentioned moisture as the most common cause of fungal contamination. Most of them explained that when crops are either stored for a long

time or when is stored not fully dry; they always develop moulds or fungus. Participants said that in most cases, it is moisture that causes this fungus to develop or when you store crops which are not fully dry and also when these crops stay in the “*ghala*” grains store for a very long time. Also, participants were quoted saying when the cloud is heavy with signs of rainfall; storing crops during such time precipitates a high chance for the crops to be contaminated with fungus. Other participants were quoted saying, it is a thing caused by lack of seriousness when stored crops like maize. When stored these kind of crops, the place should be dry because if there is even little moist then these (fungi) develop.

2.4.6. Awareness of the health effects of fungus found in crops or grains

Participants who were aware of fungus mentioned change of taste and unpleasant smell of the food as the effects of mouldy contamination. The participants were able to link these effects to human health like stomach-ache or flatulence. Participants explained that its effect is change of taste to the food and sometimes the smell becomes abnormal. Thus, it is normally important to wash the contaminated grains very well in order to reduce the smell. Also, others said that honestly, they were not aware of the health effects but what they know is that when you use such crops which have stayed for a very long time, even if you wash them the flour taste becomes sour.

Many of the qualitative themes are aligned with quantitative data. The merged responses about awareness are shown in Table 2.5.

Table 2.5: Qualitative and quantitative information about awareness of aflatoxin or fungi contamination

Qualitative findings (n=121)	Quantitative findings n=121)	Number	(%)
Aware that moulds infect cereal type of crops	Aware that aflatoxins can contaminate crops at the farm	21	(5.8)
Aware that fungi infect foods when stored in moist conditions	Aware that aflatoxins can contaminate crops in storage	28	(7.7)
Change of taste and unpleasant smell of the food	Aware that aflatoxins can contaminate food	23	(6.3)
Change taste of flour and becomes sour	Aware that aflatoxins can contaminate complementary food	26	(7.1)
Aware that contaminated food can cause health problem	Aware that aflatoxins can affect human health	19	(5.2)
Stomach ache or flatulence	Aware of one or more health effects of aflatoxins	4	(1.4)

2.5 Discussion

This study investigated the level and factors of awareness of aflatoxin health risks among parents who prepare and feed infants with cereal-based complementary foods in Central Tanzania. The study showed that 82.0% of parents were not aware of aflatoxin contamination in complementary foods and their health effects. The finding from this study is similar to those reported in Malaysia (Sabran *et al.*, 2012) and Ethiopia (Ephrem *et al.*, 2014), Benin, Ghana, and Togo (James *et al.*, 2007); and India (Rajendra *et al.*, 2014). As it was previously reported about Kenya and Mali; awareness and knowledge of potential danger posed by aflatoxin contamination in foodstuffs is extremely low (Narrood *et al.*, 2011). Also, in Uganda, the majority of farmers, traders and consumers are not aware of the aflatoxin contamination in foods (Kaaya. and Warren, 2005). However, these results contradict the finding of another study from Malawi where the level of awareness

was higher, 65% (Smith and Moss, 1985; ICRISAT and NASFAM, 2009) and Lower Eastern Kenya where the level of awareness was 59% (Daniel *et al.*, 2011; Marechera and Ndwiga, 2014). The higher level of awareness in Malawi and Kenya was attributed to high literacy levels of the communities and many outbreaks experienced in the countries. Also in Malawi, 80% of the farmers had experienced aflatoxin problems in their households (Monyo *et al.*, 2010).

The study also sought to explore whether people who were aware of aflatoxins know the types of crops which are highly susceptible to aflatoxin or fungal/mouldy contamination. Of the Parents or caregivers who were aware, 5.8% showed that cereal type of crops are the most vulnerable to fungal or mould. Participants in the FGDs who indicated that they were aware mentioned cereal type of crops as the most vulnerable to fungus/mould (aflatoxin) contamination including maize, sorghum, and millet. Similar finding was reported by a study conducted in Kenya where farmers were found to be aware of the main crops mostly affected by aflatoxins namely maize, sorghum, cassava, and millet (Marechera and Ndwiga, 2014). In Kenya, however, farmers were highly aware of the aflatoxin problem and knew that the contamination in maize was caused by *Aspergillus* spp arising from high moisture content either during harvesting or in storage (Marechera and Ndwiga, 2014). Of the parents or caregivers who were aware, 7.7% were aware that crops could be contaminated during storage in moist conditions. In general, most people in the FGDs explained that when crops were either stored for a long time or when stored not fully dried, they always developed moulds or fungus. These results are similar to those reported by studies performed in other developing countries (Smith and Moss, 1985; Williams *et al.*, 2004) which revealed that farmers were aware that *Aspergillus* spp growing in maize arise from high moisture content either during harvest or in storage. In addition, the findings (Daniel *et al.*, 2011) confirm that high levels of humidity,

temperature and poor aeration during storage are important factors that may contribute to aflatoxin contamination. In another study done (Hawkins *et al.*, 2005) further emphasizes that aflatoxin contamination can occur when food commodities are stored under high moisture and temperature conditions. Of the parents who were aware of the effects of aflatoxin in foods or complementary foods, 6.3% and 7.1% of the parents respectively, were aware that change of the taste and unpleasant smell of foods were due to aflatoxin or mouldy or fungi contamination; and the minority 1.4% were aware that their health could be effected if they consumed such foods as they reported during FGDs that it could lead them to abdominal ache. This is consistent with past studies conducted by Maren (2002) who reported that bad smell and decay might be due to activities of *Aspergillus* fungi which normally lead to food decomposition, and that members of *Aspergillus* genus are more heat tolerant and xerophilic than other fungal species. In addition, Williams *et al.* (2004) reported that chronic dietary exposure to low doses of aflatoxins is a risk factor for liver cancer and may also affect protein metabolism and immunity, hence worsening infectious diseases and malnutrition. Consuming highly contaminated foods with aflatoxin results in acute exposure known as aflatoxicosis and the symptoms include vomiting, jaundice and abdominal pain, and can lead to liver failure and death. No specific treatment has been found for acute aflatoxicosis (Ibid).

With regard to demographic factors associated with awareness of aflatoxin contamination, the study found that parents with lower monthly income were less likely to be aware of aflatoxins contamination compared to respondents whose monthly income was higher. These results are similar to those from a study in Ghana (Jolly *et al.*, 2008) and Malaysia (Sabran *et al.*, 2012) which found that the respondents with higher income were more aware of aflatoxin contamination and its health risks than those with lower income. During focus group discussions, low household income was identified as a factor responsible for

food insecurity. The parents in the current study believed that low household income was responsible for food insecurity. Another important predictor of awareness of aflatoxin contamination was occupation. Employed participants were significantly more likely to be aware of aflatoxin contamination than farmers. These results are similar to those of the study done in Ghana Jolly *et al.* (2008) which revealed that employed (health and agricultural professionals) perceived that there were significant economic and health benefits to be obtained from reducing the level of aflatoxin contamination and were more likely to discuss the problem of aflatoxin with colleagues and subordinates.

Generally, awareness of aflatoxin contamination of crops and foods used in the preparation of complementary food and the relationship of its health effects was low among parents in the study areas. Parents' unawareness on aflatoxin contamination in this study area could be a danger to their food security and health because it creates possibilities for them and their livestock to consume aflatoxin contaminated foods. Therefore, there is a need to create public awareness of the potential harmful effects of aflatoxin. Awareness raising campaigns can be carried out through appropriate media such as radio, television, newspapers and drama, existing system of government extension workers, health workers, and existing community groups in the study areas.

2.6. Conclusion

It is concluded that the level of awareness on aflatoxin contamination and health risks is very low in the study community. Parents/caregivers with lower (less than US\$ 22.8) monthly income were less likely to be aware of aflatoxin contamination compared to those whose monthly income was higher (more than US\$ 22.8). Employed parents were significantly more aware of aflatoxin contamination than farmers. Inconsistent and low awareness of aflatoxin contamination among parents or caregivers put the children at

increased health risks in the community. As such, there is an urgent need to raise awareness and educate parents on aflatoxin risks associated with complementary foods in Central Tanzania. Awareness raising campaigns can be carried out through appropriate media such as radio, television, newspapers and drama, existing system of government extension workers, health workers and existing community groups in the study areas.

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CHAPTER THREE

3.0 Parents' Practices Associated with Aflatoxin Contamination and Control of Complementary Foods in Central Tanzania

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3.1 Abstract

Parents' practices that are associated with aflatoxin contamination and control in complementary foods were studied in Central Tanzania. A descriptive cross-sectional survey using interviewer-administered structured pretested questionnaire was conducted among 364 randomly selected parents of children aged between 6-23 months, and the mean age (SD) of the respondents was 30 (8.3) years old. The majority 33.2% of the participants harvest their crops in April followed by June which is 26.6%, March which is 21.2% and May which is 19% of all the participants. Most processing activities like dehulling, milling, drying, storage were analysed. The statistical packages SPSS (version 21) computer software packages were used to analyze the data. The results of logistic regression model for dehulling crops confirmed that respondents with less than or with US\$ 22.8 as monthly income (OR=0.250, 95% CI: 0.111-0.564) were significantly 0.3 less

likely to dehull crops ($p < 0.05$) than respondents who earned more than US\$. 22.8. On the other hand, petty trader participants (OR = 3.712, 95% CI: 1.420-9.699) were significantly almost 4 times more with a tendency of dehulling the crops ($p < 0.05$) than farmers. The study team recommends that parents should be trained on appropriate methods of drying, storage, and dehulling their crops after harvesting in order to control fungal and aflatoxin infestation. In addition, research on harvesting time, drying, storage, and dehulling practices of crops in Tanzania is needed.

Keywords: *aflatoxin, parents, post-harvest practices, complementary foods*

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3.2 Introduction

Aflatoxins are naturally occurring toxins produced by certain fungi, most importantly *Aspergillus flavus* and *Aspergillus parasiticus*, and they are widely recognized as a major health problem especially in hot, humid countries (Bennett and Klich, 2003; CAST, 2003; Williams *et al.*, 2004; Manetta, 2011; Ephrem, 2015). Major types of aflatoxins are B₁, B₂, G₁, and G₂; and metabolites of B₁ and B₂ are M₁ and M₂ respectively. AFB₁ is the most potent of the aflatoxins. Aflatoxins have been rated as class 1A carcinogens by the International Agency for Research of Cancer (IARC, 2002; 2012). They are heat stable and difficult to destroy during processing. Aflatoxins B₁ (AFB₁) and B₂ (AFB₂) produced by *A. flavus* and aflatoxins G₁ (AFG₁) and G₂ (AFG₂) produced by *A. flavus* as well as *A. parasiticus* can contaminate not only maize and other cereals such as wheat and rice, but also groundnuts, pistachios, cottonseed, copra, and spices (Rustom, 1997; Piermarini *et al.*, 2009).

In many developing countries, poor diet and multiple communicable diseases are associated with malnutrition and growth faltering in infancy and childhood. In addition, dietary staples in some of these regions are frequently contaminated with fungal toxins such as the aflatoxins

(Wild and Gong, 2010). Aflatoxin production normally occurs in the field, particularly when stimulated by drought, stress, and high temperatures or during prolonged drying (Cotty and Jaime-Garcia, 2007; Wu *et al.*, 2011; Hamidou *et al.*, 2014; Farfan *et al.*, 2015).

Aflatoxins contaminate many African dietary staples such as maize, groundnuts, rice and cassava, particularly under certain conditions like dry weather near crop maturity, high moisture during harvest, and inadequate drying and storage of crops (Hell *et al.*, 2010). Contamination can occur at any time from pre-harvest to storage of the crops (Wagacha and Muthomi, 2008; Hell *et al.*, 2010; Hell and Mutegi, 2011; Ephrem, 2015). Food contaminated by aflatoxins is one of the most serious consequences that poor post-harvest management can have (Hell *et al.*, 2011). Poor post-harvest in warm humid areas and bad storage practices lead to rapid growth of the fungi and hence higher levels of toxins can occur (Hell and Mutegi, 2011; Craufurd *et al.*, 2006). This particularly happens in developing countries where appropriate measures for preventive actions are often overlooked (ICRISAT, 2000; ICRISAT and NASFAM, 2009). Crops commonly affected by aflatoxins include maize, groundnuts, cottonseed, sorghum, millet, rice, Brazil nuts, pecans, walnuts, pistachio nuts, sesame and spices (particularly chillies), cassava, potatoes, legumes, pigeon peas, sunflower, simsim, peanuts, and products made from these crops (Strosnider *et al.*, 2006; Mkoka, 2007).

Aflatoxin contamination in cereal grains is a worldwide concern especially in sub-tropical and tropical areas (Strosnider *et al.*, 2006; Guan *et al.*, 2011). In East Africa, aflatoxin exposure has also been directly correlated with reported daily intake of maize and fumonisin exposure which occurs almost entirely from maize (Kimanya *et al.*, 2008). Another major source of exposure to aflatoxin is through the consumption of groundnuts (Liu and Wu, 2010; IARC, 2012).

Tanzania is among African the countries which lie in the latitudes between 40°N and 40°S which are susceptible to aflatoxin contamination (NBS, 2012; Abt Associates, 2012; URT, 2013). Prevalence of aflatoxins contamination in crops in Tanzania is higher than that of the European Union aflatoxin standard (4 ppb) and that of USA (20 ppb) and in many countries (Abt Associates, 2012; Shirima *et al.*, 2013; Kimanya *et al.*, 2014; Kamala *et al.*, 2016). Due to food shortage and low knowledge on fungal and aflatoxins contamination, these families sometimes eat dehulled, unsorted and mouldy crops without washing or winnowing them hence exposing themselves to high health risks of aflatoxin contamination in their diet. The specific objective of this study was to identify practices used that can contribute to levels of contamination of aflatoxins in complementary food in the households with children aged between 6-23 months in Central Tanzania. However, there is limited information on the parents' practices of post-harvest of crops which are used in the preparation of complementary food, aflatoxin contamination and control, and their associated health problems in Bahi, Mundemu, Handali, Mvumi Mission, Ikungi, Puma, Manyoni, and Maweni ward. These places are found in Dodoma and Singida regions in Central Tanzania. This study is part of a larger study with the overall objective of examining the influence of awareness, knowledge and actions of communities on childhood dietary exposure to aflatoxins in Dodoma and Singida regions.

3.3 Materials and Methods

3.3.1 Research setting

The study was carried out in four districts of Bahi and Chamwino (in Dodoma region) and Manyoni and Ikungi (in Singida region) in Central Tanzania. Figure 3.1 and Figure 3.2 show a map of the study areas. According to the Tanzania National Bureau of Statistics (NBS, 2012), the population of Bahi District was 221,645, Chamwino was 330,543, Manyoni was 296,763, and Ikungi was 272,959. The major socio-economic

activities found in the study areas are agriculture and livestock keeping. The major crops grown include maize, sorghum, millet, and groundnuts. Other crops are cowpeas, sunflower, cassava, Bambara nuts, paddy, simsim, and sweet potatoes [28, District Agricultural Officers]. The non-agricultural activities which include shop keeping, local brewing, civil services and petty trade are commonly practiced in towns and village centres (Morris *et al.*, 2001). A multistage sampling technique was used to select a total of 364 parents/caregivers with children aged between 6-23 months to participate in the study. The process involved simple random sampling at district, division, ward, village/street and sub-ward up to household level. Bahi District consists of 20 wards and out of these, two wards were selected randomly (Bahi and Mundemu) and from these wards, one village/street was selected from each (Bahi Sokoni and Mundemu respectively). Chamwino District consists of 32 wards and out of these two wards were selected randomly (Handali and Mvumi Mission) and from those two wards, one village/street was selected from each (Handali and Ndebwe respectively). Manyoni District consists of 30 wards and out of these, two wards were selected randomly (Manyoni and Maweni) and from those two wards, one village/street was selected from each (Manyoni and Maweni respectively). Ikungi District consists of 26 wards and out of these, two wards were selected randomly (Ikungi and Puma) and from these wards, one village/street was selected from each (Ikungi and Puma respectively). The areas were selected because of their semi-arid condition which is characterized by high temperature during the day (up to 35°C) and cool (to 10°C) during the night. Both temperature and humidity favour the growth of fungi (Op.cit) signalling possibility of aflatoxins production (Cotty and Jaime-Garcia, 2007).

3.3.2 Research design

The research design was cross-sectional study. It involved the collection of data at one point (households) in line with the limited resources that were available for the study. Quantitative research method was employed in this study. A structured interview with both open and close-ended questions was developed and used to gather basic socio-demographic information from the participants with regard to post-harvest activities related to aflatoxin and its contamination in complementary foods and its health effects after consumption. Hence, this design was considered to be capable of providing some base-line information that could be used for future studies in the country.

3.3.3 Data analysis

Descriptive statistics (frequencies and percentages) were calculated to give characteristics of variables by using Statistical Package for Social Sciences (SPSS version 21.0) to analyze data after data cleaning. A 5% level of significance with 95% Confidence Interval (CI) was used throughout the study and an independent variable with p-value less than 0.05 was considered as statistically (significantly) associated with outcome variable. Logistic regression was done to identify which factors predicted parents/caregivers practices to minimize aflatoxins in complementary foods of their children.

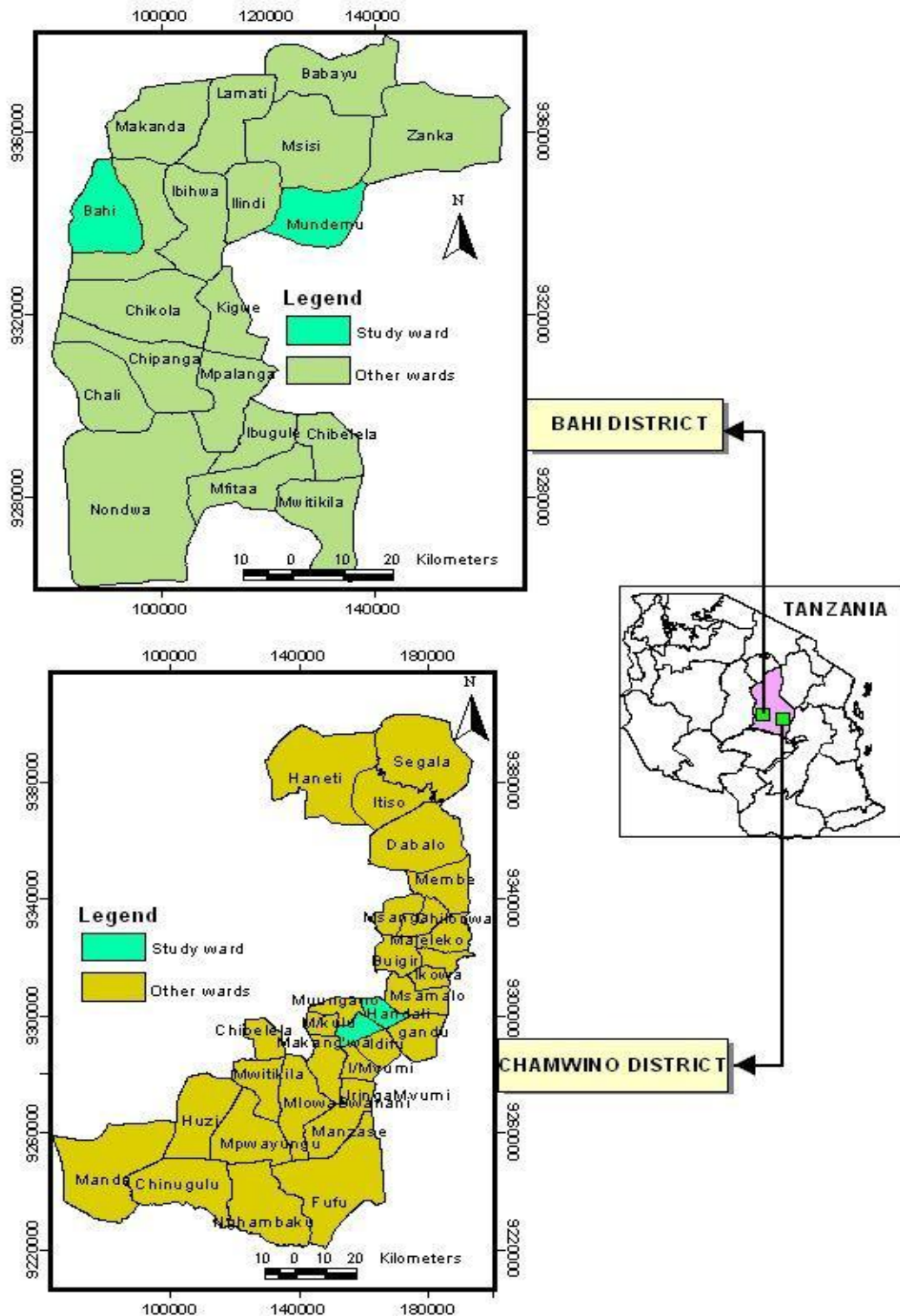


Figure 3.1: The maps of Bahi and Chamwino districts in Dodoma region showing study areas

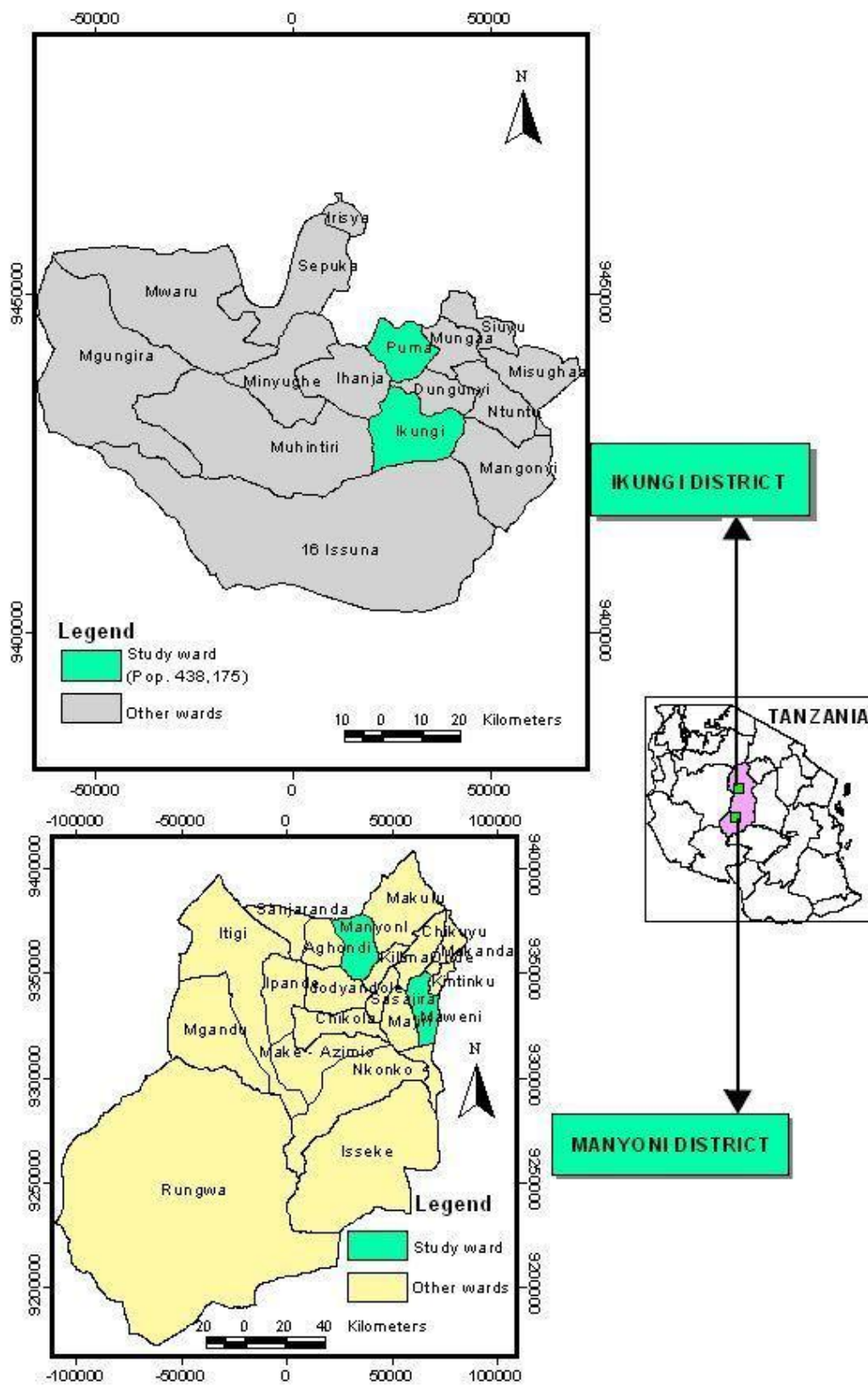


Figure 3.2: The maps of Ikungi and Manyoni districts in Singida region showing study areas

3.4 Results

3.4.1 Socio-demographic characteristics of respondents

Results in Table 1 show the distribution of parents/caregivers by socio-demographic characteristics in Bahi, Chamwino, Ikungi, and Manyoni Districts. The age of parents/caregivers ranged from 17 to 80 years with mean age (SD) of 30 (8.3) years. About 270 (74.2%) of the respondents were aged below or 34 years while respondents aged above 34 years were 94 (25.8%). Mothers made up 331 (90.9%) of the respondents.

Table 3.1: Distribution of parents/caregivers by socio-demographic characteristics

Characteristics	Number	(%)
Age Group (Years)		
≤ 34	270	74.2
> 34	94	25.8
Monthly income (US\$)		
≤22.8	256	70.3
>22.8	108	29.7
Level of education		
Never been to school	64	17.6
Partial primary	49	13.5
Primary	204	56.0
Partial secondary	18	4.9
Secondary	29	7.9
Respondent's occupation		
Farmers	287	78.8
House wives	32	8.8
Employees	10	2.7
Petty traders	35	9.6
Marital status		
In Union	272	74.7
Not in Union	92	25.3

3.4.2 Harvesting time recorded during the interview

The majority, 33.2%, of the participants harvests their crops in April followed by June which is 26.6% and March which is 21.2% of all the participants. Only 19% of the people in study areas harvest their crops in May every year depending on the type of crops planted.

3.4.3 Dehulling practices of crops used in the preparation of complementary foods

The majority, 81.3%, of the respondents were not dehulling the crops used in the preparation of complementary foods. Multiple logistic regression model was employed to find out how awareness and demographic characteristics were associated with dehulling of crops. The parameter estimates and associated odds ratios (OR) of the fitted model for dehulling the crops are presented in Table 2. The results of the logistic regression model for dehulling the crops confirmed that respondents with less than or with US\$ 22.8 as monthly income (OR=0.250, 95% CI: 0.111-0.564) were significantly less likely to dehull crops ($p < 0.05$) than respondents who earned more than US\$ 22.8. On the other hand, occupation of the respondents was also a predictor of dehulling the crops. Petty trader participants (OR =3.712, 95% CI: 1.420-9.699) had significantly more tendency of dehulling the crops ($p < 0.05$) than farmers. Though not significant ($p = 0.2673$), employed (OR=3.349, 95% CI: 0.396-28.326) and housewives ($p = 0.7789$, OR=1.161, 95% CI: 0.409-3.295) also had higher odds of dehulling the crops compared to farmer respondents. Other independent variables, namely age, education level, and marital status were not significantly associated with dehulling of crops.

Table 3.2: Parameter estimates and odds ratios for dehulling crops

Variable	Parameter Estimate (se)	OR	95% CI	P-Value
Awareness				
No	Reference	Reference	Reference	Reference
Yes	-0.2571 (0.3973)	0.773	[0.355-1.685]	0.5176
Age (Years)				
≤ 34	Reference	Reference	Reference	Reference
> 34	0.2625 (0.3199)	1.300	[0.695-2.434]	0.4120
Monthly income (US\$)				
> 22.8	Reference	Reference	Reference	Reference
≤ 22.8	-1.3873 (0.4154)	0.250	[0.111-0.564]	0.0008
Education Level				
Never been to School	Reference	Reference	Reference	Reference
Partial Primary	0.5092 (0.5416)	1.664	[0.576-4.810]	0.3471
Primary	0.6309 (0.4264)	1.879	[0.815-4.334]	0.1390
Partial Secondary	0.2819 (0.7828)	1.326	[0.286-6.148]	0.7188
Secondary	0.1586 (0.7727)	1.172	[0.258-5.329]	0.8373
Occupation				
Farmer	Reference	Reference	Reference	Reference
House wife	0.1494 (0.5322)	1.161	[0.409-3.295]	0.7789
Petty trader	1.3115 (0.4901)	3.712	[1.420-9.699]	0.0074
Employee	1.2085 (1.0894)	3.349	[0.396- 28.326]	0.2673
Marital status				
Not in Union	Reference	Reference	Reference	Reference
In Union	0.5465 (0.3540)	1.727	[0.863, 3.456]	0.1226

(se)=standard error

Reference= The first category acts as a baseline, and can interpret the other coefficients as an increase or decrease in the log odds ratio over the baseline category

3.4.4 Community practices on handling crops

In this study, the majority, 90.1%, of all participants dried their crops before storage. Only, 9.9% of them did not dry their crops. The majority of the participants, 90.9%, were milling their crops which were used in the preparation of complementary foods. During the survey, about half, 49.2%, of the respondents were seen drying their crops on roofs (their roofs were normally covered with mud), while 32.7% of the participants placed their crops directly on the soil for drying and 8.2% of them dried in traditional granaries. Only 9.9% of the respondents did not dry their crops at all (Figure 3.3).



Figure 3.3: From left and down: Drying crops directly on the soil and on the roof

The findings show that 90.9% of the respondents normally stored their crops after harvesting while the rest did not. In addition, results revealed that almost half, 50.5%, of the respondents stored their crops in bags, 40.4% stored in the traditional granaries and only 9.1% of them did not store their crops at all. Also, in the study areas, the majority, 72.5%, of the participants used their home grown crops for food consumption while 27.5% of them used them for both consumption and selling. Further results from this study revealed that more than half, 54.4%, of the respondents declared that their crops usually got mouldy during storage. Moreover, the majority, 36.5%, of the parents provided both special and family foods to their babies while others provided only family food. The study also revealed that the majority, 53.0% of the parents, after harvesting, gave the mouldy feed to animals while 38.5% of participants used it in local brewing and 8.5% consumed the mouldy food. In addition, the majority, 54.4%, of the participants said that they left their residual crops after harvest in the field while 36.5% gave them as feed to animals.

3.4 Discussion

This study investigated the parents' practices that contribute to aflatoxin contamination and control in complementary foods in Central Tanzania. A total of 364 parents with children aged between 6-23 months participated in the study. Harvesting time was very important in aflatoxins contamination and control. Timing of the harvest was a key factor in aflatoxins reduction. Harvesting crops in March or April was called early harvesting in the study areas. Early harvesting and the rapid drying of crops in moisture content levels below 15% are believed to effectively stop aflatoxin accumulation (Gourama and Bullerman, 1995). However, in these study areas, nothing was used to measure the moisture content due to lack of money to buy the instrument. In that situation, food quality was likely to be compromised. In these study areas, late harvesting was between May and June during which the crops could easily get contaminated by fungus since the crops had

over matured, cracks, birds were prone to damage and insects could infest them either on the ground soil or as standing plants. The influences of delayed harvest on contamination are most severe when crops are caught by rain just prior to or during harvest (Jaime-Garcia and Cotty, 2003). Therefore, late harvesting (after the second half of May to June) in the study areas not only causes an increase in cracking of some crops but also provides further exposure to aflatoxin production and contamination. Damage by birds, pests, shell discoloration and falling down of crops/seeds on contaminated soil surface are other risk factors associated with late harvesting (Sommer *et al.*, 1986; Doster and Michailides, 1999; Moradi, 2005; Moradi and Javanshah, 2005; Moradi and Mirabofathy, 2007). Delayed harvesting occurred in areas where farmers left the crop to dry completely on the field as it happened in some places in the study areas.

Dehulling is the process of removing the pericarp from the grain. In these study areas, dehulling was done by using stones or mortar and pestles, the same as what was reported by Fandohan (2004) in Benin, West Africa. Generally, in these study areas dehulling was done by women and this is also the same as revealed in Benin (Ibid). It has been reported that dehulling removes most of the toxins in the bran and germ fractions (Fandohan *et al.*, 2006). From the survey done by Abt Associates, (2012; 2013) in three districts (Kongwa, Bukombe, and Njombe) in Tanzania, it was revealed that farmers did not know about aflatoxins. Their level of knowledge was very low and agricultural extension officers were not trained in mycotoxin and aflatoxin, the same as it was the case in the current study. Furthermore, parents with high monthly income were more likely to dehull the crop than those with low monthly income. In addition, parents who were petty traders were more likely to dehull their crops than farmers. Therefore, it is very important to train parents about mouldy infestation in the crops and aflatoxin and its control because they use it as blended flour that is mixed with maize, groundnuts and others crops in the preparation of

complementary foods. In the study done in Kenya, the results revealed that reduction in aflatoxin levels was realized during dehulling of maize grains as evidenced by the lower levels of aflatoxins in de-hulled maize; the percentage of reduction in aflatoxin levels was between 5.5 % and 70.0 % with a mean of 46.6 % (Mutungi *et al.*, 2008). Also, in the study done in Zimbabwe, it was revealed that undehulled crops had higher percentage of aflatoxin levels when compared to the dehulled crops (Siwela *et al.*, 2005). Also these results emphasize that there is a need of dehulling the crops before eating. In Benin-West Africa, women used some unit operations like sorting, winnowing, washing, crushing, and dehulling to remove significant amounts of aflatoxins and fumonisins in maize and maize products (Fandohan *et al.*, 2005). Also Park (2002) noted the effects of processing aflatoxin reduction. Furthermore, drying, storage, and dehulling reduce aflatoxin levels and can be recommended as a decontamination method developed mainly in the African countries where it is still uncommon but these technologies should be improved to reduce women workload. Therefore, it is revealed in the current study that people might have been consuming contaminated foods through their diets because the majority does not de-hull their crops before milling or cooking in the preparation of foods for the babies or food for the family.

This study revealed that parents dried their crops on the roof (mud roofed) and others put them directly on the soil while few used traditional granaries or containers made by thatches or bamboo. These results are not similar to the study done Narrod (2013) which revealed that about half of the families surveyed in Kenya reported that they take maize home to dry in tarpaulins, which would limit direct exposure of the maize to dirt. However, this study reflects the one done in Mali because nearly half of those families surveyed reported that they dried groundnuts in large piles on the ground. Such direct contact with the soil is problematic because aflatoxins are a toxic substance emitted by

fungi that are plentiful in agricultural soil in the tropics and sub-tropics regions (Strosnider *et al.*, 2006; Abbas *et al.*, 2009).

As it was revealed in the current study, the greater part of parents normally stored their crops after harvesting while others did not. Again, as it has been explained that poor post-harvest practices and storage conditions are known to increase aflatoxin prevalence. This study revealed that almost half, 50.5%, of respondents stored their crops in the polythene (nylon) bags, 40.4% stored their harvested crops in the traditional granaries and only 9.1% of them did not store their crops. These results contrast with the study done in Kenya which observed that about 30 percent of the respondents reported that they left maize uncovered in the fields and the most common reported storage practices were either using a room in the house or an improved granary with a wooden wall (Bett *et al.*, 2012). As it is in Tanzania, Kenya and a few Malian farmers, use of the storage structures as traditional granaries is most common. As a result of poor food storage, aflatoxin levels are increased in storage and in the markets, suggesting that current crops drying and storage practices are inadequate. These may be due to not only lack of knowledge on the importance of storing crops for future use, but also some of them did not have money to buy bags for storage and did not have enough food for storage. For example, there were no pallets or wood used during storage but farmers put their crops directly on the floor making it easy for the crops to be infested by insects such as termites and rodents. It was further revealed that sometimes these farmers used nylon bags (sacks) during storage of crops because they were cheap and easily available compared to sisal sacks. Also this study revealed that almost more than half of the respondents declared that their crops got mouldy during storage but they did not know whether it was aflatoxin or not because the word “aflatoxin” was a new terminology to them. This is inconsistent with the findings Strosnider *et al.* (2006) who reported that it is suitable to keep the grains from contact with the soil, keep

them in wooden pallets or on concrete floor and ensure adequate ventilation in the storage facility which helps to prevent an increase in moisture content, insect, and rodent infestation during storage; for that is a critical measure against aflatoxin contamination. Also, Lanyasunya *et al.* (2005) in Kenya reported that the majority of the farmers did not protect their grains against scorching sunshine, termites and pests damage, and more importantly, mould colonization. Mwihia *et al.* (2008), in his study on aflatoxins level on locally grown maize from Makueni district in Kenya noted that nylon sacks (bags) were commonly used as grain storage containers by 89 % of the respondents. These sacks maintain moisture and prevent free air circulation within the grain store hence they promoting aflatoxin contamination while the sisal sacks which are known to keep minimal moisture content reduce aflatoxin contaminations and were used by only 10 % of the people.

This study shows that more than half of the parents provided animals (cow, hens and ducks) with the mouldy feeds after harvesting while some of them were using them as local brewing ingredients and others consumed the mouldy food by mixing it with good one and milling it without even sorting or winnowing. Parents used mouldy food both for special and family food for their children, thus exposing themselves to great health risks of eating contaminated foods with aflatoxins. The parents in these study areas should be trained on appropriate methods of drying, storage and dehulling their crops after harvesting in order to control fungal and aflatoxin infestation. Also, harvesting time is vital in order to manage aflatoxin contamination.

3.5 Conclusion

Most of the parents in these study areas engage in small-scale, mixed farming that includes some livestock. Maize, sorghum, and groundnuts are the primary dietary staple and the main

crops produced in these study areas. These crops are very susceptible to mouldy if inappropriately processed. At harvest, farmers store most of their crops for household consumption in poor storage facilities and sell the rest (if they harvest enough) to meet other household needs. When household crops stored are finished, farmers sell their livestock and purchase crops from the market. Due to food shortage and low knowledge on fungal and aflatoxins contamination, these families sometimes eat undehulled, unsorted and mouldy crops without washing or winnowing them hence exposing themselves to high health risks of aflatoxin contamination in their diet.

This study strongly recommends that parents in Central Tanzania need to be made aware of potential health dangers of fungal and aflatoxin production in their crops through enhanced involvement of agricultural extension services in farming communities. The agricultural extension officers and village health officers need to be trained on aflatoxin awareness and knowledge since they are also unaware of these aflatoxins. The agricultural extension officers need to know issues that can result in fungal infection and aflatoxin production and how contamination of crops can be reduced before they are used in the preparation of complementary foods. The agricultural extension officers should in turn advise farmers on good agricultural practices that can reduce aflatoxins contamination of crops such as good pre and post harvesting practices.

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CHAPTER FOUR

4.0 Perception and Attitude of Parents towards Aflatoxins Contamination in Complementary Foods and Its Management in Central Tanzania

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4.1 Abstract

Complementary foods in Tanzania are contaminated with aflatoxins (AF), a group of highly toxic metabolites produced mostly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Although intake of aflatoxins contaminated food may cause cancer of the liver, very few parents perceive aflatoxins exposure as a public health threat. This study was cross-sectional designed and used both quantitative and qualitative approaches to assess perception and attitude towards aflatoxins contamination in child foods and its management among 364 parents with children aged between 6-23 months in central Tanzania. Health Belief Model (HBM) was used to determine parents' perception and attitude of aflatoxins. Exploratory Factor Analysis identified the underlying constructs of

the Health Belief Model (HBM). The multivariate analysis indicated that mean perception score for parents aged above 34 years was significantly higher ($\beta=0.3666$, $p<0.05$) compared to those aged below or equal 34 years. The mean perception score for parents with primary education was significantly higher ($\beta=0.3730$, $p<0.05$) in comparison to mean score of those that had never been to school. The estimated mean attitude towards aflatoxins score for parents in union was significantly higher ($\beta=0.2639$, $p<0.05$) compared to those not in union. Parents with primary education and those with secondary education ($\beta=0.3405$, $p<0.05$) and ($\beta=0.5528$, $p<0.05$), respectively, were significantly important predictors of attitude for actions towards aflatoxins reduction. There was strong association between perception and attitude scores towards aflatoxin contamination and reduction to the foods. The findings were complemented by results of the focus group discussions (FGDs) which showed that people were not provided with education about aflatoxin contamination in complementary foods, although few of them were using their experience to control fungi. Health professionals and public extension officers should work together to advise the people about the problem of aflatoxins and means to prevent its occurrence in complementary foods.

Keywords: *aflatoxin, complementary foods, parents, perception, attitude, health belief model*

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4.2 Introduction

Aflatoxins, are among the most potent carcinogenic compounds found in human and animal foods (IARC, 1993). These are fungal toxin that commonly contaminates maize, nuts, and other types of cereals are notorious not only for the destruction they cause to crops but also the health effects they inflict on humans and animals (Wild and Gong, 2010). In developing countries, many people depend on consumption of largely cereal-based diets (Wu *et al.*, 2011) that in addition to being deficient in essential nutrients

contain aflatoxins. Feeding on aflatoxin contaminated food may contribute to nutritional deficiency and lead to growth failure (Gong *et al.*, 2008). In 2004, 317 people including children became ill and 125 of them died in the central provinces of Kenya by consuming contaminated food (CDC, 2004; Azziz-Baumgartner *et al.*, 2005; Strosnider *et al.*, 2006). Williams *et al.*, 2004 have indicated that over 5 billion people in developing countries worldwide are at risk of chronic exposure to aflatoxins through contaminated foods. The effects of this exposure include liver cirrhosis, intestinal dysfunction, immune suppression, and increased susceptibility to some infectious diseases including HIV-AIDS, and maternal and child health problems such as anaemia, malnutrition, stunting and wasting (Turner *et al.*, 2007).

Infection of crops with aflatoxins producing fungi is most common in the tropical regions in which humidity and temperature are high (IARC, 1993; Schmale and Munkvold, 2012). The mould is capable of attacking crops during production, harvest, storage, and even during processing and is now recognized as one of the biggest challenges to food and nutrition security, health and trade across the African continent (Cotty and Bhatnagar, 1994; Cotty and Jaime-Garcia, 2007; Cotty *et al.*, 2008). Contamination during storage of the crop can occur if moisture and relative humidity, oxygen availability, damaged or broken grain kernels are allowed to go beyond critical values (Lanyasunya *et al.*, 2005). Crops grown in warm climates conditions have greater chances of being infected by aflatoxin and in some areas, infection occurs when temperatures rise in connection with drought and influences not only the amount of aflatoxins, but also the types of aflatoxin to be produced (Cotty and Jaime-Garcia, 2007). The most critical environmental factors that determine whether or not a substrate will support mould growth are moisture content, temperature and time (FAO, 1998; FAO, 2004; Ncube *et al.*, 2010). Thus, drying, proper

storage and suitable transportation are of prime importance in prevention (Williams *et al.*, 2004).

The wide range of food products which are contaminated by aflatoxins include cereals like maize, sorghum, pearl millet, other crops as well as milk and milk products (Lopez-Garcia and Park, 1998; CAST, 2003; Jolly *et al.*, 2009). Maize is probably one of the major staple food in many countries and therefore of major concern, because it is grown in climates that are likely to have perennial contamination with aflatoxins (Marechera and Ndwiga, 2014). Most of the people living in rural areas use local products in preparation of complementary foods, mainly cereals like maize, and groundnuts, which come with an added risk of exposure to aflatoxins (WHO, 2006).

Many people in Tanzania and other East African countries produce and consume food crops which are at risk of aflatoxin contamination. The same crops are also traded directly and through networks of middle men in rural and urban markets that are not regulated for aflatoxins (Azziz-Baumgartner *et al.*, 2005; Kimanya *et al.*, 2008). There is strong agreement that aflatoxin contamination of important crops such as maize and groundnut poses a significant threat to public health, trade and livelihoods in Tanzania (Abt Associates, 2012; Abt Associates, 2013; Kimanya, 2014). Aflatoxin contamination is a public health threat since its contamination and exposure in complementary foods has been reported to be very high (Shirima *et al.*, 2013; Kimanya *et al.*, 2014; Kamala *et al.*, 2016). Therefore, it is important to know how people perceive its effects and build an attitude to seek knowledge on its contamination and management in complementary foods and its health effects to the community. It is clear that most people are not aware of the aflatoxin issue, and so do not perceive aflatoxin contamination as a problem in their production systems. They also do not have enough information on health risks associated

with the consumption of aflatoxin contaminated products including crops used in preparation of complementary foods. The study employed the Health Belief Model (HBM) to address parents' perceptions and attitude of susceptibility to aflatoxin induced diseases, seriousness of the aflatoxin problems, perception of the barriers that hinder alleviation of the problem, perception of benefits derived from reducing aflatoxin levels in foods and perceived actions necessary to reduce aflatoxin contamination in complementary foods. The HBM was chosen as the basis of the theoretical framework for this study because of its proven ability to successfully foresee the adoption of health behaviours (Hanson and Benedict, 2002). Despite the recognition that HBM is a psychosocial model and can account for those aspects of behaviour that can be explained by attitudes and beliefs, it has provided a useful theoretical framework for over thirty years and has been applied to a wide range of health-related behaviours (Nutbeam and Harris, 2004).

The health belief model was adopted being one of the models of behaviour change, usually used for studying health behaviours, and promoting the preventive health behaviours. The HBM was first developed in the 1950s by three social psychologists; Irwin Rosenstock, Godfrey Hochbaum and Stephen Kegels, working in the U.S. public health services to explain why medical screening programmes were not always successful (Steckler *et al.*, 2010). Since then, the model has been adapted to explore a variety of long and short-term health behaviours. The HBM is based on the assumptions that, a person will take a health-related action if that person feels that a negative health condition can be avoided, has a positive expectation that by taking a recommended action, he/she will avoid a negative health condition, and believes that he/she can successfully take a recommended health action.

The model holds that perceptions about a disease and strategies available to decrease its occurrence determine health behaviour (Hochbaum, 1958). The model consists of four constructs representing the perceived threat and net benefits, namely: perceived susceptibility, perceived severity (seriousness), perceived benefits, and perceived barriers. These concepts were proposed as accounting for people's "readiness to act." Subsequent amendments to the model were made whereby the concept of cues to action was added so as to activate that readiness and stimulate explicit behaviour. Another addition to the model is the concept of self-efficacy or one's confidence in the ability to successfully perform an action. This concept was added by Rosenstock and colleagues in 1988 to help the HBM better fit the challenges of changing habitual unhealthy behaviours, such as being sedentary, smoking, or overeating. The model that was found suitable as a framework for this study was Health Belief Model (HBM) which was initially developed, adopted and used to examine the structure of awareness and perceptions of groundnut aflatoxin among Ghanaian health and agricultural professionals and its influence on their actions (Jolly *et al.*, 2009; Steckler *et al.*, 2010). It has been used to assess hand hygiene knowledge, perceptions, and practices (Khateeb, 2011), breast cancer screening for female college students (Frankenfield, 2009), and influences on exercise behaviour among medical centre employees (Cychosz, 1994). Oral health education among 12 years old children was studied by Solhi *et al.*, 2010 using the HBM. Promote behaviour change among Nigerian single youth by using HBM (Oyekale *et al.*, 2010). In agriculture, a study of farmers to understand pesticide use decisions employed HBM (Khan, 2010). The modified Health Belief Model (HBM) was used in this study. The HBM is based on the perceived risks associated with the ingestion of the contaminated food. Therefore, the study aims to assess parents' perception and attitude towards aflatoxins contamination in child foods and its managements in Central Tanzania.

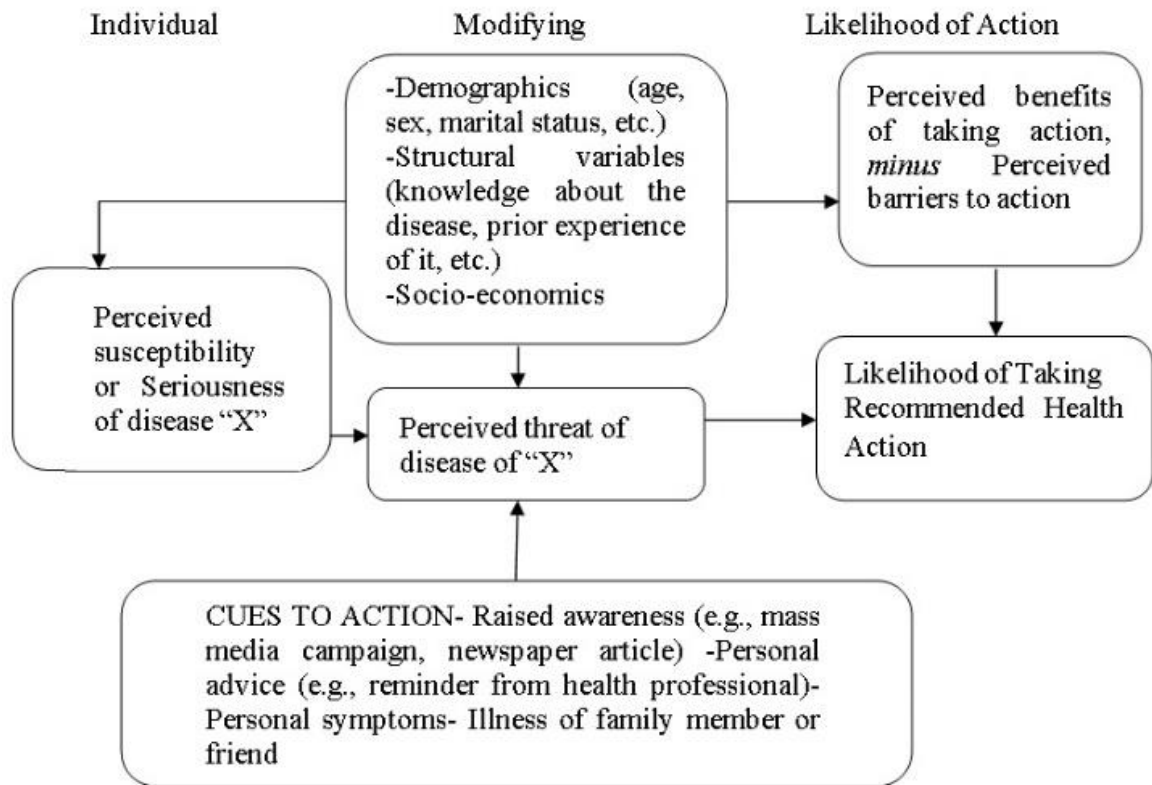


Figure 4.1: The Health Belief Model

Source: Glanz *et al.* (2002)

4.3 Materials and Methods

4.3.1. Description of the study areas

A study was carried out in four districts that are Chamwino and Bahi in Dodoma region and Manyoni and Ikungi in Singida region, which are all located in central part of Tanzania. These regions experience low rainfall and short rainy seasons which are often erratic with long periods of drought. The regions were selected because of the semi-arid condition which is characterized by high temperature during the day (up to 35°C) and cool (to 10°C) during the night. Both temperature and humidity favour the growth of fungi, thus signaling the possibility of aflatoxins production in improperly stored crops (Turner *et al.*, 2007). Descriptions of the study areas are in Ngoma *et al.* (2016).

4.3.2 Sample size

Sample size (n) of parents/caregivers in this study was determined by applying the single proportional formula by Magnani, (1997) used in the Statcalc programme of Epi Info Version 6 as follows: $n = (t^2) p (1-p) / m^2$

Where:

n = the desired total sample.

t = standard normal deviate value (set at 1.96 which corresponded to the 95% confidence interval level).

m= margin error, the degree of accuracy (taken to be 5% in this study).

p= the proportion of parents/caregivers who have been aware of aflatoxin taken to be 33%) from Jolly *et al.* (2009).

When these figures in the above formula, are substituted, it gives a minimum sample size of 340. Then the figure was rounded up to 364 so as to accommodate the unforeseen problems such as non-response to some or all questions. This sample size assumes that a household has one child aged between 6-23 months. In case a household has more than one child aged between 6-23 months, one child was randomly selected to avoid clustering of information.

4.3.3 Study methodology

A multistage sampling technique was used to select a total of 364 parents/caregivers with children aged between 6-23 months. The process involved simple random sampling at district, division, ward, village/street and sub-ward up to household level.

A descriptive cross-sectional study design was employed. Both quantitative and qualitative approaches to data collection with pre-tested questionnaires and structured interviews were used. All the 364 parents/caregivers were interviewed using Likert scale statements

to obtain their views, perceptions and attitudes about aflatoxin contamination in complementary foods and its health effects.

4.3.4 Data collection procedure

Six research assistants were selected by the principal researcher (PR) to assist in data collection. Research assistants were selected based on their previous experience in research work in communities and ability to understand and write Kiswahili and English languages. They were trained by the PR to understand the objectives of the study, the purpose and the procedure of the interview process; in order to have a common understanding of the questions in the interview schedule, and also to ask the questions to ensure that participants understood them. The questions were translated from English to Kiswahili by the PR and were explained to the participants.

Quantitative data were collected by administering an interview to the 364 parents/caregivers. The interview contained both open and close-ended questions developed and used together basic information on respondents' demographics, perception, and attitude on aflatoxin contamination in complementary foods and its actions to control in the community.

Qualitative data were collected by using the Krueger and Casey (2015) methodology for conducting focus group discussions (FGDs). A questionnaire was developed and used after pre-testing through conducting a focused group discussion and an interview involving parents or caregivers. The Focus Group Discussions were carried out after setting appointments with stakeholders (parents or caregivers) to be interviewed. The FGDs were conducted by using a checklist of the semi-structured, open-ended questions to allow the researcher guide the sessions and obtain the participants' views. Participants

(including parents or caregivers with children aged between 6 to 23 months) in Focus Group Discussion (FGD) were purposefully selected from the four randomly selected districts. These were the criteria that were used in inclusion and exclusion of participants. The research assistants had been trained on how to probe for specific issues when running FGDs. One pair of assistants (male and female) facilitated the FGDs by using a discussion guide and the principal researcher (PR) served as the assistant moderator. Another pair took notes during the FGDs. Information was collected by research assistants through 17 Focus Group Discussions (FGDs) with 121 participants (105 females and 16 males). The composition of all 17 FGDs was six (6) participants except six groups which had nine participants each and one group which had seven participants. The discussions were held nearby primary school class rooms, the office of village leaders or office of ward executive officer. The information collected was on community actions to prevent aflatoxins contamination in complementary foods and also personal experiences about aflatoxin contaminations in complementary foods. The interview lasted for approximately 40 minutes for each session. All the interviews were audio recorded, after obtaining the consent from participants and then the tapes were transcribed and translated into English by the principal researcher.

4.3.5 Data analysis

The Statistical Package for Social Sciences (SPSS) programme 21.0 version was used to analyze the data after cleaning. A 5% level of significance was used throughout the study and an independent variable with a p-value less than 0.05 was considered as statistically (significantly) associated with the outcome variable. For the qualitative part, coding was done using NVivo 7 software. The NVivo package has the ability to code and sort narrative data, interface with SPSS, and has good modelling facility and is user friendly (Hancock, 1998). It also combines best the NUD*IST computer software package with

much more flexibility (Hancock, 1998). The FGDs were analysed by using Thematic Content Analysis method.

4.3.6 Inferential statistics

In order to reach the conclusions that extend beyond the immediate sample, the inferential statistics were used in the data process. Inferential statistics are used to make an inference about a population from a sample (Tabachnick and Fidell, 2007; Meyers *et al.*, 2010; Zikmund, 2003). In this regard, the major statistical operations performed under inferential statistics, were the multivariate analysis; specifically factor analysis, Pearson chi square test and Analysis of Variance (ANOVA).

4.3.6.1 Factor analysis

Factor analysis is a group of analytical techniques used for different purposes such as data reduction, development and evaluation of tests and scales (Tabachnick and Fidell, 2007; Pallant, 2011). There are two main approaches to factor analysis that are commonly discussed in various literatures; exploratory and confirmatory. An exploratory factor analysis is used to explore the interrelationship amongst a set of variables and reduce them into a small number of factors which can easily be managed, while the confirmatory factor analysis is used to test specific hypotheses or theories regarding the structure of the underlying latent variables (Pallant, 2011). This study adopted the exploratory factor analysis to explore the relationship among a set of variables that measures perception and attitude towards aflatoxins contamination, and reduce them into few components/factors that can easily be managed for further analysis. The factors are inferred from the observed variables and estimated as linear combinations.

The general estimation of j th factor F_j is as follows:

$$F_j = \sum W_{ji} X_i = W_{j1}X_1 + W_{j2}X_2 + \dots + W_{jp}X_p \dots\dots\dots(1)$$

$i=1$

Where,

W_j = factor score coefficients

P =number of variables reply

In addition, factor loading analysis was done and the higher factor loadings suggest that more of the variance in that observed variable is attributable to the latent variable. To ensure that the sample was suitable for factor analysis, the Measure of Sampling Adequacy (MSA) Test, Kaiser–Meyer–Olkin (KMO) test and the Bartlett test of sphericity was run. Next, the eigenvalue a criterion was used to represent the amount of variance accounted for by a factor. Besides, the communality was applied to find the total amount of variance an original variable shares with all other variables included in the analysis. Furthermore, variance explained and varimax normalization was used. An exploratory factor analysis was carried out to define the underlying structure in the data matrix. Principle component analysis was used to extract factors and produce one component for each variable. Although the analysis yielded as many factors as variables, the smaller factors, in terms of accounted variable variance were dropped if the value was less than or equal to 0.5. For factor analysis to produce meaningful result, communalities of each variable retained in the factors must be greater than 0.5 (University of Texas, 2003; Field, 2005). To avoid multicollinearity, determinant should be greater than 0.00001 (Field, 2005). In this study, the recommended acceptable values was greater than 0.5, value below this, it should lead to the researcher to collect more data, otherwise the researcher would be required to rethink which variables to include (Kaiser, 1974). Respondents were asked questions about their attitudes, perceptions, susceptibility and seriousness, barriers, benefits and actions toward aflatoxins contamination and its control.

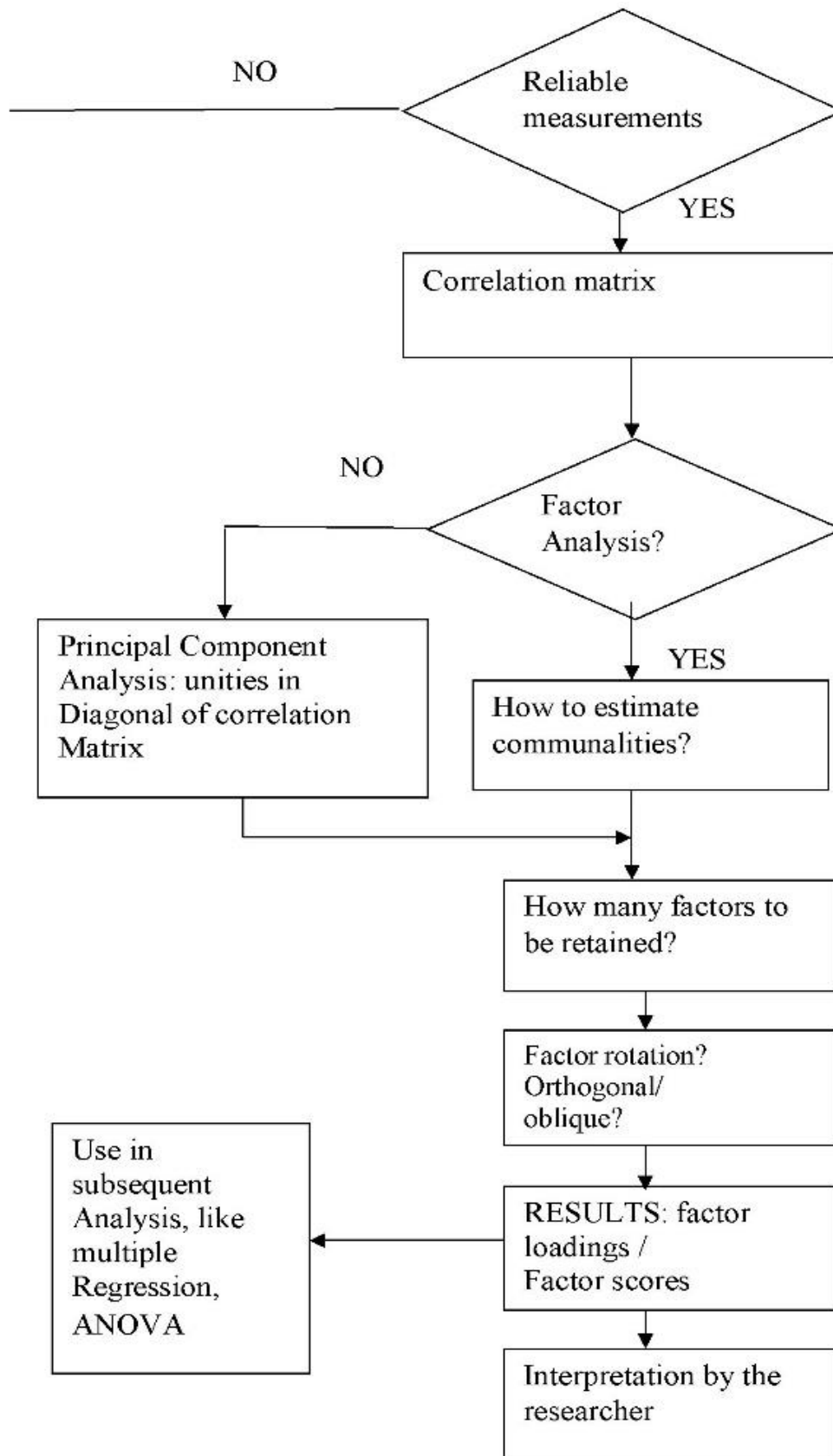


Figure 4.2: An overview of the steps in a factor analysis

4.3.6.2 Analysis of variance (ANOVA)

Multi-way Analysis of Variance was performed to compare group means of the demographic variables, specifically the respondent’s age, level of education, occupation, marital status and monthly household income which determined statistically significant difference in terms of the perception and attitude score toward aflatoxins. The perception and attitude scores were generated using factor analysis.

The general multi-way ANOVA model is given as:

$$E(Y_i) = \beta_0 + \beta_1 x_1 + \dots + \beta_p x_p \dots\dots\dots (2)$$

Where E (Y_i) is estimated mean score for perception and attitude toward aflatoxins, x_i 's are independent variables and β_i 's are their respective parameters.

The R square (R²) or coefficient of multiple determinations is one of the useful statistics used to examine the amount of variance explained in the outcome variable by the predictors in the model (Field, 2009). The R square (R²) measures the amount of variance in the outcome variable, explained by the model relative to how much variation there was to explain in the first place (SST) (Pallant, 2011). Therefore, as a percentage, it presents the percentage of variation in the outcome variable that can be explained by the model (Field, 2009). It is easily computed by dividing the model sum of square (SSM) by the total sum of square (SST).

That is, $R^2 = \frac{SSM}{SST} \dots\dots\dots (3)$

4.3.6.3 Consent

We had had a clear and free consent of each patient before starting the study.

4.4 Results

4.4.1 Socio-demographic characteristics

Results in Table 4.1 show the distribution of parents/caregivers by socio-demographic characteristics. Generally, the majority of the respondents were aged below or equal to 34 years (74.3%); earned income less than or equal to US\$ 22.8 (70.3%); had a primary education (56%); were farmers (78.8%), and in the marital union (74.7%).

Table 4.1: Distribution of parents/caregivers by socio-demographic characteristics

Characteristics	Number	(%)
Age Group (Years)		
≤ 34	270	74.2
> 34	94	25.8
Monthly income (US\$)		
≤ 22.8	256	70.3
> 22.8	108	29.7
Level of education		
Never been to school	64	17.6
Partial primary	49	13.5
Primary	204	56
Partial secondary	18	4.9
Secondary	29	7.9
Respondent's occupation status		
Farmer	287	78.8
House wife	32	8.8
Employee	10	2.7
Petty trader	35	9.6
Marital status		
In Union	272	74.7
Not in Union	92	25.3

4.4.2 Perception towards aflatoxins contamination

Perception is the process of acquiring, interpreting, selecting and organizing sensory information on a given item. The frequency analysis results of the respondent's perceptions toward aflatoxin contamination are presented in Table 2. The results show that 32.1% of the respondents strongly agreed that sorting of grains/nuts reduces aflatoxins (mouldy) contamination and were aware that washing grains/nuts reduce aflatoxins (mouldy) contamination (29.7%). While the majority of the respondents (43.7%) were not sure that washing of grains/nuts reduces aflatoxins (mouldy) contamination before milling. About 36% of the respondents strongly agreed that discoloured grains/nuts indicate the presence of aflatoxins (mouldy) but 31.9% of them were not sure that discoloured grains/nuts indicate the presence of aflatoxins (mouldy). Besides, 39.3% of the respondents strongly agreed that eating contaminated foods with aflatoxins (mouldy) can cause diseases, while 33.8% were not sure that eating contaminated foods with aflatoxins (mouldy) can cause diseases. Almost half of the respondents (46.4%) were not sure that aflatoxins (mouldy) contamination can occur any time in foods. Furthermore, most of them (37.9%) were not sure that eating contaminated foods with aflatoxins (mouldy) will cause death while (30.5%) strongly agreed.

4.4.3 Factors influencing perception toward aflatoxins contamination

Factor Analysis was carried out for different responses on questions to the respondents relating to perception toward aflatoxins contamination. After carrying out the factor analysis, information about respondents' perception toward aflatoxins' contamination was represented by one factor of perception toward threat and reducing aflatoxins contamination with the following items; Sorting of fungal infected seeds may reduce levels of aflatoxins (fungus); Washing of fungal infected seeds may reduce levels of aflatoxins (fungus); Rotten seeds indicate the presence of fungus; Foods containing fungus

may cause diseases; People may die by consuming foods that have been infected with fungus; and Fungal growth may occur at any time in foods.

Table 4.2: Perception toward aflatoxins

S/N	Perception statements	5 (n/%)	4 (n/%)	3(n/%)	2(n/%)	1(n/%)
1	Sorting of grains/nuts reduces aflatoxins (mouldy) contamination	117 (32.1)	85 (23.4)	124 (34.1)	19 (5.2)	19 (5.2)
2	Washing grains/nuts reduces aflatoxins (mouldy)contamination	108 (29.7)	63 (17.3)	159 (43.7)	19 (5.2)	15 (4.1)
3	Discoloured grains/nuts indicate the presence of aflatoxins (mouldy)	133 (36.5)	96 (26.4)	116 (31.9)	9 (2.5)	10 (2.7)
4	Eating contaminated foods with aflatoxins (mouldy) can cause diseases	143 (39.3)	74 (20.3)	123 (33.8)	13 (3.6)	11 (3.0)
5	Eating contaminated foods with aflatoxins (mouldy) can cause death	111 (30.5)	86 (23.6)	138 (37.9)	16 (4.4)	13 (3.6)
6	Aflatoxins (mouldy) contamination can occur any time in foods	83 (22.8)	67 (18.4)	169 (46.4)	29 (8.0)	16 (4.4)

5= Strongly agree, 4=Agree, 3=Undecided, 2=Disagree and 1= Strongly disagree.

Table 4.3: Components extracted from factor reduction analysis on five items of perception towards aflatoxin contamination

S/N	Variable	Component 1
1	Sorting of grains/nuts reduces aflatoxins (mouldy) contamination	0.798
2	Washing grains/nuts reduces aflatoxins (mouldy) contamination	0.806
3	Discoloured grains/nuts indicate the presence of aflatoxins (mouldy)	0.855
4	Eating contaminated foods with aflatoxins (mouldy) can cause diseases	0.835
5	Eating contaminated foods with aflatoxins (mouldy) can cause death	0.800

4.4.4 Factors analysis for perception toward aflatoxins contamination

Screening of the data was done, so as to make the remaining factors have to mean. The factors which remained measured the same dimensions i.e. correlated to each other. Therefore the variable “Fungal growth may occur at any time in foods” had a communality of 0.384. This variable was removed from the next iteration of the factor analysis since it was less than 0.5. In the second iteration, all variables were greater than 0.5 showing that they highly correlated to each other as there was no any variable with communality less than 0.5. There was only one factor retained which explain 67.2% of total variance (Table 4.4).

Table 4.4: Factor reduction analysis on five indicator variables to obtain a single variable

Component	Total variance explained					
	Initial Eigen values			Extraction sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	3.358	67.153	67.153	3.358	67.153	67.153
2	0.755	15.104	82.257			
3	0.380	7.609	89.866			
4	0.257	5.148	95.015			
5	0.249	4.985	100.000			

Since there was only a single factor, there was no complex structure existence. On the other hand, checking for multicollinearity was done which could be detected by looking at the determinant of the R-matrix. The determinant for the factor formed was 0.062 (i.e. greater than 0.00001), this implies there is no multicollinearity.

4.4.5 Kaiser-Meyer-Olkin (KMO)

The KMO statistic measures of sampling adequacy vary between 0 and 1. A value of 0 indicates that the sum of partial correlations is large relative to the sum of correlations, indicating diffusion in the pattern of correlations hence factor analysis is likely to be inappropriate. The value close to 1 indicates that patterns of correlations are relatively compact and so factor analysis should yield distinct and reliable components. For these data, the value was 0.810, which fell within an acceptable value that is greater than 0.5. This shows that factor analysis was appropriate for this data (Table 4.5).

Table 4.5: KMO and Bartlett's test for perception toward aflatoxins examination

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.810
Bartlett's Test of Sphericity	Approx. Chi-Square	1002.965
	Df	10
	Sig.	0.000

4.4.6 Bartlett's measure

For factor analysis to work there should be some relationships between variables and if the R-matrix were an identity matrix, then all correlation coefficients would be zero. Therefore, the test needs to be significant. If the test is significant, then R-matrix is not an identity matrix. The probability associated with the Bartlett test was <0.000, which satisfies the requirement, thus Bartlett's measure was highly significant since <0.001 (Table 5) and therefore factor analysis was appropriate for perception toward aflatoxins examination.

4.4.7 Factor loadings and eigen value for perception toward aflatoxins examination

The Eigen value associated with perception toward Aflatoxins Examination has represented the variance explained by that particular linear factor. The Eigen values were displayed in terms of the percentage of variance explained. Factor loadings for each variable which were based on the common variance are presented in Table 4.6, where all were greater than 0.5.

Table 4.6: Factor loadings and Eigen value for respondents' perception toward aflatoxins contamination

S/N	Variable	Factor Loadings	Eigen Value
1	Sorting of grains/nuts reduces aflatoxins (mouldy) contamination	0.798	
2	Washing grains/nuts reduces aflatoxins (mouldy) contamination	0.806	
3	Discoloured grains/nuts indicate the presence of aflatoxins (mouldy)	0.855	
4	Eating contaminated foods with aflatoxins (mouldy) can cause diseases	0.835	
5	Eating contaminated foods with aflatoxins (mouldy) can cause death	0.800	3.358

4.4.8 Analysis of variance (ANOVA) model for perception toward the aflatoxins contamination

The multi- way analysis of variance was also employed to find out how the perception scores from factor analysis vary with respect to demographic characteristics. The results of the fitted ANOVA model for perception revealed that the estimated mean perception score for respondents aged above 34 years was significantly higher by 0.3666 compared to subjects aged below or equal to 34 years ($p < 0.05$). The mean perception score for

respondents with primary education was significantly higher by 0.3730 in comparison to mean score of the respondents that had never been to school ($p < 0.05$). Though not statistically significant, participants with partial primary education (partial secondary education) and secondary or higher education (were also found to have higher mean perception score than that of the participants with no education. Other independent variables, namely monthly income, occupation and marital status were significantly important predictors of perception toward aflatoxins contamination. The R^2 of this model was 0.0573, which implies that about 6% of the variability in perception toward the aflatoxins contamination can be explained by the independent variable included in the model.

Table 4.7: Parameter estimates of fitted ANOVA model for perception toward aflatoxins contamination (n=364, $R^2=0.0573$)

Variable	Parameter Estimates(β)	Standard Error	T-Value	P-Value
Age (Years)				
≤ 34	Reference	Reference	Reference	Reference
> 34	0.3666	0.1211	3.03	0.0026
Monthly Income (US\$)				
≤ 22.8	Reference	Reference	Reference	Reference
> 22.8	-0.0522	0.1234	-0.42	0.6725
Education Level				
Never been to School	Reference	Reference	Reference	Reference
Partial Primary	0.3283	0.1879	1.75	0.0814
Primary	0.3730	0.1426	2.62	0.0093
Partial Secondary	0.4843	0.2697	1.8	0.0734
Secondary	0.3583	0.2634	1.36	0.1746
Occupation				
Peasant	Reference	Reference	Reference	Reference
House wife	0.3232	0.1857	1.74	0.0827
Petty trade	0.1978	0.1931	1.02	0.3064
Employed	0.6805	0.3932	1.73	0.0844
Marital Status				
Not in Union	Reference	Reference	Reference	Reference
In Union	-0.0386	0.1250	-0.31	0.7576

Reference= The first category acts as a baseline, and can interpret the other coefficients as an increase or decrease in the log odds ratio over the baseline category

4.4.9 Attitude toward aflatoxins contamination factors

Results on respondents' attitudes towards aflatoxins are presented in Table 8. The results show that the majority of the respondents (37.1%) strongly agreed that good agricultural practices would minimize aflatoxins (fungus) in crops and were aware that sorting of discoloured (mouldy) crops reduces contamination of aflatoxins (fungus) in the child's foods (27.5%).

However, about 36.3 percent of them were not sure that sorting of discoloured (mouldy) crops reduces contamination of aflatoxins. Half of the respondents (50.0%) stated that clean grains/nuts always sell faster. Moreover, most of them (51.1%) strongly believed that sorting of grains/nuts is hygienic. Around 39.3 percent were not sure that washing of grains/ nuts before milling can reduce aflatoxins (mouldy) contamination. Nearly 47.5 percent of the respondents indicated that clean grains/nuts attract better prices. However, 28.0% of them revealed that sorting of damaged grains/nuts is too costly and also 33.5% of them showed that sorting of damaged grains/nuts is time consuming. Large proportions (43.7%) of the respondents were not sure that aflatoxins could be removed during cooking of child's foods. These were very true because aflatoxins can be minimally reduced during cooking but not eliminated.

Table 4.8: Attitude toward aflatoxins

S/N	Attitudinal statements	5 (n/%)	4 (n/%)	3 (n/%)	2 (n/%)	1 (n/%)
1	Good agricultural practices will minimize aflatoxins in crops	135 (37.1)	89 (24.5)	117 (32.1)	14 (3.8)	9(2.5)
2	Sorting of discoloured (mouldy) crops will minimize contamination of aflatoxins in the child's foods	100 (27.5)	97 (26.6)	132 (36.3)	24 (6.6)	11(3.0)
3	Washing of grains/ nuts before milling can reduce aflatoxins (mouldy) contamination	90 (24.7)	88 (24.2)	143 (39.3)	27 (7.4)	16(4.4)
4	Aflatoxins can be removed during milling of child's foods	65 (17.9)	44 (12.1)	169 (46.4)	29 (8.0)	57(15.7)
5	Sorting of damaged grains/nuts is time consuming	68 (18.7)	63 (17.3)	122 (33.5)	37 (10.2)	74(20.3)
6	Aflatoxins can be removed during cooking of child's foods	53 (14.6)	43 (11.8)	159 (43.7)	30 (8.2)	79 (21.7)
7	Sorting of damaged grains/nuts is too costly	102 (28.0)	62 (17.0)	81 (22.3)	42 (11.5)	77(21.2)
8	Sorting of grains/nuts is hygienic	186 (51.1)	65 (17.9)	88 (24.2)	14 (3.8)	11 (3.0)
9	Clean grains/nuts attract better prices	173 (47.5)	71 (19.5)	66 (18.1)	25 (6.9)	29 (8.0)
10	Clean grains/nuts always sell faster	182 (50.0)	52 (14.3)	78 (21.4)	17 (4.7)	35 (9.6)

5= Strongly agree, 4=Agree, 3=Undecided, 2=Disagree and 1= Strongly disagree.

4.4.10 Factors analysis for attitude toward aflatoxins contamination and management

The first iteration in this case, revealed that communalities for each variable were greater than 0.5. There was also no complex structure for the formed factors hence no any variable dropped. Checking for multicollinearity was done which could be detected by looking at the determinant of the R-matrix. The determinant for the factor formed was 0.009 (i.e. greater than 0.00001), this implies that there is no multicollinearity.

4.4.11. Factors analysis for attitude toward aflatoxins

The factor analysis of the 10 attitudinal statements was conducted and the factors are ranked according to the proportion of variance explained and are named to reflect the latent stimuli underlying parents' attitude about aflatoxin contamination and its management in foods. The analysis identifies three latent factors influencing parents' opinions about aflatoxin contamination and its management in foods. After carrying out the factor analysis, information about respondent's attitude toward aflatoxins was represented in three factors here under:

Factor I (Barriers versus Benefits)

Items/Variables: Fungal may be removed by milling the child's food; Sorting of spoiled seeds is time wasting activity; Fungus/aflatoxins may be removed during cooking of child's food, and Sorting of spoiled seeds is costly in terms of money.

Factor II (Benefits)

Items/Variables: Sorting of spoiled seeds is a good hygienic practice; Clean seeds offer a better price, and Clean seeds are marketable

Factor III (Actions to Reduce Aflatoxins)

Good agricultural practices will minimize problems associated with aflatoxins in crops; Sorting of discoloured (mouldy) crops will minimize contamination of aflatoxins in the child's foods and Washing of seeds before milling helps to reduce the chance of having fungal/aflatoxin in foods fed to children.

Table 4.9: Factor reduction analysis on ten indicator variables to obtain a single variable

Component	Total Variance Explained								
	Initial Eigen values			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.602	36.022	36.022	3.602	36.022	36.022	2.609	26.094	26.094
2	2.458	24.580	60.602	2.458	24.580	60.602	2.450	24.497	50.591
3	1.177	11.773	72.375	1.177	11.773	72.375	2.178	21.784	72.375
4	.604	6.036	78.411						
5	.497	4.975	83.386						
6	.478	4.776	88.161						
7	.371	3.714	91.875						
8	.352	3.522	95.397						
9	.300	3.005	98.402						
10	.160	1.598	100.000						

Extraction Method: Principal Component Analysis.

For test attitude toward aflatoxins, Kaiser-Meyer- Olkin (KMO) value was 0.782, which fell within the acceptable value. Likewise, the probability associated with the Bartlett test was <0.000, which satisfies the requirement, thus Bartlett's measure was highly significant since $P < 0.001$. Therefore, factor analysis was appropriate (Table 4.10).

Table 4.10: KMO and Bartlett's test attitude toward aflatoxins

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		0.782
Bartlett's Test of Sphericity	Approx. Chi-Square	1675.096
	Df	45
	Sig.	0.000

4.4.13 Factor loadings and Eigen values for attitude toward aflatoxins

From factor extraction, the Eigen values associated with each linear component before extraction, after extraction and after rotation were computed using SPSS software. Before

extraction, finding indicated ten factors were identified. The Eigen values associated with each factor represented the variance explained by that particular factor. The Eigen values were displayed in terms of the percentage of variance explained. First factor explained 36.02% of the total variance whereas second factor explained 24.58% of the total variance. The third factor explained 11.77% of the total variance. Rotation has the effect of optimizing the factor structure and it tends to make the three factors with equal relative importance. Therefore, after rotation, the first component accounted for 26.09%, the second accounted for 24.5% and the third accounted for 21.78% of the total variance. All three factors retained were able to explain 72.4% of the total variance (Table 4.10).

Table 4.11: Factor loadings and Eigen value for respondent's attitude toward aflatoxins

S/ N	Variable	Factor Loadings	Eigen Value
1	Aflatoxins can be removed during milling of child's foods	0.781	
2	Sorting of damaged grains/nuts is time consuming	0.825	
3	Aflatoxins can be removed during cooking of child's foods	0.800	
4	Sorting of damaged grains/nuts is too costly	0.766	3.602
5	Sorting of grains/nuts is hygienic	0.781	
6	Clean grains/nuts attract better prices	0.903	
7	Clean grains/nuts always sell faster	0.915	2.458
8	Good agricultural practices will minimize aflatoxins in crops	0.794	
9	Sorting of discoloured (mouldy) crops will minimize contamination of aflatoxins in the child's foods	0.829	
10	Washing of grains/ nuts before milling can reduce aflatoxins (mouldy) contamination	0.755	1.177

The results of the fitted ANOVA model for attitude toward aflatoxins reveal that the estimated mean attitude toward aflatoxins score for respondents in union was significantly higher by 0.2639 compared to those not in unions. Although not statistically significant, respondents aged above 34 years ($\beta = -0.0732$, $p = 0.5512$), were having low estimated

mean score to attitude toward aflatoxins compared to the respondents aged below or equal to 34 years. On other hand, although not significant, respondents with partial primary education ($\beta = 0.1610, p = 0.3984$), Primary education ($\beta = 0.1987, p = 0.17$) and secondary education or higher ($\beta = 0.0333, p = 0.9007$) were having higher estimated mean score attitude toward aflatoxins compared to the respondents who had never been to school. Also, respondents with partial secondary education ($\beta = 0.0299, p = 0.9129$) were having higher mean score of attitude toward aflatoxins contamination compared to those who had never been to school. Likewise, occupations of the respondents were having different impacts to the estimated mean score of attitude toward aflatoxins. Attitude towards aflatoxins for Housewife ($\beta = 0.0894, p = 0.6349$) and employed ($\beta = 0.3061, p = 0.4429$) being higher compared to peasant respondents whereas that of petty traders was lower compared to that of peasants although all occupation categories were not statistically significant.

Table 4.12: Parameter estimates of fitted ANOVA model for attitude factor 1 (barriers versus benefits) toward aflatoxins contamination (n=364, R²=0.0321)

Variable	Parameter Estimates	Standard Error	T Value	P-Value
Age (Years)				
≤ 34	Reference	Reference	Reference	Reference
> 34	-0.0732	0.1227	-0.60	0.5512
Monthly Income (US\$)				
≤ 22.8	Reference	Reference	Reference	Reference
> 22.8	0.0556	0.1250	0.45	0.6565
Education Level				
Never been to School	Reference	Reference	Reference	Reference
Partial Primary	0.1610	0.1904	0.85	0.3984
Primary	0.1987	0.1445	1.37	0.1700
Partial Secondary	0.0299	0.2733	0.11	0.9129
Secondary	0.0333	0.2669	0.12	0.9007
Occupation				
Peasant	Reference	Reference	Reference	Reference
House wife	0.0894	0.1882	0.48	0.6349
Petty trade	-0.2283	0.1957	-1.17	0.2441
Employed	0.3061	0.3984	0.77	0.4429
Marital Status				
Not in Union	Reference	Reference	Reference	Reference
In Union	0.2639	0.1199	2.20	0.0284

Reference= The first category acts as a baseline, and can interpret the other coefficients as an increase or decrease in the log odds ratio over the baseline category

Table 4.13 presents the parameter estimate, standard error and p-value for the predictors towards the factors III (actions to reduce aflatoxins) attitude to aflatoxins contamination. Respondents with primary education and those with secondary education or higher ($p < 0.05$) were significantly important predictors of attitude for factor III toward aflatoxins contamination. Although not significant, respondents with partial primary education ($\beta = 0.0579$, $p = 0.7595$) and partial secondary education ($\beta = 0.4584$, $p = 0.0922$) were having higher estimated mean score of factor III (actions to reduce aflatoxins) attitude toward aflatoxins contamination compared to the respondents who had never been to school.

The results of the fitted ANOVA model for factor III (actions to reduce aflatoxins) attitude reveals that the estimated mean attitude score for the respondents aged above 34 years was significantly higher by 0.2124 compared to subjects aged below or equal to 34 years. However, estimated mean of factor III attitude score for subjects having monthly household income of US\$ above 22.8 was lower by 0.1154 compared to the respondents with monthly income less or equal to US\$ 22.8 although not significant.

Although not significant, housewives ($\beta = 0.1439, p = 0.4417$), petty traders ($\beta = 0.1563, p = 0.4218$) and employed respondents ($\beta = 0.053, p = 0.8935$) had higher estimated mean attitude for factor III score compared to peasants. However, although not significant, respondents in union ($\beta = -0.0933, p = 0.4585$) had lower mean score of estimated factor III attitude towards aflatoxins contamination compared to those not in unions.

Table 4.13: Parameter estimates of fitted ANOVA model for attitude factor iii (actions to reduce aflatoxins) toward aflatoxins contamination (n=364, R²= 0.0452).

Variable	Parameter Estimate	Standard Error	T-Value	P-Value
Age (Years)				
≤ 34	Reference	Reference	Reference	Reference
> 34	0.2124	0.1219	1.74	0.0823
Monthly Income (US\$)				
≤ 22.8	Reference	Reference	Reference	Reference
> 22.8	-0.1154	0.1242	-0.93	0.3531
Education Level				
Never been to School	Reference	Reference	Reference	Reference
Partial Primary	0.0579	0.1891	-0.31	0.7595
Primary	0.3405	0.1435	-2.37	0.0182
Partial Secondary	0.4584	0.2714	-1.69	0.0922
Secondary	0.5528	0.2651	-2.09	0.0378
Occupation				
Farmer	Reference	Reference	Reference	Reference
House wife	0.1439	0.1869	0.77	0.4417
Petty trade	0.1563	0.1943	0.8	0.4218
Employed	0.0530	0.3957	0.13	0.8935
Marital Status				
Not in Union	Reference	Reference	Reference	Reference
In Union	-0.0933	0.1258	-0.74	0.4585

Reference= The first category acts as a baseline, and can interpret the other coefficients as an increase or decrease in the log odds ratio over the baseline category

4.4.14 Socio-demographic variables, perception and attitude

The Pearson's correlations in Table 4.15 suggest that age of the respondents did not correlate to the education levels in mouldy reduction or health effects. However, age of the respondent correlated positively to the perception (p=0.005), and negatively to occupation (p=0.041). The analysis shows that monthly income positively correlated to occupation and education towards aflatoxin reduction (p=0.000). On the other hand, occupation negatively correlated to marital status (p=0.006). The correlations also show that there is association between perception and attitude scores towards aflatoxin (mouldy) reduction and its health effects to the community (p=0.000).

Table 4.14: Correlation matrix of the socio-demographic variables, perception and attitude

	Age in Years	Monthly Income	Education Level	Occupation	Marital Status	Perception score	Attitude score
Age in Years	1.000						
Monthly Income	0.043(0.416)	1.000					
Education Level	-0.001(0.980)	0.201**(0.000)	1.000				
Occupation	-0.107*(0.041)	0.305**(0.000)	0.379(0.000)	1.000			
Marital Status	-0.009(0.871)	-0.061(0.243)	-0.062(0.236)	-0.145**(0.006)	1.000		
Perception score	0.147**(0.005)	0.007(0.893)	-0.033(0.530)	0.089(0.091)	0.017(0.750)	1.000	
Attitude score	0.010(0.851)	-0.041(0.439)	-0.081(0.124)	0.010(0.849)	0.071(0.179)	0.597**(0.000)	1.000

* $p < 0.05$, ** $p < 0.01$

4.4.15 Qualitative results

Several themes with supporting quotes were obtained from focus group discussions.

4.4.15.1 Community actions to prevent aflatoxins contamination in complementary foods

When asked about things which they were doing to prevent aflatoxins contamination in the complementary food, most people mentioned the same kind of precautions they were usually taking to prevent aflatoxins in general. Participants mentioned drying of crops before taking them inside for storage, use of treatments (traditional materials) for example ash and mud sprayed on the crops before and during storage. It was noted that very few participants were buying and using fungicides/insecticides from the shops. Also, minorities of participants were taking grains/nuts outside regularly in between storage time and storing crops in the rooms which were well ventilated and sun rays normally got in and out daily and easily. Some participants mentioned that, storage inside or outside depended on the type of crop where with some crops, they never took them inside because even if they would take them while dry, they could easily get moisture so those kinds of

crops were normally stored outside on the roofs during summer. Another challenge they were facing is a lack of money with which to buy storage materials.

4.4.15.2 Education about aflatoxin contamination and control

When asked about perception or attitude on education which they had received about mouldy (aflatoxin) contamination and prevention in complementary food, almost all participants in four districts mentioned that there were no any formal or informal education provided about fungi contamination, and even control, rather they were generally preventing (mouldy) aflatoxins through experiences.

4.5 Discussion

Contamination of complementary foods by aflatoxin is a serious public health problem that requires attention to ensure that proper measures are taken to limit its health effects. This study has investigated the parents' perception and attitude towards aflatoxins contamination in complementary foods and its management in Central, Tanzania. The findings of the study indicate that parents who are responsible for preparation of complementary foods in central Tanzania do not fully perceive that aflatoxins are harmful to human and animals and their attitude towards their control was low, in general. They were, however, of a strong belief that sorting and washing of grains or nuts before milling may reduce the level of (mouldy) aflatoxins in foods. A similar study in Benin reported that most aflatoxins contamination occurs on relatively in a few grains or nuts; therefore sorting of damaged grains can reduce toxins loads in stored grains (Fandohan *et al.*, 2005; Afolabi *et al.*, 2006). These results are not similar to the study done by Shabani *et al.* (2015) in Handeni, Tanga which revealed that 67% of the farmers did not sort their defective maize. However, such study contrasts with the study done in Tabora, Kilimanjaro and Iringa in Tanzania, that more than 90% of the maize users were sorting

their maize before use (Kimanya *et al.*, 2008). Though there was disagreement that washing of grains or nuts would minimize the level of aflatoxins contamination, the parents strongly agreed that eating of contaminated foods could cause diseases. Almost half of the parents 46.4% were not sure that aflatoxins (mouldy) contamination could occur any time in foods. This result contrasts with the study done by Kumar *et al.* (2010) in India which revealed that farmers disagreed that the contamination could occur at any time during pre-harvest stages 55.5% and post-harvest stages 60%.

After carrying out the factor analysis, information about parent's perception toward aflatoxins contamination was represented as one factor of perception toward reducing aflatoxins and threat (seriousness and susceptibility) of contamination. All factor loadings were statistically significant and valid. The result of this factor suggests that parents pay attention to aflatoxin contamination and its threat from different aspects and they are generally positive towards aflatoxins reduction on foods if they will be provided with education. These results are almost similar to the study done by Jolly *et al.* (2009) in Ghana which showed that all factor loadings in seriousness and susceptibility towards aflatoxin contamination were statistically significant at the 95% confidence level which indicates that the variables are good indicators of their particular basic constructs and hence those constructs are valid.

The role of socio-demographic characteristic was also examined in the study. The results were obtained with regard to the effects on perception, attitude and actions towards aflatoxins contamination and its management. It was revealed that the parents aged above 34 years old were more likely to have higher perception towards aflatoxins threat and reduction compared to subjects aged below or equal to 34 years old. This may be due to the experience on crops which are used in the preparation of complementary foods and

also which are susceptible to fungal infection. This result concurs with Bektas *et al.* (2011) in Turkey who revealed that the families with elderly people aged 50 years and above were more sensitive to food safety than others. The parents with primary education were more likely to have higher perception towards aflatoxins threat and its reduction in comparison to respondents that had never been to school. This may be due to the reason that majority of the participants in this study had a primary level of education. However, parents with partial primary education, partial secondary education and secondary or higher education were found to have no effects on perception towards aflatoxins contamination and its management than those participants who had never been to school. Meanwhile, participants in focus group discussion claimed that no any information or education was given to them about aflatoxin regardless of their education levels. These results are in contrast with the previous study done by Jolly *et al.* (2009) in Ghana and (Baker, 2003) that people with higher levels of education are likely to be better informed on some risks of food additives or pesticides in food than people with less education (Khan, 2010; Khan *et al.*, 2013). Therefore, they are more likely to better understand aflatoxin and its threat to humans and animals. Furthermore, monthly income, occupation and marital status were significantly important predictors of perception toward aflatoxins contamination and managements though they did not affect the parents' perceptions. Hence, there were no significant differences in their perceptions on this aspect.

On the other hand, parents' attitude towards aflatoxin contamination and its management showed that some of the parents strongly agree that good agricultural practices would minimize aflatoxins (fungus) in crops and also were aware that sorting of discoloured (mouldy) crops would minimize contamination of aflatoxins (fungus) in the child's foods. These findings correlate with the study done in Benin revealed that aflatoxins reduction was observed after sorting, winnowing and washing of the raw maize (Fandohan *et al.*,

2005). However, about 36.3 percent of them were not sure that sorting of discoloured (mouldy) crops would minimize contamination of aflatoxins. Around 39.3 percent were not sure that washing of grains or nuts before milling can reduce aflatoxins (mouldy) contamination. However, 28.0% of the parents revealed that sorting of damaged grains or nuts are too costly and also 33.5% of them shows that sorting of damaged grains or nuts is time consuming. These findings contradict with the study done in Benin revealed that aflatoxins reduction was observed after sorting, winnowing and washing of the raw maize (Fandohan *et al.*, 2005).

In this study, the socio-demographic factors were influenced by the attitude towards aflatoxin contamination. The study found that in factor one which includes barriers and benefits towards aflatoxin contamination, only marital status influenced parents' attitude towards aflatoxin contamination and its control to the foods. Respondents in the union were more likely to have a positive attitude on barriers and benefits of aflatoxin contamination compared to those not in a union. This finding was similar to the study done by Bektas *et al.* (2011) in Turkey who reported that married individuals were more sensitive to food safety than unmarried; this may be due to family responsibility. In factor two the benefits towards aflatoxins reduction showed that all age predictors monthly income, education level, occupation and respondents' marital status were not statistically significant with the attitude towards aflatoxins ($P > 0.05$).

Hence, there were no significant differences in their attitude on this aspect. However, parents aged above 34 years were likely to have a higher attitude toward aflatoxins compared to those aged below or equal to 34 years. Also, parents' attitude towards aflatoxins contamination was lower to the respondents' with higher income compared to the subjects having lower monthly income.

Education levels have different impacts to the attitude towards aflatoxins. Having partial primary education, primary education, partial secondary education and secondary education or higher made it more likely for the respondents to have a higher positive attitude toward aflatoxins contamination and its control compared to the parents who had never been to school. In the study done in Canada by Dosman *et al.* (2001) reported that people who have high educational level are more likely to give preference to the healthiness of food than those who have a lower level of education. Furthermore, with regard to occupation, the housewives, petty traders and employed had a higher positive attitude toward aflatoxins contamination compared to farmers. Parents who were in the union also were more likely to have a higher attitude towards aflatoxins contamination compared to those who were not in a union. This might be that most of the time they discuss issues of quality of food with their partners. While in the third factor of actions towards aflatoxin reduction, parents with primary education and those with secondary education or higher ($p < 0.05$) were significantly important predictors of attitude toward aflatoxins contamination and control. Although not statistically significant, respondents with partial primary education and partial secondary education were having a higher attitude toward aflatoxins contamination compared to the respondents who had never been to school.

The respondents aged above 34 years were more likely to have higher positive attitude compared to subjects aged below or equal to 34 years old. However, the parents having higher monthly income had a lower attitude in action to reduce aflatoxin compared to the parents with lower monthly income. These results contrast with those of the study done by Sabran *et al.* (2012) in Malaysia which revealed that people with high income were more likely to take precaution about food and were willing to pay for food safety (Baker, 2003, Dosman *et al.*, 2001) than those with lower income. Furthermore, housewives, petty

traders, and the employed respondents were more likely to have a higher attitude towards reducing aflatoxin compared to farmers. However, respondents in the union had a lower attitude towards aflatoxins contamination compared to those not in a union.

Parents strongly agreed that sorting and washing of crops before milling would minimize aflatoxin contamination and believed that eating contaminated food could cause diseases and even death. This was similar to the study done by Fandohan *et al.* (2005) in Benin that sorting, washing, and winnowing minimize aflatoxin level in grains. Age, the level of education and marital status of the respondents were the most significant variables to the perception and attitude of aflatoxin contamination and control in this study area. The respondents' age and level of education were mostly determinants of participants' perception of aflatoxin contamination and control in the foods. However, in general, the parents had a low level of perception and attitude as it was reflected on the total scores of perception and attitude. Inequality of age, marital status, and level of education might be the reasons for the respondents to have a lack of attitude and perception about aflatoxin contamination and control.

The Pearson's correlations show that there was a strong association between perception and attitude scores towards aflatoxins contamination and control in the community. However, the age of the respondent correlated positively to the perception, and also monthly income was positively correlated with occupation and education towards aflatoxin reduction and its threat to the health of the people. Similar findings were obtained by Jolly *et al.* (2009) that income and education are highly correlated and people with high income are willing to pay for food safety (Baker, 2003). This study used standard methods. Therefore one must be very careful in the interpretation of the results. Again, further research is also needed to analyze the sub-constructs of constructs from

exploratory factor analysis so as to be submitted to a confirmatory factor analysis to study each factor if it illustrates individual items.

4.6 Conclusion and Recommendations

Contamination of food by aflatoxin is a serious public health threat that requires attention to ensure that proper actions are taken to limit its health effects. The findings showed that socio-demographic variables had an effect on parents' perception and attitude on aflatoxin contamination and its management in the foods either directly or indirectly. This study focused on communities and its findings do not represent the whole populations including health and agricultural professionals on their levels of perception and attitude regarding aflatoxin contamination and control in the foods. Thus, when the communities are educated and perceived about the threat associated with aflatoxin, they will build an attitude on its actions to reduce contamination and that information will be easily diffused to the public.

Though the analysis revealed a number of factors that reflect the parents' attitude about aflatoxin risks, the study suggests that there is little variation in actions to reduce aflatoxin levels in complementary foods. The study observed perception and attitude divided into sub-constructs after using exploratory factor analysis, this is not the limitation of the study rather than those sub-constructs should be submitted to confirmatory factor analysis for further studies. The study team recommends public extension services to deliver information on aflatoxins and its control to the communities in a more timely and effective way. Such information can be disseminated through radio, existing system of government extension workers and communities groups which exist in the study areas where information can be distributed in an appropriate manner. Also, aflatoxin contamination and control should be taught in schools as special courses, not only in the medical schools

but in all tertiary institutions. These may be important strategies to increase public attention on aflatoxins contamination and adoption methods of control towards health effects of human and animals.

4.7 Conflict of Interest

All the authors have declared that they do not have any potential conflicts of interest.

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CHAPTER FIVE

5.0 Parents' Barriers and Actions Associated with Reducing Aflatoxins

Contamination in Complementary Foods in Central Regions of Tanzania

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5.1 Abstract

Contamination of complementary foods by aflatoxin is a serious public health threat that requires attention to ensure that proper actions are taken to limit its health effects. This study used a cross-sectional design to assess parents' local barriers and actions associated with reducing aflatoxins contamination in complementary foods among parents with children aged between 6-23 months in the central regions of Tanzania. The tools used in the survey included semi-structured questionnaire (364 respondents and focus group discussion (FGD) with 121 respondents. The information collected included socio-demographic variables, parents' barriers, and actions to mitigate aflatoxin contamination and reduction strategies. The results of the fitted model revealed that among proposed predictors of barriers to proper processing of grains/nuts to reduce spoilage/aflatoxin/mould contamination at home, only the number of children that a participant had was

statistically significant. The family with 3-7 children, the estimated odds that the barrier is time consuming rather than costly, was 0.305 times the estimated odds for the family with 1-2 children. This means that households with 3-7 children were less likely to report that time consumed was the barrier rather than the costs involved in comparison to subjects with 1-2 children. On the other hand, the estimated odds for subjects with 3-7 children that the barrier reduces food quantity instead of costs, was equal to 2.389 times the estimated odds for subjects with 1-2 children. The results of multiple logistic regression model for applying traditional fungicides/pesticides for storing crops showed that respondents aged above 34 years (OR=0.576, 95% CI:0.342-0.969) were significantly less in applying traditional fungicides/pesticides for storing crops than those respondents aged less or equal to 34 years old. The respondents with partial primary education (OR=2.872, 95% CI: 1.283-6.427) and primary education (OR=2.256, 95% CI: 1.194-4.264) significantly applied traditional fungicides/pesticides for storing crops than those never been to school respondents. FGDs revealed that, drying of crops before taking them inside for storage, use of traditional treatment herbs such as ash and mud applied/smeared/sprayed on the grain before and during storage were used to preserve crops/grain against fungi. Very few of the parents use good post harvest practices hence crops might get mould easily. It was revealed that no any formal or informal education had been provided about fungi contamination, and even control, rather, the respondents were generally preventing (mould) aflatoxins through experience. Therefore, there is a need to educate community on good agricultural practices for the better food safety and health of the community in general.

Key words: aflatoxin, parents, barriers, complementary foods, central Tanzania

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5.2 Introduction

Aflatoxin, a fungal toxin that commonly contaminates nuts, maize and other types of cereals is notorious not only for the havoc it wreaks on crops but those it inflicts on humans

and animals as well (Wild and Gong, 2010; CAST, 2003). The fungus is capable of attacking crops during production, harvest, storage, and even during processing and is now recognized as one of the biggest challenges to food and nutrition security, health and trade across the African continent (Bennett and Klich, 2003; Strosnider *et al.*, 2006; Wild and Gong, 2010; Ephrem, 2015). It is estimated that more than 5 billion people in developing countries worldwide are chronically exposed to aflatoxins (Williams *et al.*, 2004). The effects to this exposure includes liver cirrhosis, intestinal dysfunction, immune suppression and increased susceptibility to some infectious diseases including HIV-AIDS, and maternal and child health problems such as anaemia, malnutrition, stunting and wasting (Williams *et al.*, 2004; WHO, 2006; Gong *et al.*, 2008; Jolly *et al.*, 2011, 2013).

According to researchers carried out on animals (Joens *et al.*, 1981; Fernandez *et al.*, 1997; CAST, 2003; Kang'ethe and Lang'a, 2009; Caloni and Cortinovis, 2010), aflatoxins reduce productivity of healthy livestock through ingestion of contaminated feed; once ingested, the fungus causes a decrease in production of milk and eggs and it also leaves toxic residues in dairy, meat and poultry products and causes serious illness in almost all animals. The occurrence of aflatoxins is influenced by certain environmental factors; hence the extent of contamination will vary with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during pre-harvest, storage, and/or processing periods (Strosnider *et al.*, 2006; Cotty and Ramon, 2007; NTP, 2011; Kamala *et al.*, 2016). Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenic effect in susceptible laboratory animals and their acute toxicological effects in humans (CDC, 2004; William *et al.*, 2004; Probst *et al.*, 2013; Caloni and Cortinovis, 2010). As it is realized that absolute safety is never achieved, many countries have attempted to limit

exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed (De Koe, 1999; Creppy, 2002; CEC, 2006; EMAN, 2012).

The aflatoxins B₁, B₂, G₁, G₂ plus two additional metabolic products, aflatoxins M₁ and M₂, are of significance as direct contaminants of foods and feeds (IARC, 2002; NTP, 2011). Aflatoxin B₁ is the most potent of the aflatoxins. Aflatoxins have been rated as class 1A human carcinogens and OTA as a possible human carcinogen (group 2B; 1) by the International Agency for Research of Cancer (IARC, 1993; 2002; 2012). These toxins have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds (Ibid).

Aflatoxin contamination of crops used in preparation of complementary foods in fields of farmers in the central regions of Tanzania can be significantly reduced by following good agricultural practices, implemented from planting, drying, storage, and to plate. The studies (Kamala *et al.*, 2015; 2016) have focused on multiple mycotoxin contamination in stored maize and the association between aflatoxins (AFs) and fumonisins (FBs) contamination of maize and traditional post-harvest practices in rural Tanzania. Results from the mentioned study indicated high levels of aflatoxins (AFs) and fumonisins (FBs) among other mycotoxins with the co-occurrence in 45% of samples. AFs and FBs were detected in 50% at levels of up to 1081 µg/kg and 73% at levels up to 38 217 µg/kg, respectively. Other studies in Tanzania have reported occurrence of these toxins at significant levels (Kimanya *et al.*, 2008, 2009). Kimanya *et al.* (2008) reported that 18% of home-grown maize samples were contaminated with aflatoxins at levels up to 158 µg/kg, with 12% of these have above the Tanzanian limit of 10 µg/kg. Other studies assessed aflatoxin and fumonisin exposures using validated exposure biomarkers and estimated their associations with growth (Shirima *et al.*, 2015). In addition, studies in

Tanzania reported high exposure of infants and young children to AFs and FBs through maize-based diet (Shirima *et al.*, 2014; Magoha *et al.*, 2014c) and AFM₁ (Magoha *et al.*, 2014a) and FB₁ (Ibid) through breast milk from mothers whose main diet was maize.

Despite the above mentioned incidences at high levels of mycotoxins and associated health effects of aflatoxins in human, no study has been conducted in Tanzania to assess the association between awareness, local barriers of reducing aflatoxin contamination of complementary foods and traditional practices used to control aflatoxin (fungi) in crops. This knowledge is vital to the community in order to develop strategies for reducing aflatoxins in crops that are used as ingredients in preparing complementary foods. This study was undertaken to explore associations between awareness, local barriers and traditional practices in reducing risks of aflatoxin contamination in grains which are used in the preparation of complementary foods in rural Tanzania.

5.3 Research Methodology

5.3.1 Study areas

This study was carried out in four districts of Chamwino and Bahi in Dodoma Region (Fig. 5.1) and Manyoni and Ikungi in Singida Region (Fig. 5.2), in Central Tanzania. These regions experience low rainfall and short rainy seasons which are often erratic with long periods of drought. The two regions were selected because of the semi-arid condition which is characterized by high temperature during the day (up to 35°C) and low temperature (as low as 10°C) during the night. Both high temperature and humidity favour the growth of fungi thus signalling possibility of aflatoxins production in improperly stored crops (Cotty and Ramon, 2007).

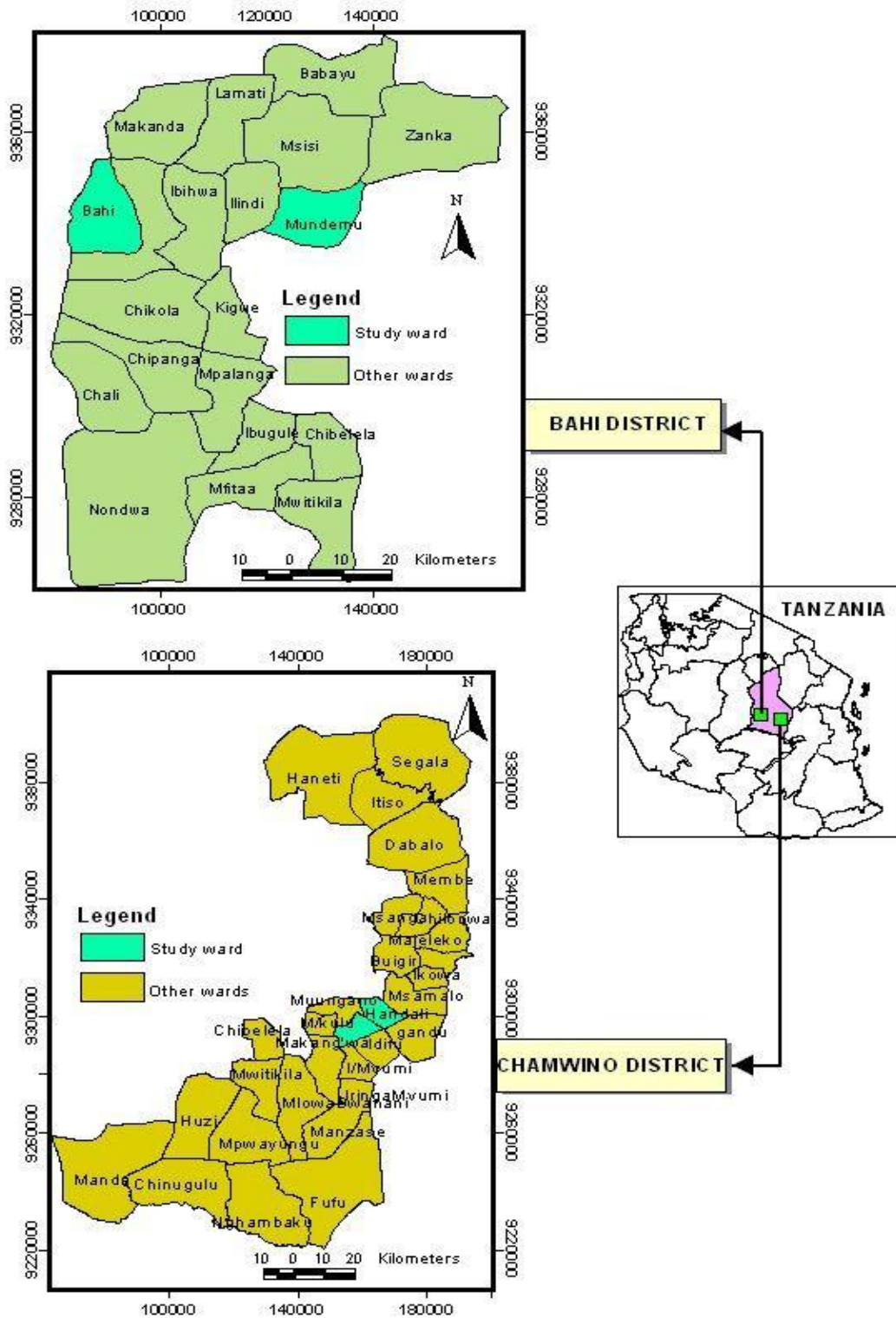


Figure 5.1: The maps of Bahi and Chamwino districts in Dodoma Region showing study areas

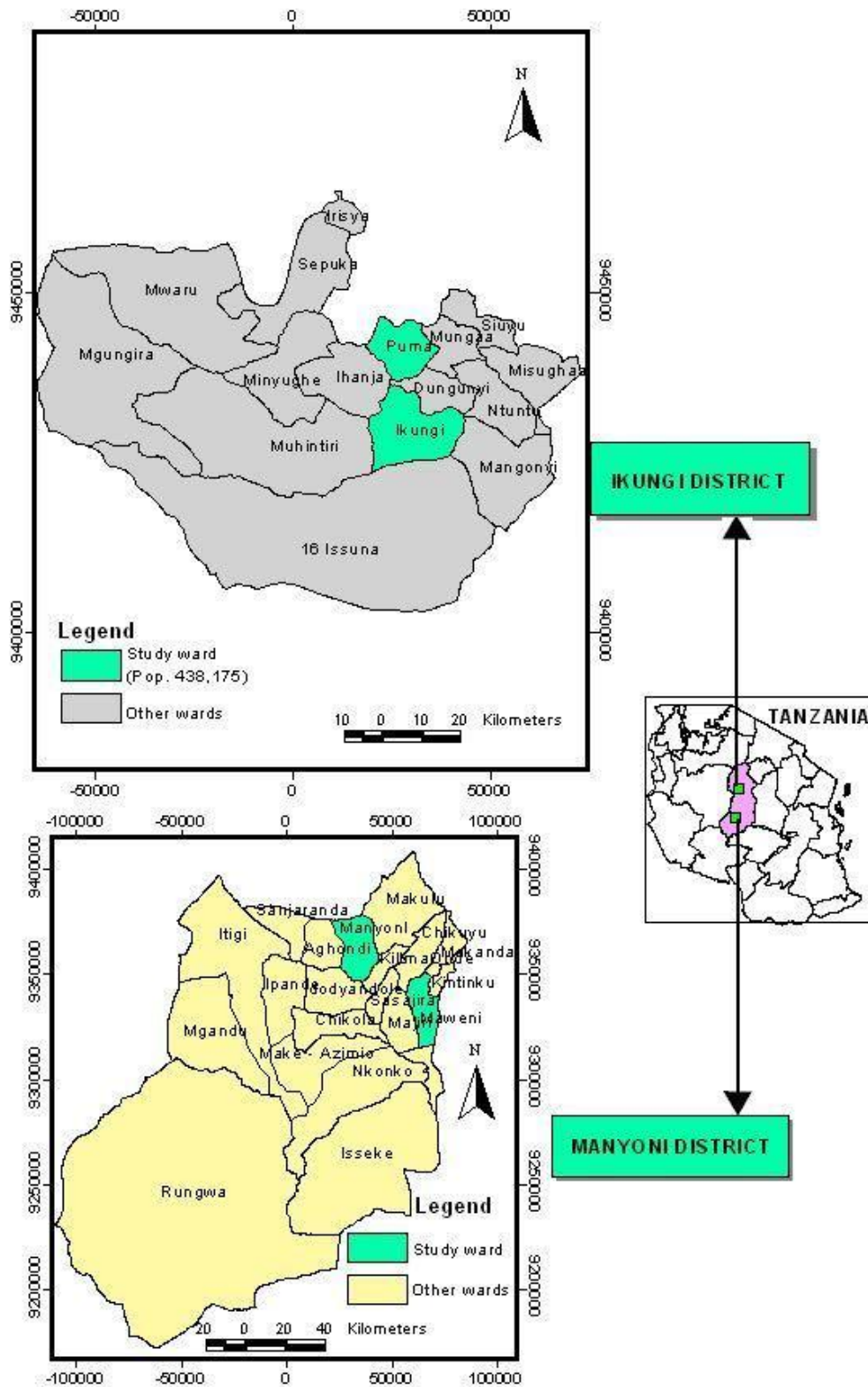


Figure 5.2: The maps of Ikungi and Manyoni districts in Singida Region showing study areas

5.3.2 Study design

The study design was cross-sectional which involved data collection at one point (households). The cross-sectional design also allows one to either use the entire population or a subset. Therefore, data were collected from 364 people to help answer research questions. Both quantitative and qualitative approaches to data collection with pre-tested questionnaires and structured interviews were used. The pre-tests were carried out in an area not selected for the study. The questionnaire was developed after being pre-tested through focused group discussion and interview involving parents or caregivers. All components of the study received University Research and Publication approval SUA/CB/26.

5.3.3 Study population and sampling

The study population consisted of males and females who were parents/caregivers of children aged between 6-23 months found in the household and who reside in the randomly selected villages, streets, or hamlets in the study areas. A multistage sampling technique was used to select 364 households. The households with children aged 6-23 months were identified after which the sampling was done. In Dodoma region a total of 182 households with children were identified while Singida region 182 households were identified for the study. In each household either men or women who are parents or caregivers of children aged between 6 to 23 months in the household was recruited from each village or street.

Sampling procedure

The procedures followed six steps:

1. List of 13 districts from each region forming the framework.
2. Four out of the thirteen districts were selected randomly.
3. Four out of the eighteen divisions were selected randomly from four districts.

4. Out of 109 wards four wards from each of the four divisions were selected at randomly.
5. Among 62 villages or streets/hamlets in these four wards, (4) villages, (8) streets and (20) hamlets were randomly selected for the study. All the villages or streets/hamlets in the selected wards were listed before random sampling made.
6. Finally, from each village, street or hamlet selected randomly the lists of ten cell leaders were obtained. With the assisted of the local government leaders and Village health workers all households which has one or more than one child aged between 6-23 months were identified.

5.3.4 Sample size

Sample size (n) of parents/caregivers in this study was determined by applying the single proportional formula by Magnani, (1997) used in the Statcalc programme of Epi Info Version 6 as follows: $n = (t^2) p (1-p) / m^2$

Where: n = the desired total sample.

t = standard normal deviate value (set at 1.96 which corresponded to the 95% confidence interval level).

m= margin error, the degree of accuracy (taken to be 5% in this study).

p= the proportion of parents/caregivers who have been aware of aflatoxin taken to be 33%) from Jolly *et al.* (2009).

When these figures in the above formula, are substituted, it gives a minimum sample size of 340. Then the figure was rounded up to 364 so as to accommodate the unforeseen problems such as non-response to some or all questions. The sample size assumes that a household has one child aged between 6-23 months. In case a household has more than one child aged between 6-23 months, one child was randomly chosen to avoid clustering of information.

5.3.5 Data collection procedure

Research assistants were selected to aid in data collection. They were selected based on previous experience in research work in communities and ability to understand and write Kiswahili and English languages. They were trained to understand the objectives of the study, the purpose and the procedure of the interview process in order for them to have a common understanding of the questions in the interview schedule, and also to ask the questions to ensure that participants understand them. The questions were translated into Kiswahili language. Quantitative data were collected by administering an interview schedule to a random sample of 364 households parents/caregivers of children aged between 6 to 23 months. The schedule contained both open and close-ended questions.

Qualitative data were collected by using the Krueger methodology for conducting focus group discussions (FGDs) (Krueger and Casey, 2015). The FGDs were conducted by using a checklist to allow the researcher guide the sessions and obtain the participants' views. Participants (including parents or caregivers with children aged between 6 to 23 months) in Focus Group Discussion (FGD) were purposefully selected from the four randomly selected districts. These were the criteria that were used in inclusion and exclusion of participants. One pair of research assistants (male and female) facilitated the FGDs by using a discussion guide and the principal researcher served as the assistant moderator. Another pair took notes during the FGDs. Information was collected by research assistants through 17 Focus Group Discussions (FGDs) with 121 participants (105 females and 16 males). The composition of all 17 FGDs was six (6) participants except six groups which had nine participants each and one group which had seven participants. The discussions were held in class rooms, office of village leaders or office of ward executive officer. The information collected was on community actions to prevent aflatoxins (mould) contamination in crops, education provided about aflatoxin/fungus contamination and

control to the community and also personal experiences about aflatoxin contaminations in complementary foods. The interview lasted for approximately 40 minutes for each session. All the interviews were audio recorded after obtaining the consent from participants and then the tapes were transcribed and translated into English by the principal researcher.

5.3.6 Data analysis

The Statistical Package for Social Sciences (SPSS) programme 21.0 version was used to analyze the data after cleaning. A 5% level of significance was used throughout the study and an independent variable with a p-value less than 0.05 was considered as statistically (significant) associated with outcome variable. For the qualitative part, coding was done using NVivo 7 software. The NVivo package has the ability to code and sort narrative data, interface with SPSS, and has good modelling facility and is user friendly (Hancock, 1998). It also best combines the NUD*IST computer software package with much more flexibility (Hancock, 1998). The FGDs were analysed by using Thematic Content Analysis method.

5.3.6.1 Baseline logit models

Baseline Logit models (BLM) are part of the broad family of Multi-categorical Response Models for unordered (nominal) multi-level category response variable (Agresti, 2002). Multi-categorical response models are used when the response variable has more than two categories. In this study, the variable barriers for proper processing of grains/nuts to reduce spoilage/aflatoxin/mould (*fangasi*) at home had three categories (Time, food quantity and Cost), hence the BLM was used to study the association between the barriers and the proposed predictors. Therefore, from the three categories mentioned above;

Let J be the number of response categories for variable Y and $\pi_1, \pi_2, \dots, \pi_J$ be the probabilities for a randomly chosen individual to fall into categories 1 . . , J , respectively.

Then $\sum_1^J \pi_i = 1$ (1)

In baseline logit model we are interested in finding if certain predictors have an effect on the probabilities $\pi_1, \pi_2, \dots, \pi_J$.

Assume that the last category (J) is the baseline category, then, the baseline category logits are

$$\text{Log} \left(\frac{\pi_i}{\pi_J} \right), \text{ for } i = 1, \dots, J - 1. \dots\dots\dots(2)$$

Given that the response falls in category i , this is the *log odd* that the response is i . For $J=3$, for instance, the model uses;

$$\text{Log} \left(\frac{\pi_1}{\pi_3} \right) \text{ and } \text{Log} \left(\frac{\pi_2}{\pi_3} \right) \dots\dots\dots(3)$$

The models using the baseline-category logits with a set of predictors x has form

$$\text{Log} \left(\frac{\pi_i}{\pi_J} \right) = \alpha_i + \beta_i x, \text{ for } i = 1, \dots, J - 1. \dots\dots\dots(4)$$

5.3.6.2 Baseline logit model for barriers for proper processing of grains/nuts to reduce spoilage/aflatoxin/mould (*fangasi*)

In this study the BLM was adopted to study the association between the barriers for proper processing of grains/nuts to reduce spoilage at home and set of proposed predictors. The outcome variable (barrier) had three categories: Time consuming; its ability to reduce food quantity and that it was costly.

5.3.6.3 Application of traditional fungicides/pesticides for storing crops

The multiple logistic regression model (LRM) was employed to determine the association between respondents' applying traditional fungicides/pesticides for storing crops to reduce aflatoxin/ fungi contamination to the crops during storage and set of proposed predictors in this study.

5.4 Results

5.4.1 Socio-demographic characteristics of respondents

Results in Table 5.1 show the distribution of parents/caregivers by socio-demographic characteristics in Bahi, Chamwino, Ikungi, and Manyoni Districts. The age of parents/caregivers ranged from 17 to 80 years with mean age (SD) of 30 (8.3) years 270 (74.2%) of the respondents were aged 34 years or below while respondents aged above 34 years were 94 (25.8%).

Table 5.1: Distribution of parents/caregivers by socio-demographic characteristics

Characteristics	Number	(%)
Age group (Years)		
≤ 34	270	(74.2)
> 34	94	(25.8)
Number of children		
1-2	180	(49.5)
3-7	184	(50.5)
Monthly income (US\$)		
≤ 22.8	256	(70.3)
> 22.8	108	(29.7)
Level of education		
Never been to school	64	(17.6)
Partial primary	49	(13.5)
Primary	204	(56)
Partial secondary	18	(4.9)
Secondary	29	(7.9)
Respondents' occupation		
Farmers	287	(78.8)
House wives	32	(8.8)
Employees	10	(2.7)
Petty traders	35	(9.6)
Marital status		
In Union	272	(74.7%)
Not in Union	92	(25.3%)

5.4.2 Analysis of barriers for proper processing of grains/nuts to reduce spoilage/ aflatoxins

Out of the 364 respondents, 242 (66.5%) reported that the cost involved is the barrier to proper processing of grains/nuts to reduce spoilage/aflatoxin/mould (which they call *fangasi*) at home while time consumed and reducing food quantity had equal proportion (16.8%). The results of the fitted model (Table 5.2) revealed that among proposed

predictors of barriers for proper processing of grains/nuts to reduce spoilage/aflatoxin/mould at home, only the number of children that a participant had was statistically significant. For households with 3-7 children, the estimated odds that the barrier is time consuming rather than costly was 0.305 times the estimated odds for subjects with 1-2 children. This means that subjects with 3-7 children were less likely to report that time consumed was the barrier instead of costs involved in comparison to subjects with 1-2 children. On the other hand, the estimated odds for subjects with 3-7 children that the barrier is reduced food quantity instead of costs involved was equal to 2.389 times the estimated odds for subjects with 1-2 children.

Table 5. 2: Parameter estimates and odds ratios (OR) of barriers for proper processing of grains/nuts to reduce spoilage/aflatoxin

Variable	Cases	Barriers	Estimate (SE)	OR	P-Value
Awareness	Yes	Time consuming	0.4240 (0.3669)	1.528	0.2478
	Yes	Reduces food quantity	-0.6489 (0.4554)	0.523	0.1541
	No	It is costly	Reference	Reference	Reference
	Yes	Time consuming	-0.0836 (0.4184)	0.920	0.8416
Number of Children	No	It is costly	Reference	Reference	Reference
	3-7	Time consuming	-1.1885(0.3264)	0.305	0.0003
	3-7	Reduces food quantity	0.8711(0.3155)	2.389	0.0058
Age (Years)	1-2	It is costly	Reference	Reference	Reference
	>34	Time consuming	-0.5190 (0.3784)	0.595	0.1701
	>34	Reduce food quantity	-0.0348 (0.3386)	0.966	0.9182
Monthly Income (US\$)	≤34	It is costly	Reference	Reference	Reference
	>22.8	Time consuming	-0.3242 (0.3732)	0.723	0.3850
	>22.8	Reduces food quantity	0.1706 (0.3499)	1.186	0.6258
Education Level	≤22.8	It is costly	Reference	Reference	Reference
	Partial Primary	Time consuming	-0.0152 (0.5395)	0.985	0.9776
	Partial Primary	Reduces food quantity	0.0608 (0.5009)	1.063	0.9035
	Primary	Time consuming	-0.2471(0.4095)	0.781	0.5462
	Primary	Reduces food quantity	-0.1829 (0.3902)	0.833	0.6392
	Partial Secondary	Reduces food quantity	-0.0172(0.7546)	0.983	0.9819
	Secondary	Time consuming	0.6592(0.5642)	1.933	0.2426
	Secondary	Reduces food quantity	-1.8133 (1.0933)	0.163	0.0972
Marital Status	Never been to School	It is costly	Reference	Reference	Reference
	In Union	Time consuming	0.1710(0.3484)	1.187	0.6235
	In Union	Reduces food quantity	0.1586(0.3591)	1.172	0.6587
	Not in Union	It is costly	Reference	Reference	Reference

Reference= The first category acts as a baseline, and can interpret the other coefficients as an increase or decrease in the log odds ratio over the baseline category

5.4.3 Analysis for application of traditional fungicides/pesticides for storing crops

It was reported that 221(60.7%) of 364 participants were applying traditional pesticides for storing crops. The results of multiple logistic regression model for applying traditional fungicides/pesticides for storing crops (Table 3) showed that respondents aged above 34 years (OR=0.576, 95% CI:0.342-0.969) were significantly less in applying traditional fungicides/pesticides for storing crops than respondents aged less or equal to 34 years. The respondents with partial primary education (OR=2.872, 95% CI: 1.283-6.427) and primary education (OR=2.256, 95% CI: 1.194-4.264) were significantly more in applying traditional fungicides/pesticides for storing crops than never been to school. However, respondents with partial secondary education $p=0.7852$, (OR=0.845, 95% CI: 0.253-2.830) and secondary education $p=0.8381$, (OR=0.882, 95% CI: 0.266-2.931) were not statistically significant, had less responses in applying traditional fungicides/pesticides for storing crops compared to respondents who had never been to school.

Other independents variables, namely awareness, number of children in the household, monthly income, occupation and marital status were not significantly associated with applying traditional pesticides/fungicides for storing crops since $p>0.05$.

Table 5.3: Parameter estimates and odds ratios (OR) for applying traditional fungicides/pesticides for storing crops

Variable	Parameter Estimate(se)	OR	95% CI	P-Value
Awareness				
No	Reference	Reference	Reference	Reference
Yes	0.3056(0.2976)	1.357	[0.758-2.433]	0.3045
Number of Children				
1-2 Children	Reference	Reference	Reference	Reference
3-7 Children	-0.1980(0.2222)	0.820	[0.531-1.268]	0.3729
Age (Years)				
≤ 34	Reference	Reference	Reference	Reference
> 34	-0.5524(0.2656)	0.576	[0.342-0.969]	0.0376
Monthly Income (US\$)				
≤ 22.8	Reference	Reference	Reference	Reference
> 22.8	0.2772(0.2791)	1.319	[0.764-2.280]	0.3206
Education Level				
Never been to School	Reference	Reference	Reference	Reference
Partial Primary	1.0549(0.4111)	2.872	[1.283-6.427]	0.0103
Primary	0.8138(0.3247)	2.256	[1.194-4.264]	0.0122
Partial Secondary	-0.1680(0.6165)	0.845	[0.253-2.830]	0.7852
Secondary	-0.1252(0.6125)	0.882	[0.266-2.931]	0.8381
Occupation				
Peasant	Reference	Reference	Reference	Reference
House wife	0.1445(0.3929)	1.155	[0.535-2.496]	0.7130
Petty trade	0.0577(0.4086)	1.059	[0.476-2.360]	0.8877
Employed	0.0217(0.9043)	1.022	[0.174-6.014]	0.9809
Marital Status				
Not in Union	Reference	Reference	Reference	Reference
In Union	-0.3620(0.2675)	0.696	[0.412-1.176]	0.1760

Reference= The first category acts as a baseline, and can interpret the other coefficients as an increase or decrease in the log odds ratio over the baseline category

5.4.4 Qualitative results

Basis for themes with supporting quotes were obtained from focus group discussions.

5.4.4.1 Community actions to prevent aflatoxins (mould) contamination in crops

During focus group discussions (FGDs) participants were asked about practices which they were doing to prevent aflatoxins contamination in the complementary food. Most of them mentioned the same kind of precautions they practice to prevent aflatoxins in general. These included drying of crops before storage, use of treatment materials such as ash and mud which were sprayed on the crops before and during storage. It was noted that very few participants buying fungicides/insecticides. Also, minority of participants were taking grains/nuts outside regularly in between storage time and storing crops in the rooms which were well ventilated and sun rays normally got in and out daily and easily. Some participants mentioned that, storage inside or outside depended on the type of crop. Such that some crops, they never took them inside because even if they took them while dry, they could easily get moist so those kinds of crops were normally stored outside on the roofs during the hot and dry season. In addition, the challenge which they were facing was lack of money with which to buy storage materials like polythene bags and sisal bags.

5.4.4.2 Community education about aflatoxin/fungus contamination and control

Participants in FGDs were asked about the education which they had received about mould (aflatoxin) contamination and prevention in complementary food. Almost all of the participants in the four districts mentioned that there were no any formal or informal education provided to them either from agricultural officers or from health officers about fungi contamination, and even control, rather they were generally preventing aflatoxins (mould) through experience.

5.5 Discussion

The study has identified a number of issues related to parents' local barriers that may cause lack of action, and the benefits of controlling aflatoxin contamination in complementary foods. In the current study, the respondents who were aged above 34 years old were less likely to apply traditional fungicides/pesticides for storing crops than parents aged less or equal to 34 years old. The relatively less number of times in applying traditional fungicides/pesticides by parents aged above 34 years old could be partially attributed to the fact that a larger proportion of this group also indicated attainment of no or low levels of formal education. This finding is similar to a study done by Ngongi (2013) in Tanzania which showed that as age of the respondents' increases, food insecurity at household level also increases. These findings are also in line with those from a study by Babatunde, (2008) which show that vulnerability to food insecurity increases as the age of the household head increases. Again, a study done by Idrisa (2008) also showed that age, in relation with farming experience has a significant influence on the decision making process of farmers with respect to risk aversion, implementation of improved good agricultural skills, and other production related decisions. It was concluded that, poor storage methods and structure and poor treatment of food crops during storage resulted to loss of food stored due to pest and molds/fungi could be one of factors leading to food insecurity and safety in the study area.

Contamination of complementary foods by aflatoxin is a serious public health threat that requires attention to ensure that proper actions are taken to limit its health effects. Although aflatoxin is a controllable risk factor in the diets, people are still not aware about its presence and consequences to health. Ngoma *et al.* (2016b) showed that 82.0 % of the parents were not aware of aflatoxin contamination in complementary foods and its health effects. The similar scenario was reported by Magembe *et al.* (2016) that awareness of

mould infection in stored maize and groundnuts was low in Kilosa, Tanzania. Recent findings by Ngoma *et al.* (2017) also indicate that parents who are responsible for preparation of complementary foods in the central regions of Tanzania do not fully perceive aflatoxins as being harmful to human and animals and their attitude towards their control generally was low.

Cost involved is a barrier for proper processing of grains/nuts to reduce spoilage/aflatoxin/mould (*fangasi*) at home while time consumed and reduced food quantity had equal proportions. Parents felt that proper processing like winnowing, washing, sorting, and dehulling of grains/nuts to reduce aflatoxin was too costly in terms of money. Families with 3-7 children were less likely to report that time consumed in the process was the barrier rather than the costs involved in comparison to the families with 1-2 children. On the other hand, families with 3-7 children reported that processing of grains/nuts to reduce aflatoxin was reducing food quantity to the family in comparison with families of 1-2 children. In the study areas, families were known to have food shortage and that fertility rate was high therefore the number of people in the families was revealed to be a great concern with regard to foods availability and safety. Parents claimed that washing, winnowing, sorting and dehulling of crops might reduce food quantity to the family. Normally, the larger the family size, the more likely the farmer is to become successful as the household has more labour to work on the farm. However, this would only work if all family members are old enough to perform the farm work, otherwise if the household size consists of majority of young children who cannot be employed as family labour, it would not work. Household size can influence food security at household level. Food insecurity increases as household size increases. Household with one or two members have the least percentage of food insecurity as long as the members are not elderly or small children. In a study by Chantesa *et al.* (2003) it was reported that

households with 7 members are more vulnerable to food insecurity compared to those with fewer members. The big number of children in the families and food shortage as found in this study may lead to eating contaminated foods with aflatoxins which are harmful to their health.

In this study, parents with partial and primary school education were more willing to use local pesticides or fungicides during storage of crops to control fungi/ mould than never been to school parents. Dosman *et al.* (2001) in Canada reported that people with higher levels of education are likely to be better informed, and therefore, may be more aware of some types of risk of food additives or pesticide residues in food than people with less education. People with higher education levels generally demand for food safety and security. Baker (2003) revealed that those with the highest levels of education were more willing to pay for food safety. Furthermore, education is very important in raising awareness; however in the current study, no formal or informal education were provided in the community about aflatoxin contamination, its health effects or control in crops used as ingredients in preparation of complementary foods. Therefore, in this study, very few parents were using good post-harvest practices, but majority of them were using poor post-harvest practices hence making them eat susceptible crops which may be contaminated with aflatoxins. These findings are similar to a study done by Barago (2013) in Mtwara, Tanzania, which indicated that majority of the household respondents, (86.7%) did not receive any extension services (education) for the whole season. Lack of extension services to the farmers on agriculture development and food security in Tanzania was also reported (Amani, 2004). In this case it constrained access to inputs and timely advice to stakeholders, particularly smallholder farmers and to a large extent impedes progress in the intensification of agriculture.

5.6 Conclusion

Aflatoxin contamination in complementary food in central Tanzania can be significantly reduced by following good agricultural practices, implemented from planting to drying and storage. Parents who aged above 34 years old were less likely to apply pesticides or fungicides during storage of crops than aged below or equal to 34 years old. Parents who have partial or primary level education were willing to use pesticides or fungicides than never been to school while high number of members in the families with food shortage may lead them to eat contaminated food to fungi or aflatoxins. In this study cost was a barrier for proper processing of grains/nuts which were used as ingredients in the preparation of complementary foods to reduce spoilage/aflatoxin/mould (*fangasi*) at home. Parents felt that proper processing like winnowing, washing, sorting, and dehulling of grains/nuts to reduce aflatoxin was too costly in terms of money. Although some of the practices indicated by the parents were conducive to mitigate aflatoxins infestation and contamination in crops which used as ingredients in preparation of complementary food, the majority of the storage practices described was unfavorable to aflatoxins reduction in their stored crops. The use of local practices like mad or ash during storage of crops may reduce aflatoxin contamination to the food. Therefore, the government should help in the management of aflatoxins and improvement of food security for the community. Comprehensive public awareness efforts should be made on the food safety and prevention of food borne diseases especially those which are caused by fungi/aflatoxins. However, further studies need to be conducted to assess the burden of aflatoxicosis in this community and also the common storage practices, as well as chemicals involved. Thus, it is important that the people continue to be educated on good agricultural practices, awareness and health behaviours with respect to food safety.

Limitations of the study

This study was limited to rural areas in Dodoma and Singida regions which ignored the peri-urban and urban communities, which might have led to under or overestimation of the findings. Hence the conclusions relate to those study areas.

Implication of the study

Although the results were from two regions, the findings are informative to the body of awareness, knowledge, perceptions and attitude towards aflatoxin contamination and its health effects. Also, enabled to determine levels of aflatoxins in ready-to-cook complementary foods in the study areas.

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CHAPTER SIX

6.0 The Influence of Awareness, Knowledge, Perception, and Practices of

Community on Childhood Dietary Exposure to Aflatoxins in Central, Tanzania

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6.1 Abstract

This study was cross-sectional, designed to assess the influence of awareness, knowledge, perception, and actions among 228 parents with children aged between 6-23 months on childhood dietary exposure to aflatoxins. The study has shown that complementary foods in Tanzania in the study area of central regions of Dodoma (Chamwino and Bahi districts) and Singida (Manyoni and Ikungi districts) are prone to aflatoxin (AF) contamination heavily contaminated with aflatoxin (AF), a group of highly toxic metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Total aflatoxins contamination in randomly selected 228 samples of ready-to-cook complementary foods had level of up to

60.284 $\mu\text{g}/\text{kg}$. Samples 52.6% were positive for aflatoxin with 9.65% of the positive samples exceeding concentration of allowed limit of 10 $\mu\text{g}/\text{kg}$. Samples with aflatoxin B₁ had level up to 38.184 $\mu\text{g}/\text{kg}$ and 15.4% of all samples tested positive with 7.02% having above the allowed limit of 5 $\mu\text{g}/\text{kg}$. Manyoni and Chamwino districts had equal proportions of complementary food samples with aflatoxins B₁ contamination of 19.3%. Ikungi district, on the other hand was noted to have the least prevalence of aflatoxins B₁ at only 7%. The results of the Univariate analysis showed that, awareness, knowledge, perception, attitude and dehulling were all significantly associated with aflatoxins (AFB₁) contamination ($p < 0.05$). The multiple logistic regression model presented that awareness of aflatoxins B₁ contamination, the chance of having food with aflatoxins B₁ contamination was significantly higher among subjects not aware of aflatoxins (OR=2.929, $p=0.015$). In this study, people with no knowledge of aflatoxins (OR =2.739, $p=0.019$) had significantly greater odds of having food contaminated with aflatoxins B₁ in comparison to people with knowledge of aflatoxins. The risk of having ready-to-cook complementary foods contaminated with aflatoxins B₁ were also found to be significantly higher among respondents un-dehulling the crops used to make children's food than those who were de-hulling (OR=2.763, $p=0.028$). Complementary foods examined in the study areas were seen to be heavily contaminated with aflatoxins; hence the children were at higher risks of exposure to aflatoxins. It was clearly established that peoples in the study area were not aware of the aflatoxin issues and therefore did not perceive aflatoxin contamination as a problem in their production systems. Also, they did not have information on health risks associated with the consumption of aflatoxin contaminated products including grain or nut flours used in preparation of complementary foods. Parents' awareness, knowledge, perception and attitude on aflatoxins contamination and their effects on health through affected complementary foods is necessary; is an

important step towards using practices that would help to reduce its exposure to the children in rural Tanzania.

Keyword: *aflatoxins, exposure, complementary foods, awareness, parents, children, Tanzania*

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6.2 Introduction

Aflatoxins are toxic substances naturally produced by moulds (fungi) that may contaminate agricultural commodities by growing on them, (Wild and Hall, 2000; IARC, 2002; 2012; Seetha *et al.*, 2017). They are one of the most potent natural carcinogenic compounds present in nature (IARC, 1993). These are a group of structurally related polyketide mycotoxins that contaminate field crops such as groundnuts, maize, cottonseed, cassava, cashew nuts, sorghum, millet and rice (Williams *et al.*, 2004; Strosnider *et al.*, 2006; Liu *et al.*, 2012). Contamination starts in the field and is exacerbated when crops are damaged by drought or insect infestation, or when the produce comes into contact with soil and is not properly dried (Wild and Gong, 2010; Kew, 2013). The most toxic of the aflatoxins is B₁, which is poorly degraded in the rumen and it is quickly excreted in milk as metabolite aflatoxin M₁ (Strosnider *et al.*, 2006). Despite the efforts made to control fungal contamination, toxigenic fungi are everywhere in nature and they can contaminate a wide range of agricultural products due to mould infestation both before and after harvest wherever humidity and temperature are sufficient (IARC, 1993; 2002; 2012; Strosnider *et al.*, 2006; Cotty and Jaime-Garcia, 2007; Cotty *et al.*, 2008; Schmale and Munkvold, 2012). The most critical environmental factors that determine whether or not a substrate will support mould growth are moisture content, temperature and time (FAO, 1998; 2004; Ncube *et al.*, 2010). Thus, drying, proper storage and suitable transportation are of prime

importance in the prevention of contamination (Williams *et al.*, 2004; Hell and Mutegi, 2011; Kamala *et al.*, 2016).

Six out of 18 different types of aflatoxins that have been identified and are considered important and are designated as B₁, B₂, G₁, G₂, M₁, and M₂, respectively, (Dors *et al.*, 2011). The order of acute and chronic toxicity is AFB₁>AFG₁>AFB₂>AFG₂, reflecting the role played by epoxidation of the 8,9-double bond and also the greater potency associated with the cyclopentenone ring of the B series, when compared with the six-membered lactone ring of the G series. Among these compounds, AFB₁ is normally predominant in concentrations in cultures as well as in food products (IARC, 1993; 2002; 2012). AFM₁ and AFM₂ are hydroxylated forms of AFB₁ and AFB₂ (Dors *et al.*, 2011).

Awareness of the potential danger posed by aflatoxins contamination of foodstuffs and the basic knowledge of aflatoxin is extremely low among communities in Kenya and Mali and Tanzania (Narrood *et al.*, 2011; Ngoma *et al.*, 2016b). Lack of awareness of aflatoxin, lack of tolerant varieties, non-availability of cheap diagnostic kits and inadequate monitoring skills all exacerbate the problem (Jolly *et al.*, 2009). Dietary exposure to aflatoxin B₁ has been identified as a major etiological risk factor for the development of hepatocellular carcinoma (Bbosa *et al.*, 2013).

Previous studies conducted in Tanzania have focused on incidences and levels of contamination in regions other than those covered in this study where recent outbreaks have been reported. According to Kimanya *et al.* (2008) people consuming maize-based food, which is also the main ingredient in formulation of complementary foods, are at high risk of exposure to multiple mycotoxins because 10% of the total samples tested detected the presence of aflatoxin and fumonisins at the levels up 90µg/kg and 6125 µg/kg,

respectively which are well above the US Food and Drug Authority (FDA) and European Union (EU) regulatory limit for aflatoxins in foods which are 20 and 4 ppb, respectively.

According to the Tanzania Government Report an outbreak of sickness in Dodoma and Manyara in 2016 that also resulted in deaths has been associated with the consumption of cereals contaminated with aflatoxins. Preliminary results from the cereals (maize, sorghum and millet) tests by the CDC showed aflatoxins contamination about 200 ppb which was remarkably above the tolerant limit to human being (Buguzi, 2016). In Dodoma region (Kondoa, Chemba, Dodoma, Chamwino districts) and Manyara (Kiteto District) the outbreak of aflatoxin contamination in cereals was revealed where 54 people were reported to have been exposed to the poison. Out of those exposed 14 deaths were reported due to liver failure with the majority of the affected being children aged below 12 years old (Ibid). In the context of this study, the selected regions for this study are characterized by semi-arid conditions, high temperature during the day (up to 35°C) and cool during the night (10°C). These conditions favour growth of fungi thus signaling possibility of aflatoxins production (Cotty and Jaime-Garcia, 2007). However, according to Ngoma *et al.* (2016b) the level of awareness of aflatoxins contamination and its health effect was revealed to be low among the community in Dodoma and Singida regions whereby only 18.1% of people interviewed were aware of such contamination. Previously, Narrod *et al.* (2011) had associated the lack of awareness, inadequate knowledge about aflatoxins contamination with high rate of exposure to aflatoxins. It is not known in Tanzania whether consumer's awareness, perception, attitude, and practices as well as challenges faced in dealing with aflatoxin problems have any association with levels of exposure. The knowledge of association between these factors and levels of aflatoxins found in foods (in particular, the complementary foods) could point to which of the factors should be prioritized in intervention measures.

This study therefore aims to determine aflatoxin levels in household ready-to-cook complementary foods in Dodoma and Singida regions, test association between awareness, knowledge and perception on aflatoxins, and practices of aflatoxin contamination and its reduction and contamination levels. Results from this study will be useful for understanding the levels of aflatoxin in complementary foods and possibly motivate policy makers on policy review, enforcement of laws and regulations and formulation of guidelines on aflatoxins management in Tanzania.

6.3 Materials and Methods

6.3.1 Description of the study areas

This study was done in Dodoma (Fig. 6.1) and Singida (Fig. 6.2) regions. These regions form part of the central zone of Tanzania which experiences low rainfall and short rainy seasons which are often erratic with long periods of drought. The regions were selected because of semi-arid condition which is characterized by high temperature during the day up to 35°C and cool to 10°C during the night. Both temperature and humidity favour growth of fungi thus signaling the possibility of aflatoxins production (Cotty and Jaime-Garcia, 2007; NBS and Macro, 2011). The details of descriptions of the study areas are described in Ngoma *et al.* (2016a). These are the areas where a recent aflatoxin contamination outbreak was reported.

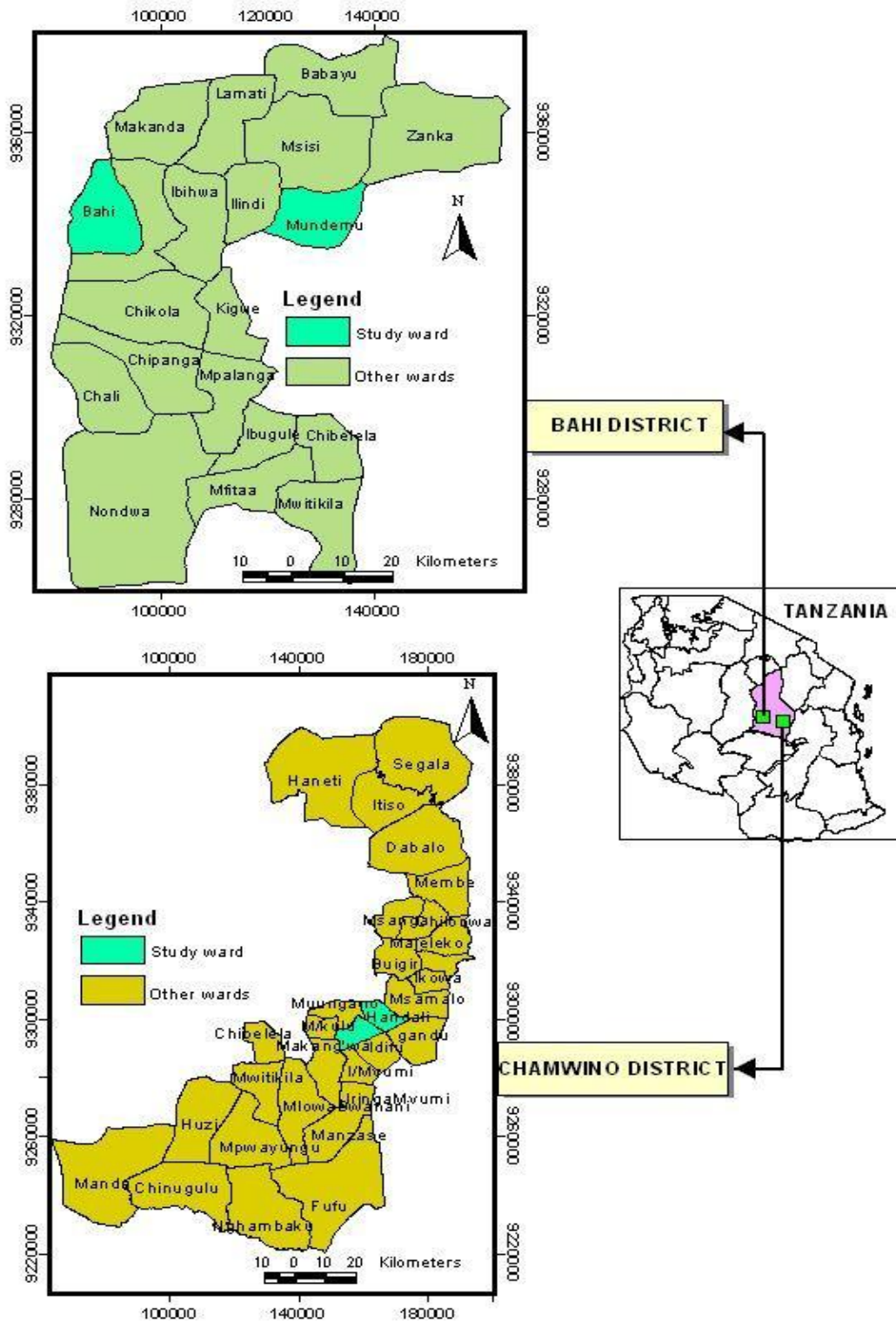


Figure 6.1: The maps of Bahi and Chamwino districts in Dodoma Region showing study areas

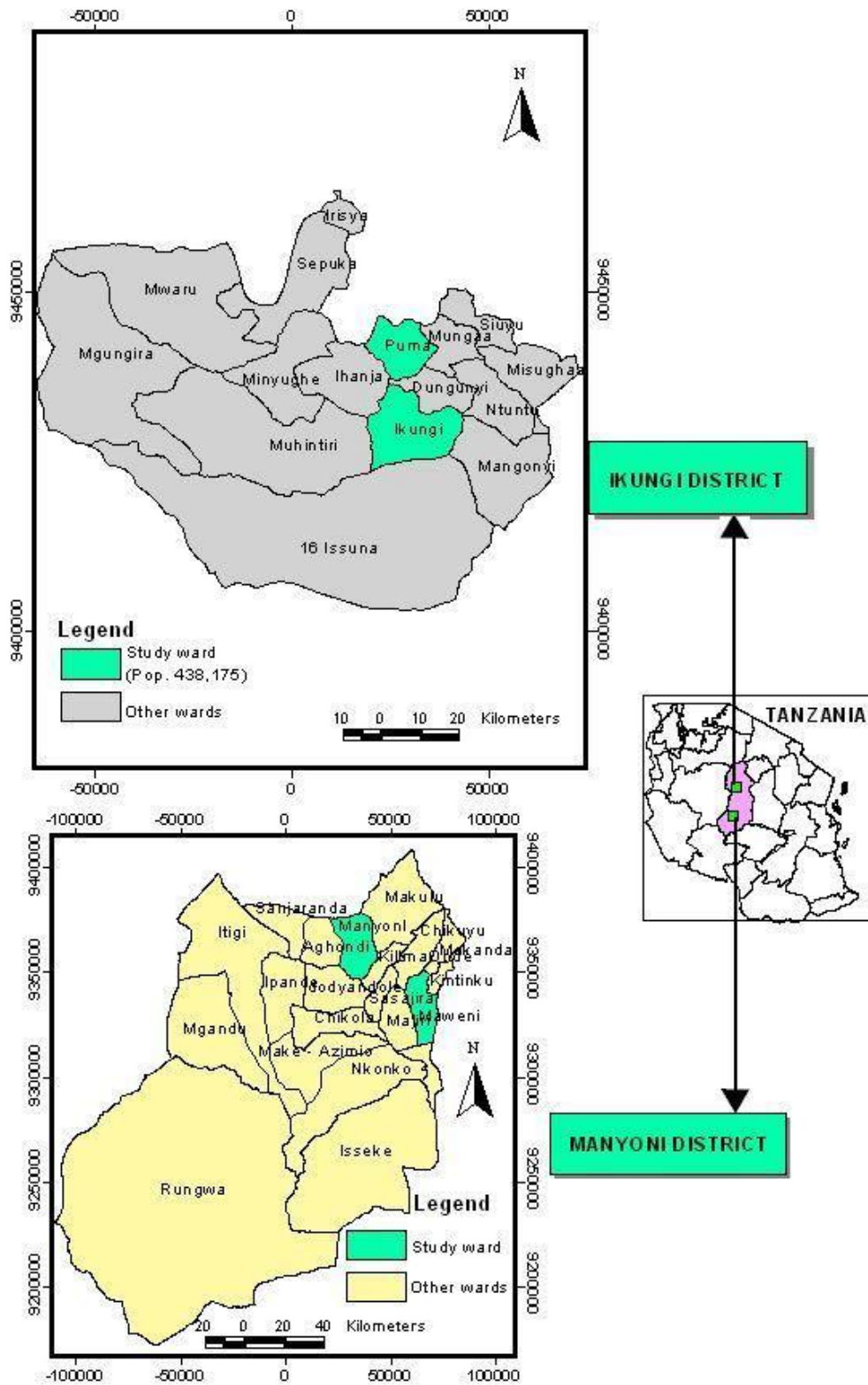


Figure 6.2: The maps of Ikungi and Manyoni districts in Singida Region showing study areas

6.3.2 Research design

The research design was cross-sectional study. It involved the collection of data at one point in time (households). Quantitative research design was employed in this study.

6.3.3 Sampling and sample size

Sample size of this study was 228 randomly selected households with parents/caretakers of the children aged between 6 to 23 months. This is the age bracket of children where stunting rate is high and a period of introduction of complementary foods (NBS & ICF Macro, 2011). Also, it is a period of transition from exclusive breastfeeding to family foods, referred to as complementary feeding and therefore a very vulnerable period. It is the time when malnutrition starts in many infants, thus contributing to the high prevalence of malnutrition in children less than two years of age (FAO/EU, 2011). Correspondingly, 228 samples of complementary foods were taken for analysis of aflatoxins.

Sample size (n) of parents/caretakers-child pairs in this study was determined by applying the single proportional formula by Magnani, (1997) used in the Statcalc programme of Epi Info Version 6 (Dean *et al.*, 1996) as follows:

$$n = (t^2) p (1-p) / m^2$$

Where: n = the desired total sample.

t= standard normal deviate value (set at 1.96 which corresponded to the 95% confidence interval level).

m = margin error, the degree of accuracy (taken to be 5% in this study).

p= the proportion of aflatoxin prevalence for complementary foods, taken to be 18% from baseline data (Kimanya *et al.*, 2008).

Substituting these figures in the above formula gives a minimum sample size of 226.8. Then the figure was rounded up to 228 so as to accommodate the unforeseen problems such as non response to some or all questions and other errors. This sample assumes that a household has one child aged between 6-23 months. In case a household had more than one child aged between 6-23 months, one-household-one child was randomly selected to avoid clustering of information.

6.3.4 Sampling procedures

A multistage sampling technique was used to select 228 households. The households with children aged 6-23 months were identified after which the sampling was done. In Dodoma and Singida regions, respectively there were 114 households for the study. In each household either men or women who were parents or caregivers of children aged between 6 to 23 months were recruited from each village or street.

Steps for sampling procedure

Procedures for sampling was conducted using the following steps:

1. List of 13 districts from each region forming the framework.
2. Four out of the thirteen districts were selected randomly.
3. Four out of the eighteen divisions were selected randomly from four districts.
4. Out of 109 wards four wards from each of the four divisions were selected randomly.
5. Among 62 villages or streets/hamlets in these four wards, (4) villages, (8) streets and (20) hamlets were randomly selected for the study. All the villages or streets/hamlets in the selected wards were listed before random sampling was made.

Finally, from each village, street or hamlet selected randomly, the lists of ten cell leaders were obtained. With the assistance of the local government leaders and Village health workers, all households which had one or more than one child aged between 6-23 months were identified.

6.3.5 Recruitment and Training of Research Assistants

Six research assistants were recruited and trained for four days before the work of data collection commenced. Selection was based on their previous experience in research work in communities. The six research assistants were three males and three females, who were High School leavers residing in Dodoma but were familiar with the study area. The training focused on the general overview of the study, interviewing skills and procedures and finally, familiarization with the study instruments. The training also emphasized on how to obtain consent, maintain neutrality, privacy issues, personal relation and ethics.

6.3.6 Pretesting

The pre-test survey using twenty structured interviews was carried out in Nzuguni village, Dodoma urban District. Pretesting was done in order to test the clarity of questions and study logistics. It also helped the research assistants to exercise flexibility in the wording of questions and probing. This exercise was carried out in an area not selected for the study and was done before the actual study took place.

6.3.7 Data collection

The data were collected from 228 households of mothers with children aged between 6-23 months during in-home visits and observational assessments of foods and liquids present in the household. Survey data and weighing of ready-to-cook flour used for complementary food preparation were collected during the in-home visit. The survey

included socio-demographics both for child and mother, awareness, knowledge, perception, and attitude on aflatoxin contamination and control, cultural beliefs and practices associated with aflatoxins (fungi) contamination in complementary food, handling and storage of crops used in preparation of complementary foods. All measures were translated into Swahili using translation-back translation method with the following steps: 1) translation of the original English material into Swahili, by ensuring that the English meaning was maintained; 2) back-translation into English by principal investigator; and 3) resolution of any discrepancies. Research assistants verified translation accuracy and appropriateness to ensure semantic, conceptual, and normative equivalence. All survey was conducted in Swahili, which was the language spoken in the homes of all participants and ready-to-cook complementary foods were collected at the same time.

6.3.8 Analysis of aflatoxins in ready-to-cook complementary foods

Samples were collected by asking permission from parents to pour the available flour onto a mat, hand-mixed thoroughly and took a portion each from two locations in the batch. The collections were then pooled and mixed to make a homogenous representative sample. 250g of the homogeneous sample was packed and transported to the Tanzania Bureau of Standard (TBS) laboratory for aflatoxins analysis. Determination of total aflatoxin and aflatoxins, B₁, B₂, G₁ and G₂ were determined in the ready-to-cook complementary food in accordance with the method described by Stroka *et al.* (2000) with a slightly modification from Romar's all purpose laboratory analysis. All the procedures and modification used are outlined in appendix III.

6.3.9 Instrumentation

Chromatographic separations were performed on a HPLC system, Agilent Technologies SL 1200 Series (Waldbronn, Germany) composed of a binary pump equipped with micro vacuum degasser, thermostated auto sampler, column compartment and fluorescence detector (model G1321A). All the separations were performed by using a ZORBAX Eclipse XDB C18 column (150 × 4.6 mm particle size 5 μm, Agilent Technologies) operating at a flow-rate of 0.8 mLmin⁻¹ in isocratic elution with a mixture of water, methanol and acetonitrile (50:40:10, v/v/v). The injection volume was 20 μL and the column temperature was set at 40°C. The fluorescence detection was carried out at a λ₁₆₀ excitation and λ emission of 365 and 450 nm, respectively. The system was interfaced, via network chromatographic software (Agilent ChemStation), to a personal computer for instrumentation control, data acquisition and processing.

6.3.10 Validation of the analytical method

In order to analyze aflatoxins B₁, B₂, G₁ and G₂ contamination levels in the ready-to-cook complementary food samples, a high performance liquid chromatographic (HPLC) method was used. The method was validated in terms of linearity, limit of detection (LOD) and limit of quantification (LOQ), selectivity, precision and recovery. The selectivity of the method was evaluated by adding standard solutions of aflatoxins to samples without any traces of the four aflatoxins. These were then submitted to extraction and quantified by HPLC-FLD, 1200 Series Agilent Technology Using Chemstation Software with the corresponding peaks and concentrations identified in the chromatogram. The method was considered to be selective when it presented no interfering peaks coinciding with the retention times of the aflatoxins. The linearity was studied by external standardization, using analytical curves built up from 4 different concentrations of the aflatoxin standards in acetonitrile. Each curve, for the aflatoxins B₁, B₂, G₁ and G₂, was prepared in

quintuplicate and injected on 5 different days. The coefficient of determination (R^2) was considered appropriate when > 0.99 .

The limits of detection (LOD) and quantification (LOQ) were found by adding decreasing concentrations of standard solution containing the four aflatoxins in the samples, and then submitted to extraction and quantification, up to the lowest detectable concentration (LOD) and the lowest quantifiable concentration (LOQ), under suitable conditions of repeatability ($n = 10$, $RSD < 15\%$). The recovery studies were conducted on samples in which the presence of the four aflatoxins was not detected, starting with the addition of two different concentrations ($5\mu\text{g}/\text{kg}$ and $10\mu\text{g}/\text{kg}$) for each aflatoxin (B_1 , B_2 , G_1 and G_2). Five samples were contaminated for each concentration and stored at room temperature for 12 hours, prior to the extraction procedure. To evaluate the recovery parameter, the values set by the EC to determine the aflatoxin at levels up to $10\mu\text{g}/\text{kg}$, corresponding to 70-110% recovery (EC 2006), were adopted as reference. Precision was expressed by the Relative Standard Deviation (RSD %) and calculated according to the repeatability of the recovery experiments for each concentration ($5\mu\text{g}/\text{kg}$ and $10\mu\text{g}/\text{kg}$). The maximum variation was set at $\leq 15\%$.

In consequence of different legal limits for aflatoxins in foodstuff consumption, different sets of standard solutions and spiked samples were used for foodstuffs (maize based complementary food). The linearity test in foodstuffs was performed by five series of analyses on five different days, by injecting four standard solutions of aflatoxins B_1 , G_1 , B_2 and G_2 each at concentrations of $1\mu\text{g}/\text{kg}$, $5\mu\text{g}/\text{kg}$, $10\mu\text{g}/\text{kg}$ and $15\mu\text{g}/\text{kg}$. (Appendix III).

6.3.11 Requirements for a derivatization

Pre-column derivatization enhances the detection and recoveries of aflatoxin (Wang *et al.*, 2008), and was done as follows: 400 μ L from the eluent was taken and mixed with 600 μ l of derivatizing reagent (70:20:10 H₂O: Trifluoroacetic acid (TFA): Acetic acid). The sample mixture was finally vortexed for 20 s then; the mixture was conditioned at 65⁰C for 15 minutes, allowed to cool and then injected into HPLC.

6.3.12 Data management

At the end of each day of data collection, the Principal Investigator (PI) assessed the completed interviews to look for omissions and inappropriate responses. This helped to improve the quality of data collection. There were also frequent debriefing meetings to discuss the progress of data collection. All the completed interviews were stored in a locked cabinet and destroyed at the end of the study. Only one person had the key to that cabinet and laboratory data were saved in personal computer and also in external drive. HPLC results were saved into PI software instruments.

6.3.13 Statistical analysis

The data collected was analyzed using simple descriptive statistics, Microsoft excel correlation analysis, Chi-square test and multiple regression equations. In this regard, the major statistical operations performed under inferential statistics, were the Univariate analysis specifically Pearson chi square test and Multiple Logistic regression analysis. A regression equation was used to show the relationship between aflatoxin exposures in relation to output. Laboratory analysis results were entered and analysed in Microsoft Excel®2003. The Statistical Package for Social Sciences (SPSS version 21.0) was used to analyze both the surveyed and laboratory data. A 5% level of significance was used

throughout the study, an independent variable with p-value less than 0.05 was considered as significantly associated with outcome variable.

6.3.13 Research clearance and ethics

Approval to do this research was obtained from the Research and Publications Committee of the Sokoine University of Agriculture (SUA), through the Department of Food Technology, Nutrition and Consumer Sciences. Thereafter, permission to carry out this study was obtained from District Executive Director (D.E.D) office of each study district and the respective local government leaders in the study area. Each respondent in the study was briefed on what the study was all about and they were informed that their participation was voluntary; nobody was forced to participate in the study. Also the respondents were assured of confidentiality and anonymity. Before the interview, participants were requested to give their consent for participation. Eligible respondents were informed about the purpose of the study and a verbal informed consent was obtained. Each participant was assured that all the information given would be confidential and code numbers were used instead of the respondent's name.

6.4 Results

6.4.1 The distribution of aflatoxins contamination by districts

The results of aflatoxin contents in 228 food samples taken from 228 household for analysis of aflatoxins B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁) and G₂ (AFG₂) are shown in Table 6.1. Total aflatoxin was detected in 120 (52.6%) samples for all four districts. The overall prevalence of AFB₁ and AFB₂ were 15.4% and 24.6%, respectively. On the other hand, the overall prevalence of AFG₁ and AFG₂ were 3.9% and 8.8% respectively. With respect to district, the findings revealed that, Manyoni and Chamwino had equal proportion of food samples with aflatoxins B₁ contamination (19.3%). Ikungi district was noted to have

the least prevalence of aflatoxins B₁ (7%). A similar trend of AFB₂ was noted among the four districts, in which Manyoni had highest prevalence of AFB₂ (35.1%) followed by Chamwino (29.8%) and Bahi (17.5%). The range for total aflatoxin and aflatoxin B₁ contamination in the study areas were 0.22-60.28µg/kg and 0.27-38.18µg/kg, respectively. The limits of detection (LOD) and quantification (LOQ) found were 0.1µg/kg and 0.4µg/kg, respectively, for the four aflatoxins. LOD=Mean of lowest conc +3sd. The limit of detection for aflatoxin in µg/kg were G₁=0.213775; B₁=0.219909; G₂=0.22146; B₂=0.21942 while limit of quantification in µg/kg were G₁=0.23238; B₁=0.23976; G₂=0.287868; B₂=0.24279. The results establish recoveries in the range 91-121% and 95-117% for the spiked concentrations of 5 µg/kg and 10 µg/kg respectively.

Table 6.1: Distribution of aflatoxins contamination by districts

District	N	AFs n (%)	AFB _{1n} (%)	AFB ₂ n (%)	AFG _{1n} (%)	AFG ₂ n (%)
Manyoni	57	40(70.2)	11(19.3)	20(35.1)	1(1.8)	8(14.0)
Chamwino	57	37(64.9)	11(19.3)	17(29.8)	2(3.5)	7(12.3)
Bahi	57	22(38.6)	9(15.8)	10(17.5)	1(1.8)	2(3.5)
Ikungi	57	21(36.8)	4(7)	9(15.8)	5(8.8)	3(5.3)
Total	228	120(52.6)	35(15.4)	56(24.6)	9(3.9)	20(8.8)

6.4.2 The overall Occurrence and levels of aflatoxins in ready-to-cook

complementary foods

Chamwino district had a highest concentration range of aflatoxin B₁ contamination (0.3-38.2 µg/kg) than other districts followed by Bahi with 0.5-20.9 µg/kg. In the other hand, Manyoni district has highest concentration range 2.8-14.5 µg/kg compared to Ikungi with 0.6-12.4 µg/kg in Singida region. Chamwino has highest total aflatoxin concentration range of 0.3-60.3 µg/kg than all other districts in the regions. The overall concentration

ranges for total aflatoxin and aflatoxin B₁ contamination in the study areas were 0.02-60.3µg/kg and 0.3-38.2µg/kg, respectively.

Table 6.2: Occurrence and levels of aflatoxins in ready-to-cook complementary food in study areas

Aflatoxin		N	Occurrence (%)	Aflatoxin (µg/kg)	
				Range	Mean ± SE
AFB ₁	Manyoni	57	31.4	1.7-14.5	1.02±0.36
	Chamwino	57	25.7	0.3-38.2	2.34±0.90
	Bahi	57	31.4	0.05-20.9	1.09±0.53
	Ikungi	57	11.4	0.6-12.4	0.31±0.22
	Total	228	15.4	0.3-38.2	1.19±0.28
Total Aflatoxin	Manyoni	57	27.1	0.02-15.1	2.35±0.52
	Chamwino	57	18.8	0.3-60.3	5.24±1.72
	Bahi	57	34.1	0.5-37.8	2.17±0.94
	Ikungi	57	20.0	0.1-39.4	1.52±0.79
	Total	228	52.6	0.02-60.3	2.82±0.55

Values are means of Aflatoxin B₁ and total aflatoxin levels for positive complementary food samples from each district.

N is the total number of analysed samples

SE is standard error

Mean values of aflatoxin B₁ and total aflatoxins

The highest aflatoxin mean value for complementary food samples was found in Chamwino districts. The lowest aflatoxin mean value for complementary food samples was found in Ikungi districts. All districts had detection above limit of detection (LODs).

6.4.4 Association between selected variable and aflatoxins (AFB₁) contamination

Table 6.3 presents results of association between aflatoxins (AFB₁) contamination and each of the selected independent variables analysed using chi-square test. Variables associated with p-value less than or equal to 0.25 by Univariate analysis were included as

candidates in the multiple logistic regression model. P-values were estimated by two-sided tests. Eventually, variables with p-value < 0.05 in multiple logistic regression models were retained in final model for interpretation. Results of the Univariate analysis showed that, the included independent variable were all significantly associated with aflatoxins (AFB_1) contamination. The proportion of AFB_1 was significantly higher in households where the mothers were not aware (25.5%) compared to mothers who were aware of aflatoxins contamination (12.7%). The percentage prevalence of aflatoxins (AFB_1) among ready-to-cook complementary food samples of mother with knowledge of aflatoxins contamination was 12.1% while that of mothers with no knowledge was 25.5%. With respect to perception toward aflatoxins contamination, the results showed that, those with at least 22.23 score had lower prevalence of AFB_1 (7.4%) compared to those with less than 22.23 score had prevalence of (19.7%). Individuals with less than 36 attitude score toward aflatoxins contamination had higher percentage of complementary foods with AFB_1 (20.2%) in comparison to subjects with at least 36 score who had 10.5% prevalence. Aflatoxins B_1 contamination was higher in ready-to-cook complementary food samples for people that were using un-dehulling the crops that they were using to make children's food (18.9%) than those who were pumping or de-hulling (8.8%).

Table 6.3: Association between AFB₁ and selected independent variables

Variables	Total Frequency	Frequency with Toxic	Chi square	P-value
Awareness			4.723	0.030
No	47	12(25.5)		
Yes	181	23(12.7)		
Knowledge			5.694	0.017
No	55	14(25.5)		
Yes	173	35(12.1)		
Perception toward Aflatoxins Contamination Score			6.101	0.014
Less than 22.23	147	29(19.7)		
At least 22.23	81	6(7.4)		
Attitude toward Aflatoxins Contamination Score			4.084	0.043
Less than 36	114	23(20.2)		
At least 36	114	12(10.5)		
Dehulling Crops			4.132	0.042
Yes	80	7(8.8)		
No	148	28(18.9)		

6.6.3 Potential predictors of aflatoxins B₁ contamination in children's food

To determine the predictors of aflatoxins B₁ contamination in children's food, all independent variables were subjected to multiple logistic regression model. With other covariates in the model controlled, the results of multiple logistic regression model presented (Table 6.3) showed that the effect of attitude toward aflatoxins contamination ($p=0.072$) was no longer significantly associated with aflatoxins B₁ contamination, hence was removed from the final model. On the other hand, awareness ($p=0.015$), knowledge ($p=0.019$), perception toward aflatoxins contamination ($p=0.022$) and de-hulling of crops used to make children's food ($p=0.028$) were found significantly to be predictors of aflatoxins B₁ contamination. Compared to respondents aware of aflatoxins B₁ contamination, the chance of having food with aflatoxins B₁ contamination was significantly higher among subjects not aware of aflatoxins (OR=2.929, $p=0.015$). In this

study, people with no knowledge of aflatoxins (OR =2.739, p=0.019) had significantly greater odds of having food contaminated with aflatoxins B₁ in comparison to those with knowledge of aflatoxins. The odds of having food with aflatoxins B₁ contamination for individual with less than 22.23 perception score was almost 3 times that of people with at least 22.23 perception score (OR =3.101, p=0.022). Those subjects with less than 22.23 perception score toward aflatoxins contamination were significantly more likely to have food with aflatoxins B₁ contamination. The risk of having ready-to-cook complementary foods contaminated with aflatoxins B₁ was also found to be significantly higher among respondents un-dehulling the crops used to make children's food than those who were pumping or de-hulling (OR=2.763, p=0.028).

Table 6.4: Parameter estimates and adjusted odds ratios (OR) of the logistic regression model for aflatoxins B₁ (AFB₁) contamination

Variable	Parameter Estimate	Standard Error	Odds Ratio(OR)	P-value
Awareness				
Yes	Reference	Reference	Reference	Reference
No	1.075	0.443	2.929	0.015
Knowledge				
Yes	Reference	Reference	Reference	Reference
No	1.008	0.429	2.739	0.019
Perception toward aflatoxins examination score				
Less than 22.23	1.132	0.494	3.101	0.022
At least 22.23	Reference	Reference	Reference	Reference
Dehulling				
Yes	Reference	Reference	Reference	Reference
No	1.016	0.463	2.763	0.028

Reference= The first category acts as a baseline, and can interpret the other coefficients as an increase or decrease in the log odds ratio over the baseline category

6.5 Discussion

Aflatoxin levels in foods are likely to provide a good indication of aflatoxin exposure (Azziz-Baumgartner *et al.*, 2005; Lewis *et al.*, 2005; Moss, 1998). Studies have shown that AFB₁ concentration in food above a certain limit is considered hazardous and a threat to health and food security (Lewis *et al.*, 2005). This study has shown that aflatoxin contamination levels in ready-to-cook complementary foods have been found to be a significant problem and they are likely to indicate aflatoxin B₁ exposure to the children. Chamwino and Manyoni districts have high prevalence and level of aflatoxin B₁ compared to Bahi and Ikungi; this could be contributed by warm and dry climatic conditions prevailing in these study areas during the survey. There was disparity in levels of mycotoxin exposure between sampling times, a pattern reflecting the combining effect of increased consumption of contaminated family food, and the seasonal variation of mycotoxins contamination which was previously reported (Wild and Hall, 2000; Turner *et al.*, 2003).

6.5.1 Feeding practices and complementary food

The practices of feeding children with any semi-solid or solid foods were seen to start early in life by majority of the children in the study areas which is contrary to the recommendation of six months after birth (WHO, 1999). It was revealed that 31.1% of the children were receiving complementary food at the early age of less than three months. Similarly, 51.8% of the children had been introduced to complementary food earlier than the recommended age of six months while only 22.8% had received the solid food at the recommended age. The early solid food introduction to children before six months can contribute to breastfeeding failure, malnutrition since solid food has no enough calories for child development and most importantly, may be contaminated with aflatoxin. According to the health records in the study areas, it was observed that the major diseases pestering

children included fever and/or malaria (62.7%), diarrhea (22.4%) and coughing (10.5%). These diseases may lead to exposure of the children to infectious diseases and toxic chemicals caused by pollution and other sources since their guts are so delicate for any contamination. Inadequate food diversity and food security forces them to eat complementary food whose main ingredient are maize-based and also susceptible to contamination with mycotoxin (Kulwa, 2016; Kimanya *et al.*, 2008). The target range for complementary feeding is generally taken to be 6 to 23 months of age, (WHO, 2004) even though breastfeeding may continue beyond two years (WHO, 2009).

6.5.2 Aflatoxin contamination in complementary food, practices and effects on health

The mean total aflatoxin content in ready-to-cook complementary food in the four study districts was 60.3µg/kg with 52.6% prevalence and 38.2µg/kg of aflatoxins B₁ with prevalence of 15.4%. The maximum concentration of total aflatoxin was higher than MTL of 10µg/kg by East African standards (EAC, 2011a). However, the maximum level was lower than 158 µg/kg as reported by Kimanya *et al.* (2008) but highest than that reported by Nyangi (2014) from other areas of Tanzania. Also the occurrence and levels of aflatoxins reported by this study are lower than previously reported from other parts of the country (Kimanya *et al.*, 2014) for maize based complementary food samples. About 53% samples were contaminated by aflatoxins and 24.1% of the samples exceeding the maximum permissible levels of 10µg/kg. Samples with aflatoxin B₁ had level up to 38.2 µg/kg and 15.4% of all samples were contaminated. Samples that were contaminated, 45.7% had levels above 5µg/kg which is the recommended limit (TBS, 2012). Some other studies have shown an association between dietary exposure to aflatoxins and child growth (Gong *et al.*, 2003; Turner, 2013; Shirima *et al.*, 2013; 2015). This alarming observation shows that people were consuming mouldy grain or nuts. While studying the fate of fumonisins and aflatoxins during processing of maize to food products in Benin, Fandohan

et al. (2005) found that sorting and winnowing reduced mean aflatoxin level in maize from 6.57 $\mu\text{g}/\text{kg}$ to 2.67 $\mu\text{g}/\text{kg}$ which is used as an ingredient in preparation of children food. Therefore, it is likely that parents or care-givers in the study area were using damaged and unclean seeds without sorting in preparing complementary foods.

6.5.3 Effects of awareness and knowledge on aflatoxin contamination

Aflatoxin contamination is highly associated with level of awareness, knowledge, perception and attitude of community towards its threat and reduction at the household level. From the study, it was clearly observed that people were not aware of the aflatoxin issue, and so did not perceive aflatoxin contamination as a problem in their production systems (Ngoma *et al.*, 2017). They also did not have information on health risks associated with the consumption of aflatoxin contaminated products including crops used in preparation of complementary foods. The odds of having food with aflatoxins B₁ contamination for individual with less perception score was almost three times that of people with at least perception score. Those respondents with less perception score toward aflatoxins contamination were significantly more likely to have food with aflatoxins B₁ contamination. Therefore, awareness, knowledge, perception toward aflatoxins contamination of the parents and dehulling of crops used to make children's food were significantly predictors of aflatoxins B₁ contamination. The low level of awareness and knowledge of aflatoxin contamination and its reduction were associated with high level of contamination in all four districts (Ngoma *et al.*, 2016b). These findings are similar to the study done by Lee *et al.* (2017) in Vietnam that there was a variation of awareness of aflatoxin contamination between the study areas. This is the first study to report awareness, perception and knowledge of aflatoxins contamination in relation to complementary food aflatoxins exposure in the central regions of Tanzania. Therefore, in order to reduce the exposure and negative impacts of aflatoxins contamination, it is also

important that people become aware and knowledgeable of aflatoxin issues and put into action good practices. From the survey done by Abt Associates, (2012, 2013) in three districts (Kongwa, Bukombe, and Njombe) in Tanzania, it was revealed that farmers did not know anything about aflatoxins. Moreover, the agricultural extension officers were not trained in mycotoxin and aflatoxin, a similar observation as made in the current study. To address the problem, the intervention programme may include management actions related to timing of planting, proper irrigation and pest management for pre-harvest, proper drying, moisture, insect and rodent control for post-harvest storage and hand sorting, winnowing, sorting and washing for post-harvest operations before cooking complementary food. Although knowledge, perception and awareness of aflatoxins and the means to control them may help change behaviour, there is a need for using aflatoxin-low products in preparation of children's food.

6.5.4 Effects of dehulling of crops on aflatoxin contamination

Dehulling is the practice that is associated with significant reduction of aflatoxin exposure to complementary food (Fandohan *et al.*, 2006). The risk of having ready-to-cook complementary foods contaminated with aflatoxins B₁ were also found to be significantly higher among people not pumping or un-dehulling the crops used to make children's food than those who were pumping or dehulling. It has been reported that dehulling removes most of the toxins in the bran and germ fractions (Ibid). A study in Zimbabwe revealed that undehulled crops had higher percentage of aflatoxin levels when compared to the dehulled crops (Siwela *et al.*, 2005). Also these results emphasize that there is a need of dehulling the crops before using or incorporation in food formulation. Based on the high levels of aflatoxins contamination observed in complementary food, it indicate that people using undehulled maize before milling or cook foods for the babies or the family hence be susceptible to aflatoxins exposure.

6.6 Conclusions and Recommendations

This study has found the presence of AFB₁ in ready-to-cook complementary foods and parent's low levels of awareness, knowledge on aflatoxins contamination and associated health risks to children aged between 6-23 months. Ready-to-cook complementary food in the four study districts in Dodoma and Singida regions had high levels up to 60.284µg/kg of total aflatoxin and prevalence of up to 52.6% and 38.184µg/kg of aflatoxins B₁ with prevalence of 15.4% that is higher than MTL of maximum limit of 10µg/kg for total aflatoxin and 5 µg/kg set in Tanzania for AFB₁. From the study, it is clearly concluded that people were not aware of the aflatoxin issues, and so did not perceive aflatoxin contamination as a problem in their production systems. They also did not have information on health risks associated with the consumption of aflatoxin contaminated products including crops used in preparation of complementary foods. Consequently, it has been established that awareness, knowledge, perception toward aflatoxins contamination of the parents and dehulling of crops used to make children's food were significantly predictors of aflatoxins B₁ contamination.

To address the problem of aflatoxin and moulds it is recommended that:

1. The government through ministry of agriculture and health hold training sessions to mothers, farmers, leaders, extension officers, community development officers and nutritionists on actions to reduce aflatoxin contamination. Such intervention programme may include management actions related to timing of planting, proper irrigation and pest management for pre-harvest, proper drying, moisture, insect and rodent control for post-harvest storage and hand sorting, winnowing, sorting and washing for post-harvest operations before cooking complementary food.

2. Although knowledge, perception and awareness of aflatoxins and the means to control them may help change behaviour, there is a need for using aflatoxin-low products in preparation of children's food.
3. The government should make available cheap diagnostic kits and institute monitoring skills in grain sales centre and in communities

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CHAPTER SEVEN

7.0 General Conclusions and Recommendations

7.1 Conclusions

Contamination of food by aflatoxin is a serious public health threat that requires attention to ensure that proper actions are taken to minimize its undesirable health effects. It is concluded that home processed ready-to-cook complementary foods in Central Tanzania are heavily contaminated with aflatoxins beyond the allowable limits. It has been established that the level of awareness on aflatoxin contamination and health risks is very low in the study community. That low awareness of aflatoxin contamination among parents or caregivers put children at increased health risks. The findings showed that socio-demographic variables, either directly or indirectly, had an effect on parents' perception and attitude on aflatoxin contamination and its management in the foods. Thus, if communities are well educated, they will perceive and understand better the threat associated with aflatoxin; they will build appropriate attitude towards proper actions in order to reduce contamination; hence information will be easily diffused to the general public.

Though analysis revealed a number of factors that reflect parents' attitude about aflatoxin risks, the results suggest that there is little variation in actions to reduce aflatoxin levels in complementary foods. Apart from low level of awareness on aflatoxin issues including their occurrence and management strategies, it has become clear that practices and food security factors increase chances of aflatoxin exposure. Most parents in these study areas engage in small-scale, mixed farming that includes some livestock. Maize, sorghum, and groundnuts are the primary dietary staple and the main crops produced in these study areas. These crops are very susceptible to mould if inappropriately handle and processed.

At harvest, farmers store most of their crops for household consumption in poor storage facilities and sell the rest (if they harvest enough) to meet other household needs. When household stored crops are used up, farmers sell their livestock and purchase foodstuffs from the market. Due to food shortage and low knowledge on fungal and aflatoxins contamination, these families sometimes eat undehulled, unsorted and mouldy crops without washing or winnowing them hence exposing themselves to high health risks of aflatoxin contamination in their diet. Such malpractices along the supply chain of grown crops especially maize and groundnuts need to be addressed. Based on this, it is further concluded that actions to reverse the situation can reduce significantly the levels of contamination to allowable maximum limits. Aflatoxin contamination is highly associated with level of awareness, knowledge, and perception of community towards its threat and reduction at the household level. Dehulling is the practice that is associated with significant reduction of aflatoxin exposure to complementary food. This study focused on communities, however, the findings do not represent the entire population particularly health and agricultural professionals so as to establish their levels of perception and attitude regarding aflatoxin contamination and control in foodstuffs. Their views will further increase knowledge and awareness-raising efforts to the public especially women.

7.2 Recommendations

This study revealed high magnitude of ready-to-cook complementary food exposure to aflatoxin together with low level of awareness of parents or caregivers of its contamination and health risks to the children aged between 6-23 months in central Tanzania. It is therefore recommended to undertake appropriate measures to address the prevailing situation. While recommending for intervention to minimise human exposure to aflatoxins, it is important to consider that the problem of aflatoxins is basically cross-cutting. In view of this, effective control measures need to be comprehensive and

participatory, involving multidisciplinary partners and joint strategies by government, the private sector, farmers, consumers and all other players in the food value chain. In addition, intervention measures to prevent exposure of children to aflatoxins should be considered as one of key initiatives for improving child health and nutrition in Tanzania. In view of the findings and subsequent discussions above, the following are specific strongly recommendations suggested from this study for reducing dietary exposure of children to aflatoxins:

- i) Parents in the central regions of Tanzania must be made aware of the potential health dangers of fungal and aflatoxin in their food crops through enhanced involvement of agricultural extension services and educational programmes. Awareness raising campaigns can be carried out through appropriate media such as radio, television, newspapers and drama, existing system of government extension workers, health workers and existing community groups in the study areas.
- ii) Agricultural extension officers and village health officers must be trained on aflatoxin awareness and knowledge since they are also unaware of these aflatoxins.iii The agricultural extension officers need to know issues that can result in fungal infection and aflatoxin production and how contamination of crops can be reduced before they are used in the preparation of complementary foods.
- iii) Agricultural extension officers should in turn advise farmers on good agricultural practices that can reduce aflatoxins contamination of crops such as good pre and post harvesting practices.
- iv) Institute public extension services to deliver information on aflatoxins and its control to the communities in a more timely and effective way. Such information

can be disseminated through radio, existing system of government extension workers and communities groups which exist in the study areas where information can be distributed in an appropriate manner.

- v) Aflatoxin contamination and control should be taught in schools as special courses, not only in the medical schools but in all tertiary institutions to include important strategies to increase public awareness on aflatoxins contamination and adoption methods of control towards health effects of human and animals.

The study further recommends research in the following areas;

- i) What barriers prevent new technologies from reaching local farmer on aflatoxin?
- ii) What can be done to improve the spread of information on new technologies that can control aflatoxin?
- iii) Can farmers access training on the use of new technologies on aflatoxin? What more can be done to ensure that training reaches farmers in the remote areas?
- iv) What hinders farmers from accepting and adopting new farming methods to control aflatoxins?
- v) How can technology be better designed to meet the needs of smallholder farmers in Tanzania
- vi) How can farmers get more involved in the design and adaptation of technologies, drawing on their own experience and expertise?

APPENDICES

Appendix 1: Questionnaire for Examine the Influence of Awareness, Knowledge and Practices of Community on Childhood Dietary Exposure to Aflatoxins in Central Tanzania

Interview with the parents /caretakers

Questionnaire number.....

Date of interview.....

Name of Village/Street

Name of District.....

PART I: PARTICULARS

A. CHILD PARTICULARS

1. Child name

2. What is your relationship with this child?

(1) Mother

(2) Father

(3) Caretaker

3. Date of birth..... (*From child clinic card*)

4. Gender

01= Boy 02= Girl

5. Place of birth of your child

(1) Hospital

(2) Home

(3) Assisted by Traditional Birth Attendant (TBA)

(4) Others mention

6. Is the child breast feeding? (If YES, skip question 7&46)

- (1) Yes
- (2) No.

7. If no, at what age did the child stop breastfeeding?months

8. At what age was the child introduced to complementary food?months

9. Child food information (1) Has special food (2) Eats the family food (3) Both

10. How many meals did you give your child yesterday

11. Out of the meals you gave your child yesterday, how many had any maize ingredient?

12. In the last one week, how many days did you give your child the food that contain groundnuts?

13. What is the source of maize used in preparation of child's food?

- (1) Home grown
- (2) From the market
- (3) Others (Specify).....

14. How do you process maize used for preparation of child's food?

- (1) Un-dehulled
- (2) Dehulled
- (3) Others (Specify).....

15. Out of the meals you gave your child yesterday, how many have groundnut?
.....

16. In the last one week, how many days did you give your child the food that contain groundnuts?

17. What is the source of groundnuts used in preparation of child's food?

- (1) Home grown
- (2) From the market
- (3) Others (Specify).....

18. How do you process ground nut used for preparation of child's food?

- (1) Sorting
- (2) roasting
- (3) boiling

19. Out of the meals you gave your child yesterday, how many have fingermillet?
.....

20. In the last one week, how many days did you give your child the food that contain
fingermillet?

21. What is the source of fingermillet used in preparation of child's food?
(1) Home grown (2) From the market
(3) Others (Specify).....

22. How do you process fingermillet used for preparation of child's food?
(1) Sorting (2) roasting (3) boiling

23. What are the major diseases facing children in this village?
(1) Fever
(2) Diarrhea
(3) Coughing
(4) Urinary tract infection (U.T.I)
(5) Malaria
(6) Flu
(7) Kwashiorkor
(8) Stunting growth
(9) Cancer
(10) Other diseases (specify).....

B. RESPONDENT PARTICULARS

24. Age of respondent (years)

25. What is your level of education?
(1) Never attended school/no formal education
(2) Did not complete primary school education

- (3) Completed primary school education
- (4) Did not complete secondary school education (ordinary)
- (5) Completed secondary school education (ordinary)
- (6) Did not complete high school education (advanced)
- (7) Completed high school education (advanced)
- (8) Post secondary/tertiary education

26. What is your marital status?

- (1) Single (2) Married/living together (3) Divorced (4) Widowed (5) Separated
- (6) Cohabiting

27. What is your main occupation?

- (1) Student (2) Peasant (3) House wife (4) Petty trade
- (5) Employed (specify)
- (6) Others (specify)

28. What is the occupation of your spouse?

- (1) Peasant (2) House wife (3) Petty trade (4) Business
- (5) Employed (6) Others (specify)

29. What are the monthly income /cash earning of your family? Choose the amount of income below; (1) Less than Tshs 50,000 (2) Less than Tshs 100,000 (3) Less than Tshs 200,000 (4) Less than Tshs 300,000 (5) Less than Tshs 400,000 (6) Greater than or equal to 400,000

S/N	SOURCE OF INCOME	AMOUNT OF INCOME
29.1	Crop Production	
1	Maize	
2	Sorghum	
3	Groundnuts	
4	Fingermillet	
5	Cassava	
29.2	Animal Production	
1	Cow	

2	Goat	
3	Sheep	
4	Milk	
29.3	Poultry Production	
1	Chicken	
2	Eggs	
29.4	Wages Employment	
1	Salary	
29.5	Business activities	
1	Shop	
2	Bar	
29.6	Others	

PART II: RESEARCH QUESTIONS

A: What are the levels of awareness, knowledge, attitude and perceptions of communities towards aflatoxins in Dodoma and Singida regions?

Awareness of aflatoxins contamination

Yes answer=1 and No answer=0

30. Have you ever heard toxins that may present in crops which can be caused by mouldy?

- (1) Yes (2) No (If yes, go question number 28)

31. Have you ever heard toxins that may present in foods which can be caused by mouldy?

- (1) Yes (2) No (If yes, go question number 28)

32. Where did you get this information?

- (1) Hospital (2) Colleague (3) Mass Media (Radio, TV, Newspapers

- (4) Others (Specify).....

33. Have you heard of the word aflatoxin before?

- (1) Yes (0) No

34. Are you aware of aflatoxins (toxins) that can contaminate crops in the farm?

- (1) Yes (0) No

35. Are you aware of aflatoxins (toxins) that can contaminate crops during storage?

- (1) Yes
- (0) No

36. Are you aware of aflatoxins (toxins) that can contaminate food?

- (1) Yes
- (0) No

37. Are you aware of aflatoxins contamination in your child's foods?

- (1) Yes
- (0) No

38. Are you aware of the effects of aflatoxins in human being?

- (1) Yes
- (0) No

38.1 If yes, please mention them;

- (1) Stunting of children
- (2) Liver cirrhosis
- (3) Cancer
- (4) Vomiting
- (5) Others specify.....

39. Are you aware of the aflatoxins effects to the animals?

- (1) Yes
- (0) No

39.1 If yes, please mention them;

- (1) Tumors
- (2) Digestive problems
- (3) Liver problem
- (4) Others specify.....

Knowledge about aflatoxins contamination

40. What is your response to the following statements in relation to knowledge of aflatoxins contamination? (Correct=1 and Incorrect=0, circle the response of respondent)

SN	Statements	Respondent	
		Correct (1)	Incorrect (0)
40.1	Hot and humid climate of this areas can promote growth of fungi		
40.2	Improper stored food can be contaminated by fungi		
40.3	Aflatoxin is a type of fungi		
40.4	Exposure to aflatoxin (mouldy) can be harmful to the health		
40.5	Aflatoxins (mouldy) are only found in crops		
40.6	Aflatoxins (mouldy) are only found in foods		
40.7	Some liver diseases can be linked to intake of aflatoxins (mouldy)		
40.8	Aflatoxins (mouldy) can cause cancer		
40.9	Aflatoxins (mouldy) can cause stunting growth of your child		
40.10	Use of pesticides can reduce fungi in storage		

Perception toward aflatoxins contamination

41. To what extent do you agree on the following statements? (Circle the answer she/he agree)

S/N	Statements	Measurement scale				
		Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
41.1	Sorting of grains/nuts reduces aflatoxins (mouldy) contamination					
41.2	Washing grains/nuts reduce aflatoxins (mouldy)contamination					
41.3	Discolored grains/nuts					

	indicate the presence of aflatoxins (mouldy)	agree (5)	(4)	(3)	(2)	Disagree (1)
41.4	Eating contaminated foods with aflatoxins (mouldy) can cause diseases	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
41.5	Eating contaminated foods with aflatoxins (mouldy) can cause death	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
41.6	Aaflatoxins (mouldy) contamination can occur any time in foods	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)

Attitude toward aflatoxins

42. To what extent do you agree on the following statements? (Circle the answer she/he agree)

SN	Statements	Measurement scale				
42.1	Good agricultural practices will minimize aflatoxins in crops	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
42.2	Sorting of discolored (mouldy) crops will minimize contamination of aflatoxins in the child's foods	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
42.3	Washing of grains/ nuts before milling can reduce aflatoxins (mouldy) contamination	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
42.4	Aflatoxins can be removed during milling of child's foods	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)

42.5	Sorting of damaged grains/nuts is time consuming	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
42.6	Aflatoxins can be removed during cooking of child's foods	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
42.7	Sorting of damaged grains/nuts is too costly?	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
42.8	Sorting of grains/nuts is hygienic?	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
42.9	Clean grains/nuts attract better prices?	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)
42.10	Clean grains/nuts always sell faster	Strongly agree (5)	Agree (4)	Undecided (3)	Disagree (2)	Strongly Disagree (1)

B: Complementary feeding practices among infants aged between 6-24 months.

43. How many children do you have or take care of?

44. Have you ever breastfed?

- (1) Yes (2) No

44.1. If No, please, what are the reasons for not breastfeeding?

- (1) The child was sick
(2) Parental separation
(3) Mother was sick
(4) Child refused
(5) Others specify.....

45. In your experience, how long did it take to breastfeed your child after birth?

(0) Immediately (1) Hours..... (2) Days.....

46. At what age does your child stopped breastfeeding?

(1) Days..... (2) Months.....

47. Does your child receive exclusive breastfeeding?

(1) Yes (2) No

48. Does your child receive solid foods?

(1) Yes (2) No

49. If your child feed on solid food, when was it introduced?

(1) After some Days..... (2) After months.....

50. Who advised you for introduction of solid foods to your child?

(1) Husband (2) Myself (Mother) (3) From traditional birth attendant

(3) From Hospital or health centers' practioners

(4) Any other specific place, mention

51. What are the main sources of solid foods of your child?

(1) Home growing (2) Purchasing (3) Getting from aid (4) Both of them

(5) Any other sources, please mention.....

52. What kind of foods frequencies given to your child?

S/N	Type of food	Which foods given to a child in week (tick)	Number of times the child eat this food per day (write)	Number of days the child eat this food per week (write)	Which foods contain maize (tick)	Which foods contain peanuts (tick)
52.1	Thin porridge					
52.2	Stiff porridge					
52.3	Rice					
52.4	Round (irish) potatoes					
52.5	Sweet					

	potatoes					
52.6	Cassava					
52.7	Banana					
52.8	Sorghum					
52.9	Finger Millet					
52.10	Makande (maize+beans)					

C: What are the practices used that can contribute to levels of contamination of aflatoxins in this area?

53. Which month did you harvest the crops used for complementary food?

(1) Months..... (2) Years

54. Do you dehull the grain/nuts during preparation of complementary foods?

(1) Yes (2) No

55. Do you mill the grain/nuts during preparation of complementary foods?

(1) Yes (2) No

56. Do you dry your crops?

(1) Yes (2) No

56.1. If yes, where do you normally dry your crops?

(1) On the ground

(2) On the roof

(3) On the traditional granaries (vichanja)

(4) Others please specify.....

57. Do you store your crops?

(1) Yes (2) No

57.1 How do you store the crops?

- (1) Traditional granaries (*vichanja*)
- (2) Darini
- (3) Roof
- (4) Bags
- (5) Others specify.....

58. What is the intended use of your grain?

- (1) Eating (2) Selling (3) Any other? Please mention.....

59. Do your crops get mouldy during storage?

- (1) Yes (2) No

60. What type of food do you give to the child?

- (1) Specific foods for child (2) The family food (3) Both

61. What are the uses of your mouldy grain?

- (1) Home consumption (2) Animal feeds (3) Traditional brews
- (4) Others? Specify.....

62. How do you dispose the crop residues?

- (1) Animal feed (2) Burn (3) Left in field (4) Ploughed in

D. What are the local barriers and practices associated with reducing aflatoxins contamination in complementary foods in the community?

63.0 Local barriers to aflatoxins reductions

63.1. What hinder proper sorting of grains in your society?

Please mention.....

63.2. What hinder regular washings of grains/nuts in the family?

Please mention.....

63.3. What limit proper handling of crops to reduce aflatoxins in your family?

Please mention.....

63.4. What limit proper processing of crops to reduce aflatoxins in your family?

Please mention.....

63.5. Is there any taboo that may hinder aflatoxins reduction in your crops?

(1) Yes (2) No

63.6. If yes what are they.....

63.7. Does your partner discuss about aflatoxins reduction in crops?

(1) Yes (2) No

63.8. If yes please explain.....

64.0 Local practices to aflatoxins reductions

64.1. What is the source of crops used in preparation of complementary foods?

Please mention.....

64.2. Do you use pesticides for crops in the farm?

(1) Yes (2) No

64.3. If yes what are they? Mention.....

64.4. Do you use pesticides on your crops during storage?

(1) Yes (2) No

64.5. If yes, Mention them.....

64.6. Do you use any local pesticides (indigenous methods) during storage of crops?

(1) Yes (2) No, if yes

64.7. What types of local pesticides (indigenous methods) do you use during storing of crops?

(1) Ash (2) Mud

(3) Others please specify.....

64.8. Do you apply any fungicides on your crops during storage?

- (1) Yes (2) No

64.9. If yes, what types of fungicides do you use in your crops?

Please mention.....

64.10. Do you use any local fungicides (indigenous methods) in your crops?

64.11. If yes, what types of local fungicides (indigenous methods) do you use in your crops? (Mention)

- (1) Ash (2) Mud

(3) Others please specify.....

Appendix 2: Guideline for Focus Group Discussion (FGDs) Questionnaires

The Focus Group Discussion guide with open ended questions will be used to collect information from the respondents. The participants will be 6 parents or caretakers/guardians, one health officer, one agricultural officer and 2 village leaders. The responses will be tape recorded and transcribed afterwards.

Date: Day.....Months.....Year.....

Group No.....Village.....Ward.....

District.....

Group Gender Composition: Male..... Female.....

No of Group Members..... Age Range.....

Briefing:

I would like to begin by thanking you very much for agreeing to participate in this discussion. The purpose is to learn and understand your personal experiences about aflatoxins (mouldy); you're awareness, knowledge, perception and attitude on this aspect. Thus, it is important for you to be as open and honest as possible in discussion, expressing and explaining your real experiences. The information you will give is important for this research due to the fact that you have enough knowledge about this area. In order to continue with this discussion I kindly request you to propose a chairperson and a secretary to lead this discussion based on this FDG guide. However, my team will be working close with the chairperson to guide the interview. The interview will be audio taped to allow me to follow the discussion and listen to you carefully and attentively and will be handled only by persons involved in this research. I hope you have understood me.

Assessing the levels of awareness, knowledge, attitude and perceptions of communities towards aflatoxins in the society.

A: Awareness about aflatoxins contamination

1. What do you understand about aflatoxins (fungi/mouldy) found in crops /grains?
Or Are you familiar with crops which are mostly contaminated by aflatoxins (fungi/mouldy) that are also used in complementary food? . *Probes, which crops, how can be infected? Probes, what kind of food contaminated? If they don't mention complementary foods, ask them and how can be contaminated.*

B: Knowledge of aflatoxins contamination

2. Do you know some causes and effects of aflatoxins (fungi) found in crops or grains that are used as food for adults or your infants? *Probes, do they found only in complementary foods?*
3. How do you ensure that your crops and grains used for food do not contain fungi/mouldy (aflatoxins)?
4. What are the major health problems facing children in this community? *Probes, How long have these problems existed; are they long time problems or recent problem; how do people in this community deal with these problems?*

C: Attitude towards aflatoxins prevention

5. What motivate you to sort and wash grains used for food of adults and your baby?
Or How do you ensure that the crops /grains used for your children's complementary food do not contain aflatoxins (fungi/mouldy)?

E: Complementary food to the children

6. What are the complementary feeding practices among infants aged between 6-24 months in your society?

7. What are the local barriers and practices hindering reduction of aflatoxins (fungi/mouldy) contamination in grains mostly used as complementary foods in the community?
 - *Local barriers to aflatoxins reductions (mention)*
 - *Local practices to aflatoxins reductions (Mention)*

Thank you for your time

**Appendix 3: Method for determination of Aflatoxin B₁, B₂, G₁, G₂, in food and feeds
by pre column derivatization using HPLC-FLD or HPLC/MS**

1.0 SCOPE AND APPLICATION

This procedure describes the method for analysis of aflatoxin B₁, B₂, G₁ and G₂ in foods and feeds by HPLC-FLD or HPLC-MS

2.0 RESPONSIBILITIES

All analysts in Food Chemistry laboratory shall be responsible for the application of this SOP.

3.0 PERSONNEL QUALIFICATIONS:

Personnel must be trained on the basic principles of the HPLC and must read the material safety data sheet of the chemicals before starting analysis.

4.0 PRECAUTION

- (i) Aflatoxin are highly toxic, use protective measures such as gloves and mask
- (ii) Decontaminate any used glassware with Sodium hypochlorite 4%
- (iii) Aflacolumn are designed for single use only
- (iv) Aflatoxin are light sensitive, handle it in a dark environment
- (v) The consumables used must be disposed in incinerator

5.0 PROCEDURE

5.1 REQUIREMENTS

5.1.1 EQUIPMENTS AND GLASS WARE

- ❖ High performance liquid chromatography (HPLC)
- ❖ 10ml syringes
- ❖ FLD detector/MS
- ❖ Erlymeyer flask-250 mL
- ❖ Vortex
- ❖ Measuring cylinder

- ❖ Vacuum filter with adapter
- ❖ Filter paper
- ❖ Brander
- ❖ shaker
- ❖ Funnel

5.1.2 CHEMICALS/CONSUMABLES

- ❖ Aflatoxin standards (B₁,B₂,G₁,G₂)
- ❖ Aflacolumn (immunoaffinity column)
- ❖ Water (HPLC grade)
- ❖ Methanol (HPLC Grade)
- ❖ Acetonitrile (HPLC Grade)
- ❖ Phosphate buffer solution (PBS) pH 6-8
- ❖ Gracial acetic acid
- ❖ Sodium hypochlorite 4%
- ❖ Trifluoroacetic acid (TFA)
- ❖ Sodium Chloride

5.2 SAMPLE PREPARATION

Four stages are involved during sample preparation

5.2.1 .EXTRACTION STAGE

- (i) Weigh out 25g of sample into 250ml Erlenmeyer flask and add 5grams of sodium chloride
- (ii) Transfer the sample in blender jar and add 100ml ml of extraction solution (60:40 methanol: water or 60:40 acetonitrile :water)
- (iii)Cover blender jar and mix on high speed for 3minutes or shake using gyratory shaker for 1hr

- (iv) Using a funnel filter extract into a sample container using filter paper (Whatman no.1)

5.2.2 DILUTION STAGE

- (i) Take 4ml of extract and add 8ml of phosphate buffer solution (PBS)
(ii) Adjust the PH to 6-8 using sodium hydroxide

5.2.3. CLEAN UP STAGE

- (i) Place the Afla-column into the adapter
(ii) Load the diluted extract using a syringe and allow it to pass through column, the flow rate should not exceed 3ml/min
(iii) Rinse the column twice with 10ml of distilled water,

Use the first rinse solution to wash the container and apply the second rinse direct to the column. In case of any remaining liquid apply slight pressure on top of column

5.2.4. ELUTION STAGE

- (i) Place the vial under the column for collection of eluent
(ii) Elute the bounded aflatoxin without the use of vacuum with 1ml of Acetonitrile HPLC grade by passing it through the column. The Acetonitrile should be left on the column for a few second before elution to allow intensive contact with the gel.
(iii) Apply slight pressure on top of column or apply vacuum in the bottom to remove any remaining liquid
(iv) Take 400µl from the eluent mix with 600µl of derivatizing reagent (70:20:10 H₂O: TFA: Acetic acid)
(V) Condition the mixture at 65⁰C for 15 minutes, allow it to cool and inject to HPLC

5.3 STANDARDS PREPARATION

Prepare a mixture of aflatoxin standard solution (B1,B2,G1,G2)of the following concentration; 1ng/ml, 5ng/ml, 10ng/ml and 15ng/ml for calibration curve.

Use derivatizing reagents as a diluents ((70:20:10) H₂O: TFA: Acetic acid) as below in **Section I.**

5.4 DETERMINATION BY HPLC

5.4.1 HPLC CONDITION

Mobile phase : 50%:40%;10% Water :Methanol :Acetonitrile

Column; C₁₈

Column temperature: 40⁰C

Flow rate: 0.8 mL/min

Injection volume:20μL

5.4.2 DETECTOR-FLD

Emission 450nm

Excitation365nm

6. CALCULATON:

Concentration of the sample, ppb =
$$\frac{\text{conc found } \left(\frac{\text{ng}}{\text{ml}}\right) \times 1\text{ml} \times 100(\text{ml}) \times 2.5(\text{dilution factor})}{4\text{ml} \times \text{weight of the sample taken (g)}}$$

The results of test sample shall be reported in one decimal place

7. REFERENCE

1. Council for agricultural science and technology-report, mycotoxin risk in plant ,animal and human systems, Jan 2013
2. Romer's all purpose methods for laboratory analysis

SECTION I: PREPARATION OF STANDARD AND EXTRACTION SOLVENT

a) Extraction solvent

- i) Acetonitrile – HPLC grade acetonitrile
- ii) Methanol/water – six part of methanol was mixed with 4 parts of water (HPLC grade) and inverted 5x to mix
- iii) Derivatizing agent – 7parts of water (HPLC grade) was mixed with 2 parts of Trifluoroacetic acid (TFA) and 1 part of glacial acetic acid. The mixture was inverted 5x to mix

b) Standards

- i) Reference standard solution (mix 9 aflatoxin) has a mixture of aflatoxin in acetonitrile with different concentrations for
 - Aflatoxin B1 = 1.06 µg/ml = 1060 ppb
 - Aflatoxin G1 = 1.03 µg/ml = 1030 ppb
 - Aflatoxin B2 = 1.06 µg/ml = 1060 ppb
 - Aflatoxin G2 = 1.00 µg/ml = 1000 ppb

ii) Stock solution

Approximated 100ppb of stock standard was prepared from reference standard solution in 1500 µl vial where 100 µl was mixed with 900 µl of acetonitrile (diluent). The vial was capped and vortexed to mix the contents

The actual concentration of each analyte in 100 ppb were as follows:

- Aflatoxin B1

$$1060 \text{ ppb} \times 100 \text{ } \mu\text{l} = C_2 \times 1000 \text{ } \mu\text{l}$$

$$C_2 = 106 \text{ ppb}$$

- Aflatoxin G1

$$1030 \text{ ppb} \times 100 \text{ } \mu\text{l} = C_2 \times 1000 \text{ } \mu\text{l}$$

$$C_2 = 103 \text{ ppb}$$

- Aflatoxin B2

$$1060 \text{ ppb} \times 100 \mu\text{l} = C_2 \times 1000 \mu\text{l}$$

$$C_2 = 106 \text{ ppb}$$

- Aflatoxin G2

$$1000 \text{ ppb} \times 100 \mu\text{l} = C_2 \times 1000 \mu\text{l}$$

$$C_2 = 100 \text{ ppb}$$

iii) Working standard

Approximated 50 ppb was prepared from stock solution in 1000 μl vial where 500 μl was mixed with 500 μl of acetonitrile.

The actual concentration of each analyte in 50 ppb were as follows:

- Aflatoxin B1

$$106 \text{ ppb} \times 500 \mu\text{l} = C_2 \times 1000 \mu\text{l}$$

$$C_2 = 53 \text{ ppb}$$

- Aflatoxin G1

$$103 \text{ ppb} \times 500 \mu\text{l} = C_2 \times 1000 \mu\text{l}$$

$$C_2 = 51.5 \text{ ppb}$$

- Aflatoxin B2

$$106 \text{ ppb} \times 500 \mu\text{l} = C_2 \times 1000 \mu\text{l}$$

$$C_2 = 53 \text{ ppb}$$

- Aflatoxin G2

$$100 \text{ ppb} \times 500 \mu\text{l} = C_2 \times 1000 \mu\text{l}$$

$$C_2 = 50 \text{ ppb}$$

iv) Calibration curve standards

Calibration curve standards were made in derivatizing agent (7H₂O:2TFA:1GAA) where 20, 100, 200 and 300 μl of 50ppb working solution were mixed with 980, 900, 800 and 700 of derivatizing agent to make calibration curve standards of concentration 1, 5, 10 and 15 ppb.

Concentration of working solution (ppb)	Volume of working solution (μ l)	Volume of diluents (TFA) (μ l)	Final volume (μ l)	Final concentration (ppb)
50	300	700	1000	15
50	200	800	1000	10
50	100	900	1000	5
50	20	980	1000	1

The actual concentration of analyte in each level of concentration were as follows:
15 ppb

Analyte	Conc of working solution (ppb)	Vol of working solution (μ l)	Volume of diluent (μ l)	Final volume (μ l)	Final concentration (ppb)
B1	53	300	700	1000	15.9
G1	51.5	300	700	1000	15.45
B2	53	300	700	1000	15.9
G2	50	300	700	1000	15

10 ppb

Anal yte	Conc of working solution (Ppb)	Volume of working solution (μ l)	Volume of diluent (μ l)	Final volume (μ l)	Final concentration (ppb)
B1	53	200	800	1000	10.6
G1	51.5	200	800	1000	10.3
B2	53	200	800	1000	10.6
G2	50	200	800	1000	10

5 ppb

Anal yte	Conc of working solution (ppb)	Vol of working solution (μ l)	Volume of diluent	Final volume (μ l)	Final concentration (ppb)
B1	53	100	900	1000	5.3
G1	51.5	100	900	1000	5.15
B2	53	100	900	1000	5.3
G2	50	100	900	1000	5

1 ppb

Analyte	Conc of working solution	Vol of working solution	Volume of diluent	Final volume (µl)	Final concentration (ppb)
	Ppb	µl		µl	ppb
B1	53	10	990	1000	1.06
G1	51.5	10	990	1000	1.03
B2	53	10	990	1000	1.06
G2	50	10	990	1000	1

c) Quality control samples

Quality control (QC) sample purchased from FAPAS UK

d) Spiking of matrix

A sample in which aflatoxin B1, G1, B2, G2 was not detected was selected for spiking. 25g of sample was weighed in triplicate, one portion was spiked with calculated volume of reference standard to get a concentration of 5 ppb and the other one 10ppb . They were then mixed thoroughly and left to stand/equilibrate for 30mins. Then all samples, unspiked and spiked samples were extracted and analysed as per procedure.

Volume taken from reference standard

i) 5 ppb

Volume of standard solution = $\frac{\text{sample weight} \times \text{spiking level}}{\text{concentration of standard}}$

concentration of standard

$$= 25.00 \times 5 / 1000$$

$$= 0.125 \text{ ml}$$

Actual concentration of analyte in the spiked sample

$$\text{concentration of individual analyte} = \frac{\text{concn of standard} \times \text{Volume to be spiked}}{\text{sample weight}}$$

Weight taken	Analyte	reference conc (ppb)	volume to be spiked (ml)	Actual conc spiked (ppb)
25.0015	AFG1	1030	0.125	5.149691
25.0015	AFB1	1060	0.125	5.299682
25.0015	AFG2	1000	0.125	4.9997
25.0015	AFB2	1060	0.125	5.299682

i) 10 ppb

$$\text{Volume of standard solution} = \frac{\text{sample weight} \times \text{spiking level}}{\text{concentration of standard}}$$

$$= 25.00 \times 10 / 1000$$

$$= 0.25 \text{ ml}$$

Actual concentration of analyte in the spiked sample

concentration of individual analyte = $\frac{\text{concn of standard} \times \text{Volume to be spiked}}{\text{sample weight}}$

Weight taken	Analyte	reference conc (ppb)	volume to be spiked (ml)	Actual conc spiked (ppb)
25.004	AFG1	1030	0.25	10.29835
25.004	AFB1	1060	0.25	10.5983
25.004	AFG2	1000	0.25	9.9984
25.004	AFB2	1060	0.25	10.5983

Recovery

Recovery was evaluated for individual analyte in each spiked concentration by using the following formula

% Recovery = $\frac{\text{conc. of spiked sample} - \text{conc. of unspiked sample}}{\text{spiking concentration}} \times 100\%$

Unspiked sample (146)

Weight taken	Analyte	HPLC CONC	Calculated conc
25.032	AFG1	0	0
25.032	AFB1	0	0
25.032	AFG2	0	0
25.032	AFB2	0	0

Spiked samples**For 5 ppb:**

Weight taken	Analyte	HPLC CONC	Calculated conc	Actual conc spiked	conc reference	volume to be spiked	% recovery
25.0015	AFG1	1.8856	4.713717177	5.149691	1030	0.125	91.53398
25.0015	AFB1	2.5747	6.436363818	5.299682	1060	0.125	121.4481
25.0015	AFG2	1.8893	4.722966622	4.9997	1000	0.125	94.465
25.0015	AFB2	2.1591	5.397426154	5.299682	1060	0.125	101.8443

For 10 ppb:

Weight taken	Analyte	HPLC CONC	Calculated conc	Actual conc spiked	conc reference	volume to be spiked	% recovery
25.004	AFG1	4.846	12.11306191	10.29835	1030	0.25	117.6214
25.004	AFB1	4.4849	11.21045633	10.5983	1060	0.25	105.7759
25.004	AFG2	3.8386	9.594964806	9.9984	1000	0.25	95.965
25.004	AFB2	4.243	10.60580307	10.5983	1060	0.25	100.0708

The results establishes recoveries in the range 91-121% and 95-117% for the spiked concentrations of 5ppb and 10ppb respectively.

SECTION II: SOME CALCULATIONS**Quality control (QC)**

This was done using QC samples of maize of known concentrations and was analyzed together with samples; the QC sample had assigned value and a range of lowest to highest value (14-34 ppb), the process was found to be in control when values were within the given range.

Calculation of RDS's, LOD, LOQ

RSD was calculated using sample of known concentration. Validation was done by injecting the sample 15 times on repeatability and reproducibility from the data obtained, mean, standard deviation and relative standard deviation were calculated. The values obtained were then compared to international standard ISO 16050:2003 from the ISO standard, different labs analyzed samples and later allowable relative standard deviation for repeatability and reproducibility was set. The obtained results when compared to the set limits of mean and RSDs were found to be satisfactory.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Different lower concentrations were run until the lowest concentration that can be detected (showed peaks) by the equipment was determined. The lowest concentration that the equipment could detect was 0.2 ppb. The determined lower concentration was injected 10 times where mean and standard deviation of each analyte/compound was calculated. LOD & LOQ were calculated from $LOD = \text{mean} + 3SD$ and $LOQ = \text{mean} + 10SD$.

Aflatoxi ns	Conc. (ppb)	Stock Solution	Conc (ppb)	1 Vol. (μ L)	1 Vol. (μ L)	2 Conc. (ppb)	2
G1	103		1.030	200	1000	0.206	
B1	106		1.060	200	1000	0.212	
G2	100		1.000	200	1000	0.200	
B2	106		1.060	200	1000	0.212	

Use the dilution formula $C_1V_1=C_2V_2$ above to know the concentration of needed for dilutions. At the beginning concentration stock solution was 1030 ppb then dropped to 100 then to 1ppb. Therefore, when 200 μ L (V_1) was taken from 1.03 ppb in G1 for example as concentration (C_1), put in the vial of 1000 μ L (V_2) then C_2 will be

$$1.03\text{ppb} \times 200\mu\text{L} = C_2 \times 1000\mu\text{L}, C_2 = 0.206 \text{ ppb}$$

S/N	AFG1	AFB1	AFG2	AFB2
1	0.206	0.211	0.200	0.210
2	0.203	0.209	0.190	0.209
3	0.205	0.211	0.200	0.211
4	0.206	0.210	0.190	0.201
5	0.201	0.212	0.200	0.212
6	0.206	0.212	0.190	0.211
7	0.206	0.207	0.200	0.208
8	0.206	0.218	0.170	0.212
9	0.208	0.212	0.19	0.212
10	0.211	0.212	0.2	0.208
Mean (M)	0.2058	0.2114	0.193	0.2094
Standard Deviation (SD)	0.0026583	0.002836	0.009487	0.00334

S/N	Aflatoxins	Limit of Detection (ppb)	Limit of Quantification (ppb)
1	G1	0.213775	0.232383203
2	B1	0.219909	0.23976273
3	G2	0.22146	0.28786833
4	B2	0.21942	0.242799933

Limit of Detection (LOD)

Limit of detection (LOD) is the lowest quantity of the analyte substance that produces a signal at least three times the average noise level of the detector

$$\text{LOD} = \text{Mean of lowest conc} + 3\text{sd}$$

Limit of Quantification (LOQ)

LOQ is the lowest concentration that can not only be detected but it can be quantified within defined limit of certainty after certain replicate are measured

LOQ=Mean of lowest conc+10sd

LOQ>LOD

REPRODUCIBILITY					
	AFG1	AFB1	AFG2	AFB2	
RANGE	0.42-0.98	13.428-31.332	0	1.65-3.85	
	0.82	19.89	0.3	2.43	
	0.69	21.5	0.1	1.98	
	0.61	24.71	0.52	2.53	
	0.79	25.72	0.1	1.78	
	0.78	20.07	0.53	1.84	
	0.69	25.62	0.2	1.98	
	0.65	22.02	0.25	2.08	
	0.69	19.89	0.4	2.87	
	0.77	22.8	0.1	2.96	
	0.64	26.37	0.3	1.73	
	0.65	23.24	0.4	2.86	
	0.69	25.71	0.22	1.97	
	0.69	26.05	0.22	1.67	
	0.64	21.62	0.5	2.03	
	0.76	21.49	0.36	2.12	
MEAN	0.704	23.11333333	0.3	2.188667	
STD	0.064343	2.39671402	0.148853	0.433357	
RSDs	9.139607	10.36940015	49.61759	19.80004	
μ		22.38	0	2.75	
0.7					
RSDs	9.5	10	51	30	

Aflatoxins	REPEATABILITY			
	AFG1	AFB1	AFG2	AFB2
Range	0.42-0.98	13.428-31.332	0	1.65-3.85
	0.65	19.5	0.29	1.78
	0.71	20.01	0.19	2.03
	0.63	19.64	0.25	2.16
	0.75	21.5	0.2	1.9
	0.74	23.5	0.29	1.98
	0.68	21.7	0.27	1.87
	0.63	20.6	0.2	2.5
	0.72	19.8	0.17	1.8
	0.75	19.3	0.16	1.92
	0.6	18.9	0.26	1.86
	0.69	19.5	0.25	1.97
	0.75	20.1	0.23	1.8
	0.74	19.6	0.24	2.1
	0.66	20.2	0.28	2.22
	0.69	20.4	0.27	2.3
MEAN	0.692667	20.28333333	0.236667	2.012667
STD	0.049924	1.173685688	0.043039	0.207896
RSDs	7.207471	5.786453682	18.18562	10.32941
	0.7	22.38	0	2.75
	9.5	5.8	19	25

Recovery

This was done by spiking the analyzed sample with known concentration, i.e. 5ppb. The sample that was taken showed 0ppb for Aflatoxin B1, G1, B2, and G2. A concentration of 5ppb was spiked onto weighed sample and both batches of samples, spiked and unspiked samples were extracted as per used procedure after calculation of final concentration from each sample, and recovery was calculated as follows:

$$\% \text{ recovery} = \frac{\text{Conc of spiked} - \text{Conc of unspiked}}{\text{Actual conc spiked}} \times 100$$

Sample Code 146			
Sample Weight (grams)	Aflatoxins		HPLC Concentrations
25.0230	AFG1		0
25.2012	AFB1		0
25.0320	AFG2		0
25.1012	AFB2		0

146 Spiked with 5.0ppb		Aflatoxins						% recoveries
25.6829	AFG1	1.8856	4.588656265	5.013063	1030	0.125	91.53398	
25.6829	AFB1	2.5747	6.2655989	5.159075	1060	0.125	121.4481	
25.6829	AFG2	1.8893	4.597660311	4.867052	1000	0.125	94.465	
25.6829	AFB2	2.1591	5.254225574	5.159075	1060	0.125	101.8443	
	TOTAL		20.70614105					

146 Spiked with 10 ppb		Aflatoxins						% recoveries
25.004	AFG1	4.846	12.11306191	10.29835	1030	0.25	117.6214	
25.004	AFB1	4.4849	11.21045633	10.5983	1060	0.25	105.7759	
25.004	AFG2	3.8386	9.594964806	9.9984	1000	0.25	95.965	
25.004	AFB2	4.243	10.60580307	10.5983	1060	0.25	100.0708	
	TOTAL		43.52428611					

SECTION III: CALBRATION CURVES

HPLC CONDITION: (1200 SERIES AGILENT TECHNOLOGY USING CHEMISTATION SOFTWARE)

Mobile phases: 50%:40%:10% Water: Methanol: Acetonitrile

Column: C18, (4.6×150mm,5µm),Eclipse XDB-C18

Column temperature: 40⁰C

Flow rate: 0.8mL/min

Injection volume:20µL

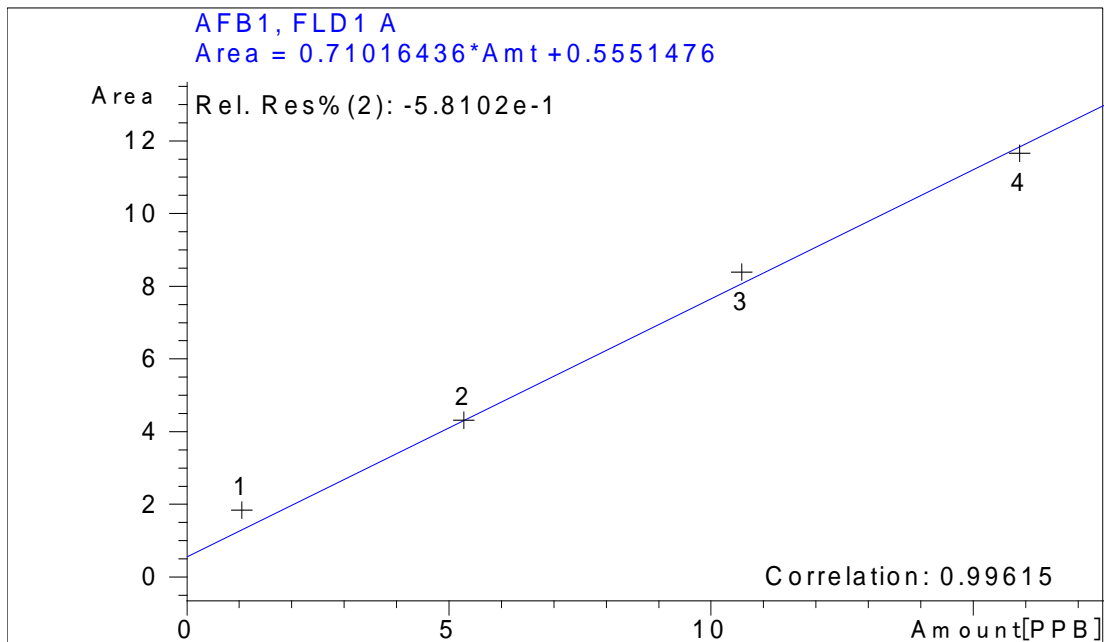
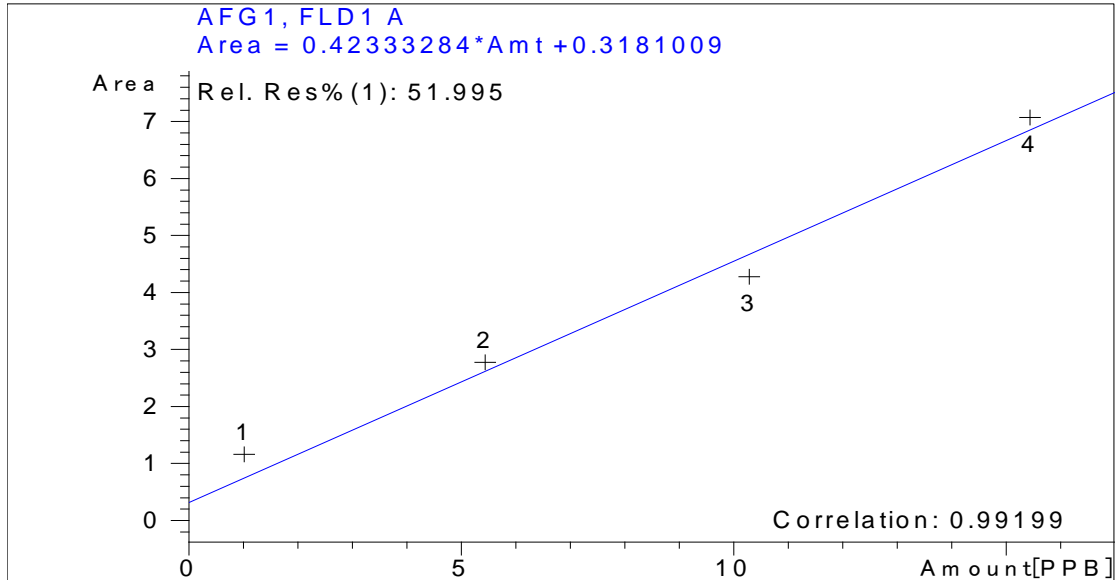
DETECTOR-FLD

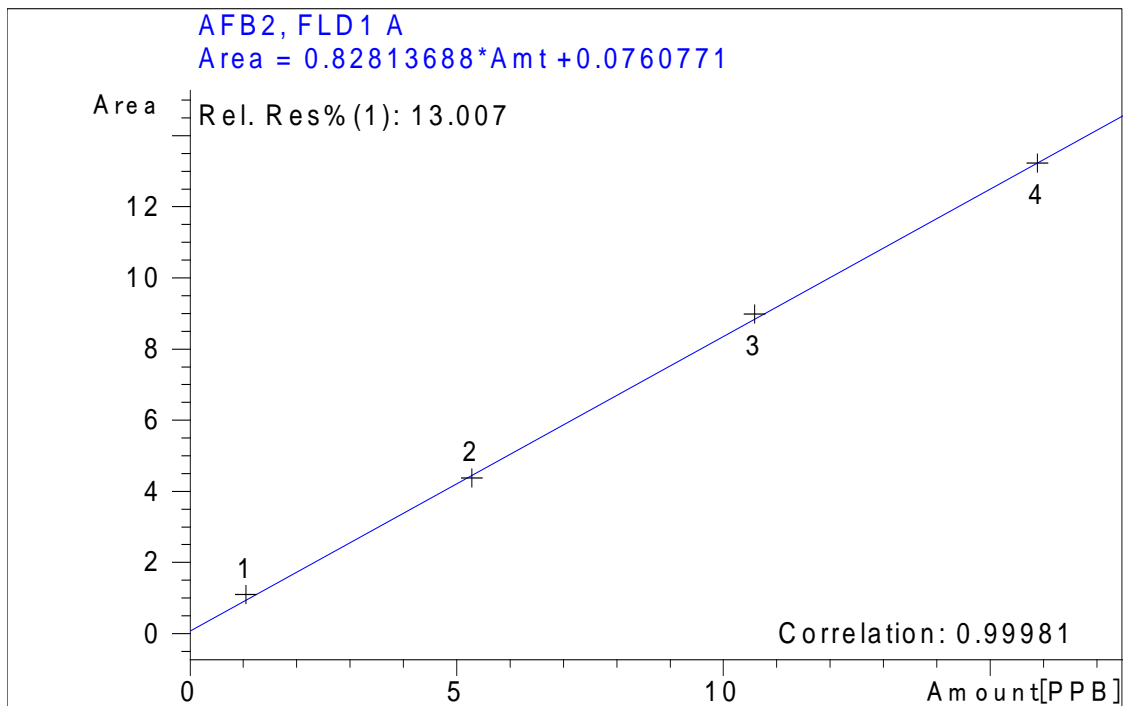
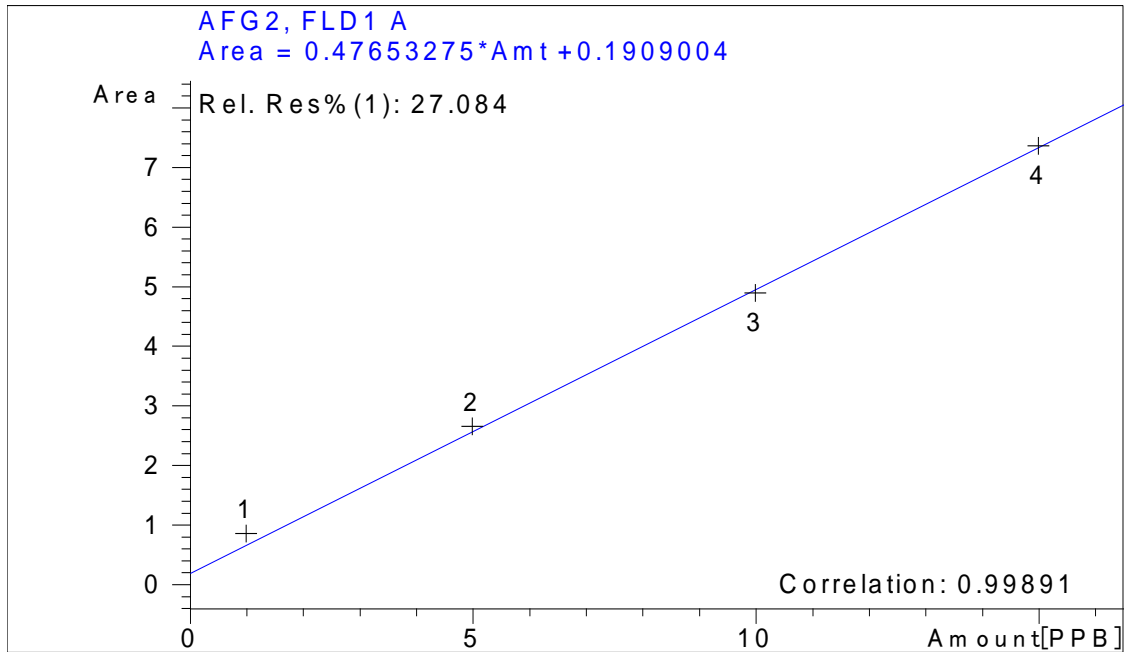
Emission 450nm

Excitation 365nm

#	RT	Signal	Compound	Lvl	Amt[PPB]	Area	Rsp.Factor
1	3.045	FLD1 A	AFG1	1	1.03	1.146	8.99E-01
				2	5.45	2.765	1.971
				3	10.3	4.263	2.416
				4	15.45	7.06	2.188
2	3.707	FLD1 A	AFB1	1	1.06	1.82	5.83E-01
				2	5.3	4.294	1.234
				3	10.6	8.366	1.267
				4	15.9	11.632	1.367
3	4.985	FLD1 A	AFG2	1	1	8.48E-01	1.179
				2	5	2.642	1.892
				3	10	4.885	2.047
				4	15	7.351	2.04
4	6.704	FLD1 A	AFB2	1	1.06	1.078	9.83E-01
				2	5.3	4.354	1.217
				3	10.6	8.958	1.183
				4	15.9	13.203	1.204

RT=Retention time, LV=Level, Amt=Amonut, RSP Factor=Response Factor

CALIBRATION CURVE FOR INDIVIDUAL STANDARDS



Appendix 4: Dodoso Swahili Version

Ukusanyaji wa taarifa kutoka kwa mzazi/mlezi

Namba ya fomu.....

Tarehe ya kusailiwa.....

Jina la kijiji/mtaa

Jina la wiliya.....

A. TAARIFA ZA MTOTO (KUTOKA KWA MZAZI AU MLEZI)

1. Jina la mtoto.....

2. Je, una uhusiano gani na mtoto huyu?

(1) Mama

(2) Baba

(3) Mlezi

3. Tarehe ya kuzaliwa.....(*Kutoka katika kadi ya kliniki*)

4. Jinsia 01= Mme 02= Mke

5. Mtoto wako umejifungulia wapi?

(1) Hospitali (2) Nyumbani

(3) Kwa msaada wa mkunga (4) Kwingine taja

6. Je, mtoto wako ananyonya? (1) Ndiyo (2) Hapana (Kama ndiyo, ruka swali la 7&46)

7. Kama hapana, ni katika umri gani mtoto wako aliacha kunyonya?miezi.

8. Ni katika umri gani mtoto wako amepewa kwa mara ya kwanza chakula mbadala?

..... miezi.

9. Taarifa ya chakula cha mtoto (1) Anachakula chake pekee (2) Anakula chakula cha familia (3)

Vyote

10. Jana umempa mtoto wako vyakula vya aina ngapi? (Idadi ya vyakula))

11. Kati ya vyakula ulivyompa mtoto wako jana, ni vingapi vyenye mchanganyiko wa

mahindi?.....

12. Katika wiki moja iliyopita, ni kwa siku ngapi umempa mtoto wako chakula chenye mahindi?.....
13. Kipi chanzo cha mahindi unayotumia kumtengenezea mtoto wako chakula?
 (1) Unayolima mwenyewe (2) Uliyonunua sokoni (3) Nyingine (taja).....
14. Je, unatarishaje mahindi unayotumia kutengeneza chakula cha mtoto wako?
 (1) Bila kukobolewa (2) Huwa unayakoboa (3) Namna nyingine (tafadhali eleza).....
15. Kati ya vyakula ulivyompa mtoto jana, ni vingapi vina karanga?.....
16. Katika wiki moja iliyopita ni kwa siku ngapi umempa mtoto wako chakula ambacho kina karanga?.....
17. Karanga unazotumia kutayarisha chakula cha mtoto huwa unazipata wapi?
 (1) Umepanda mwenyewe (2) Umenunua sokoni (3) Kwingine (Taja).....
18. Je, unatarishaje karanga unazotumia kutengeneza chakula cha mtoto?
 (1) Unachagua (2) Unazikaanga (3) Unazichemsha
19. Kati ya vyakula ulivyompa mtoto jana ni vingapi vina mtama?.....
20. Katika wiki moja iliyopita ni siku ngapi umempa mtoto wako chakula chenye mtama?

21. Mtama unaotumia kutayarisha chakula cha mtoto huwa unaupata wapi?
 (1) Umepanda mwenyewe (2) Umenunua sokoni (3) Kwingine (Taja).....
22. Je, unatarishaje mtama unaotumia kutengeneza chakula cha mtoto?
 (1) Unachagua (2) Unaukaanga (3) Unauchemsha
23. Ni magonjwa yapi makubwa kwa watoto hapa kijijini?
 (1) Homa
 (2) Kuharisha
 (3) Kukohoa

- (4) UTI
- (5) Malaria
- (6) Mafua
- (7) Kwashakoo
- (8) Kudumaa
- (9) Saratani
- (10) Mengine taja.....

B. TAARIFA YA MZAZI AU MLEZI

24. Una umri wa miaka mingapi

25. Je, unaelimu ya kiwango gani?

- (1) Hujasoma shule kabisa
- (2) Hukumaliza elimu ya msingi
- (3) Una elimu ya shule ya msingi
- (4) Hukumaliza elimu ya sekondari
- (5) Umemaliza elimu ya sekondari
- (6) Hukumaliza elimu ya kidato cha sita
- (7) Umemaliza elimu ya kidato cha sita
- (8) Una elimu ya chuo

26. Kwa sasa hali yako ya ndoa ikoje?

- (1) Uko pekee yako (2) Mnaishi pamoja(ulewa/oa) (3) Mtalikiwa (4) Mjane/mseja (5) Mmetengana (6) Mnaishi pamoja (hamjaoana)

27. Kwa sasa, Unafanya shughuli gani?

- (1) Mwanafunzi (2) Mkulima (3) Mama wa nyumbani (4) Mfanyabiashara ndogondogo (5) Mwajiriwa(wapi taja) -----
- (6) Nyingine (Taja) -----

28. Mwenza wako anafanya shughuli gani?

- (1) Mkulima (2) Mama wa nyumbani (3) Mfanya biashara ndogondogo (4) Mfanya biashara (5)Mwajiriwa (6) Nyingine (Taja)-----

29. Je, huwa mnapata shilingi ngapi kwa mwezi? Chagua katika oradha hii.

- (1) Chini ya Tshs 50,000 (2) Chini ya Tshs 100,000 (3) Chini ya Tshs 200,000 (4) Chini ya Tshs 300,000 (5) Chini ya Tshs 400,000 (6) Kubwa kuliko au sawa na Tshs 400,000

S/N	CHANZO CHA MAPATO	KIASI CHA MAPATO
29.1	Uzalishaji wa mazao	
1	Mahindi	
2	Mtama	
3	Karanga	
4	Ulezi	
5	Mihogo	
29.2	Uzalishaji wa wanyama	
1	Ng'ombe	
2	Mbuzi	
3	Kondoo	
4	Maziwa	
29.3	Uzalishaji wa kuku	
1	Kuku	
2	Mayai	
29.4	Ajira	
1	Mshahara	
29.5	Biashara zingine	
1	Duka	
2	Grocery	
29.6	Nyingine	

SEHEMU YA PILI: MASWALI YA UTAFITI

A: What are the levels of awareness, knowledge, attitude and perceptions of communities towards aflatoxins in Dodoma and Singida regions?

Awareness of aflatoxins contamination

Jibu la ndiyo=1 na Jibu la hapana=0

30. Je, ulishawahi sikia kuwa kuna sumu ambayo inaweza kuwepo kwenye mazao inayoweza sababishwa na uvundouvundo (fangasi)?

- (1) Ndiyo (2) Hapana (Kama ndiyo, nenda swali namba 32)

31. Je, ulishawahi sikia kuwa kuna sumu ambayo inaweza kuwepo kwenye chakula inayoweza sababishwa na uvundouvundo (fangasi)?

- (1) Ndiyo (2) Hapana (Kama ndiyo, nenda swali namba 32)

32. Je, ulipata wapi taarifa hizi?

- (1) Hospitali/kituo cha afya/zahanati (2) Kwa marafiki (3) Kupitia vyombo vya habari (kama Radio, TV, magazeti
(4) Sehemu
nyingine.....
33. Je, ulishawahi kusikia neno “aflatoxin” kabla ya leo?
(1) Ndiyo (0) Hapana
34. Je, unafahamu aflatoxins (sumu/uvundo) unaoweza athiri mazao shambani?
(1) Ndiyo (0) Hapana
35. Je, unafahamu aflatoxins (sumu/uvundo) unaoweza athiri mazao yakiwa yamehifadhiwa?
(1) Ndiyo (0) Hapana
36. Je, unafahamu aflatoxins (sumu/uvundo) unaoweza athiri chakula?
(1) Ndiyo (0) Hapana
37. Je, unafahamu aflatoxins (sumu/uvundo) unaoweza athiri chakula cha mtoto?
(1) Ndiyo (0) Hapana
38. Je, unajua madhara ya sumu ya aflatoxins/fangasi kwa binadamu?
(1) Ndiyo (0) Hapana
- 38.1. Kama ndiyo, tafadhali yataje;
(1) Kudumaa kwa watoto
(2) Ugonjwa wa ini
(3) Saratani
(4) Kutapika
(5) Mengine yataje.....
39. Je, unajua madhara ya sumu ya fangasi au aflatoxins kwa wanyama?
(1) Ndiyo (0) Hapana
- 39.1. Kama ndiyo, tafadhali yataje;
(1) Mauvimbe
(2) Matatizo kwenye mfumo wa chakula
(3) Matatizo ya maini
(4) Mengine yataje.....

Ufahamu wa sumu aina ya fangasi/aflatoxins

40. Je, ni nini maoni yako kuhusu ufahamu wa sumu aina ya fangasi/uvundo/aflatoxins? (kweli=1 na sikweli=0, zungushia duara maoni ya muulizwaji)

SN	Statements	Respondent	
40.1	Hali ya joto na ukungu katika eneo hili linawezasabisha fangasi au uvundo kuota.	Kweli (1)	Sikweli (0)
40.2	Kutohifadhi chakula vizuri kinaweza kupata sumu/uvundo/fangasi	Kweli (1)	Sikweli (0)
40.3	Sumu aina ya Aflatoxin ni jamii ya fangasi	Kweli (1)	Sikweli (0)
40.4	Kula chakula chenye uvundo kunaweza sababisha madhara ya kiafya kwa binadamu	Kweli (1)	Sikweli (0)
40.5	Sumu/uvundo/fangasi unapatikana kwenye vyakula tu	Kweli (1)	Sikweli (0)
40.6	Sumu/uvundo huu unapatikana kwenye mazao tu	Kweli (1)	Sikweli (0)
40.7	Baadhi ya magonjwa ya ini yanahusishwa na ulaji wa chakula chenye uvundo	Kweli (1)	Sikweli (0)

40.8	Uvundo/fangasi kwenye chakula huweza sababisha saratani	Kweli (1)	Sikweli (0)
40.9	Kula chakula chenye uvundo/fangasi kunaweza sababisha mtoto wako asikue vizuri	Kweli (1)	Sikweli (0)
40.10	Kutumia dawa za kuua wadudu kunaweza punguza fangasi/uvundo kwenye sehemu ya kuhifadha mazao	Kweli (1)	Sikweli (0)

Perception toward aflatoxins contamination

41. To what extent do you agree on the following statements? (Circle the answer she/he agree)

S/N	Statements	Measurement scale				
		Nakubali ana kabisa (5)	Naku balian a (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
41.1	Kuchambua mbegu zilizoathirika na fangasi/uvundohupunguza maambukizi ya fangasi/aflatoxin	Nakubali ana kabisa (5)	Naku balian a (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
41.2	Kuocha mbegu zilizoathirika na fangasi hupunguza maambukiza ya fangasi/aflatoxin	Nakubali ana kabisa (5)	Naku balian a (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
41.3	Mbegumbegu zilizooza ni dalili za kuwepo kwa uvundo/fangasi	Nakubali ana kabisa (5)	Naku balian a(4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
41.4	Kula chakula kilicho na fangasi huweza sababisha magojwa	Nakubali ana kabisa (5)	Naku balian a(4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
41.5	Kula chakula kilicho na fangasi huweza sababisha kifo	Nakubali ana kabisa (5)	Naku balian a(4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
41.6	Uvundo/fangasi huweza tokea wakati wowote kwenye chakula	Nakubali ana kabisa (5)	Naku balian a(4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)

Attitude toward aflatoxins

42. To what extent do you agree on the following statements? (Circle the answer she/he agree)

SN	Statements	Measurement scale				
		Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.1	Kilimo Cha Kisasa hupunguza matatizo ya uvundo/aflatoxins kwenye mazao	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.2	Kuchambua mbegu zisizo na kiwango hupunguza uathiri wa chakula cha watoto kwa fangasi/uvundo	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.3	Kuoshia mbegu kabla ya kusaga hupunguza uwezekano wa kupata fangasi kwenye chakula cha mtoto	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.4	Uvundo unaweza kutoka wakati wa kusaga chakula cha mtoto	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.5	Kuchambua mbegumbegu zilizooza kunapoteza muda	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.6	Uvundo/fangasi unaweza toka wakati wa kupika chakula cha mtoto	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.7	Kuchambua mbegumbegu zilizooza kunapoteza hela	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.8	Kuchambua mbegumbegu zilizooza ni hali ya usafi	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.9	Mbegumbegu safi zina bei nzuri	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)
42.10	Mbegumbegu safi siku zote huuzwa haraka	Nakubalian a kabisa (5)	Nakubaliana (4)	Sidhani (3)	Sikubaliani (2)	Sikubaliani kabisa (1)

B: Complementary feeding practices among infants aged between 6-24 months.

43. Una watoto wangapi?.....
44. Ulisha wahi nyonyesha?
 (1) Ndiyo (2) Hapana
- 44.1. Kama hapana, Tafadhali, ni sababu gani za kutokunyonya?
 (1) Mtoto alikuwa mgojwa
 (2) Wazazi tuliachana
 (3) Mama alikuwa mgojwa
 (4) Mtoto alikataa kunyonya
 (5) Nyingine taja.....
45. Kwa uzoefu wako inachukua muda gani kumnyonyesha mtoto wako punde tu anapozaliwa?
 (0) Hapohapo anapozaliwa (1) Baada ya masaa..... (2) Baada ya siku
46. Ni katika umri gani mtoto wako aliacha kunyonya?
 (1) Baada ya siku (2) Baada ya miezi.....
47. Je, mtoto wako amenyonya mfululizo kwa miezi sita bila chakula chochote?
 (1) Ndiyo (2) Hapana
48. Je, mtoto wako anakula chakula kingine nje ya maziwa?
 (1) Ndiyo (2) Hapana
49. Kama mtoto wako anakula chakula kigumu ni lini ulimwanzishia?
 (1) Baada ya siku (2) Baada ya miezi
50. Ni nani alitoa ushauri wa kutumia chakula kigumu kwa mtoto?
 (1) Mume (2) Mama mwenyewe (3) Mkunga wa jadi
 (4) Kutoka kituo cha afya/hospitali
 (5) Kingine taja.....

51. Chakula mbadala cha mtoto wako unakipata kutoka wapi?

- (1) Unalima mwenyewe (2) Unanunua (3) Unapata chakula cha msaada (4) Vyote hapo juu (5) Sehemu nyingine taja.....

52. Ni aina gani ya vyakula anavyompa mtoto mara kwa mara?

S/N	Aina ya chakula	Chakula kipi unampa mtoto kwa wiki (tiki)	Ni mara ngapi unampa mtoto chakula hiki kwa siku (andika)	Siku ambazo mtoto wako anakula chakula hiki kwa wiki (andika)	Chakula kipi kina mahindi (tiki)	Chakula kipi kina karanga (tiki)
52.1	Uji mwepesi					
52.2	Uji mzito					
52.3	Wali					
52.4	Viazi mviringo(ulaya)					
52.5	Viazi vitamu					
52.6	Mihogo					
52.7	Ndizi					
52.8	Mtama					
52.9	Ulezi					
52.10	Makande mahindi+maharage					

C: What are practices used that can contribute to levels of contamination of aflatoxins in this area?

53. Je, lini umeyavuna mazao yako unayoyatumia kutengeneza chakula cha mtoto?

- (1) Mwezi..... (2) Mwaka

54. Je, huwa unakoboa mazao unayotumia kutengeneza chakula cha mtoto?

- (1) Ndiyo (2) Hapana

55. Je, huwa unasaga mazao wakati wa kutengeneza chakula cha mtoto?

- (1) Ndiyo (2) Hapana

56. Je, huwa unayakausha mazao yako?

- (1) Ndiyo (2) Hapana

56.1. Kama ndiyo, huwa kwa kawaida unayakaushia wapi mazao yako?

- (1) Kwenye udongo chini
 (2) Kwenye paa
 (3) Kwenye vichanja
 (4) Sehemu nyingine taja

57. Je huwa unahifadhi mazao yako?

- (1) Ndiyo (2) Hapana

57.1 Je ni kwa namna gani unayahifadhi mazao?

- (1) Kwenye *vichanja*
 (2) Darini
 (3) Kwenye paa
 (4) Kwenye magunia
 (5) Sehemu nyingine taja

58. Je, nini matumizi ya mazao yako uliyonayo?

- 1) Kula 2) Kuuza 3) Vyote 4) Vingine taja.....

59. Je, mazao yako yanapata ukungu/fangasi yakiwa yamehifadhiwa?

- (1) Ndiyo (2) Hapana

60. Je, ni aina gani ya chakula unachompa mtoto wako?

- (1) Chakula maalumu kwa mtoto (2) Chakula cha familia (3) Vyote

61. Nini matumizi ya mazao yako yenye uvundo?

- (1) Kwa chakula (2) Chakula cha mifugo (3) Kutengenezea pombe za kienyeji (4)

Kingine taja.....

62. Je, unaharibuje masalia ya mazao yako?

- (1) Chakula cha wanyama (2) Kuchoma (3) Huacha shambani
(4) Huyalimia chini ya ardhi

D. What are the local barriers and practices associated with reducing aflatoxins contamination in complementary foods in the community?

63.0 Local barriers to aflatoxins reductions

63.1. Je, katika jamii hii kuna vikwazo vyovyote kwenye kuchambua mahindi mabovu?

Tafadhali taja.....

63.2. Je, katika familia yako kuna vikwazo vyovyote vinavyozuia kuosha mazao yako?

Tafadhali taja

63.3. Je, katika familia yako ni vitu gani vinavyozuia uwekaji vizuri wa mazao ili kupunguza uozaji/uvundo?

Tafadhali taja.....

63.4. Je, katika familia yako ni vitu gani vinavyozuia utengenezaji vizuri wa mazao ili kupunguza uozaji/uvundo?

Tafadhali taja.....

63.5. Je, kuna mila zozote ambazo zinazuia kupunguza kwa uvundo/fangasi kwenye mazao yako?

- (1) Ndiyo (2) Hapana

63.6. Kama ndiyo ni zipi?

63.7. Je huwa mnaongea na mweza wako kuhusu jinsi ya kupunguza uvundo/fangasi kwenye mazao yenu?

- (1) Ndiyo (2) Hapana

63.8. Kama ndiyo, tafadhali elezea

64.0 Local practices to aflatoxins reductions

64.1. Je, unayapata wapi mazao yanayotumika kutengeneza chakula cha mtoto?

Tafadhali taja.....

64.2. Je, unatumia dawa za kuua wadudu kwenye mazao katika shamba lako?

(1) Ndiyo (2) Hapana

64.3. Kama ndiyo ni zipi hizo zitaje.....

64.4. Je, huwa unatumia dawa za kuua wadudu kwenye mazao yako wakati wa kuyahifadhi?

(1) Ndiyo (2) Hapana

64.5. Kama ndiyo ni zipi zitaje.....

64.6. Je huwa unatumia dawa za kuua wadudu za kienyeji wakati wa kuyahifadhi mazao yako?

(1) Ndiyo (2) Hapana, Kama ndiyo....

64.7. Ni aina gani ya dawa za kienyeji unazotumia wakati wa kuyahifadhi mazao yako?

(1) Majivu (2) Matope

(3) Zingine tafadhali taja.....

64.8. Je huwa unatumia dawa ya kuua fangasi katika mazao yako?

(1) Ndiyo (2) Hapana

64.9. Kama ndiyo, Ni aina gani ya dawa ya kuua fangasi unayotumia kwenye mazao yako? Tafadhali taja.....

64.10. Je huwa unatumia dawa za kuua fangasi za kienyeji wakati wa kuyahifadhi mazao yako? (1) Ndiyo (2) Hapana, Kama ndiyo....

64.11. Ni aina gani ya dawa za kuua fangasi za kienyeji unazotumia wakati wa kuyahifadhi mazao yako?

(1) Majivu (2) Matope

(3) Zingine tafadhali taja.....

Appendix 5: Kiswahili Version Guideline for Focus Group Discussion (FGDs)

The Focus Group Discussion guided with open ended questions will be used to collect information from the respondents. There will be two groups of participants (i.e. 8 parents or caretakers/guardians in each). The responses will be recorded and transcribed afterwards.

Tarehe: Siku.....Mwezi.....Mwaka.....

Group No.....Jina la kijiji.....

Group Gender: Wanaume..... Wanawake.....

No of Group Members:..... Age Range.....

Utangulizi

Kwanza kabisa ningependa kuwashukuru kwa kukubali kwenu kuja katika mjadala huu. Kusudio kubwa ni kujifunza na kuelewa kutoka kwenu na uzoefu wenu kuhusu fangasi/uvundo/ukungu/aflatoxins katika mazao na vyakula. Unaombwa uelezee jinsi unavyojua ili kuweza kusaidia utafiti huu kukamilika vizuri. Ili kuweza kuendelea na mjadala huu naomba kwa heshima zenu tumchague mwenyekiti na katibu ili atuongozee mjadala wetu huku tukifuata kanuni za utafiti huu (FGD Guide). Pamoja na hayo, timu yetu itakua inafanya kazi pamoja na mwenyekiti kwa karibu ili tuweze kwenda kwa pamoja. Majadiliano haya yatarekodiwa ili kunifanya niweze kufuatilia vizuri mjadala na kusikiliza kwa makini. Nadhani mmenielewa vizuri.

Assessing the levels of awareness, knowledge, attitude and perceptions of communities towards aflatoxins in the society.

A: Awareness about aflatoxins contamination

1. Je, unaufahamu au uelewa wowote kuhusu aflatoxins au tuseme ukungu au fangasi au uvundo unaotokea/kuota kwenye mazao?

2. Je, kwa ufahamu wako ni mazao yapi yanayoshambuliwa sana na fangasi/uvundo/ukungu ambayo pia yanatumika kutengenezea chakula cha mtoto?

Muongozaji: Dodosa, Mazao gani, yanaathirikaje? Dodosa ni aina gani ya vyakula vinavyoathirika zaidi? Kama hajataja vyakula vya mtoto, waulize vyakula hivyo na jinsi gani vinaathirika.

B: Knowledge of aflatoxins contamination

3. Je, unajua kuwa uvundo au ukungu au fangasi unaopatikana kwenye mazao jamii ya mahindi, ulezi, uwele, mtama au karanga n.k ambayo yanatumika kama chakula kwa watu wazima au watoto wadogo husababishwa na nini? *Muongozaji: Dodosa, je huwa yanapatikana kwenye vyakula vya watoto tu?*

4. Je, mnayajua madhara ya uvundo au ukungu au fangasi unaoonekana kwenye mazao kama mahindi, ulezi, mtama, uwele, karanga n.k. ambayo hutumika kama vyakula kwa watu wazima na watoto wako? *Muongozaji: Dodosa, je huwa unapatikana kwenye vyakula vya watoto tu?*

5. Je, huwa unahakikishaje kuwa mazao yako unayotumia kama chakula hayapati uvundo au ukungu au fangasi (aflatoxins)?

6. Je, ni matatizo gani makubwa ya kiafya (yaani magonjwa) yanayowasumbua watoto katika jamii hii? *Muongozaji: Dodosa, ni kwa muda gani matatizo(magonjwa) haya yapo; ni kwa muda mrefu au muda mfupi; na je watu wa hapa wanakabilianaje na haya matatizo (magonjwa)?*

C: Attitude towards aflatoxins prevention

7. Je, ni kitu gani kinachokusukuma uchambue na kuosha mazao yako unayotumia kama chakula kwa watu wakubwa na watoto wako?

8. Je, unahakikishaje kwamba mazao ambayo unatumia kutengenezea chakula cha mtoto hayana uvundo au ukungu au fangasi (aflatoxins)? *Muongozaji: Dodosa ni kwa jinsi gani wanazuia uvundo au uvundo au fangasi hizo?*

E: Complementary food to the children

9. Je, kwa namna gani watoto wenu wanapewa chakula mbadala wenye umri kati ya miezi 6-24 katika jamii hii? *Muongozaji: Dodosa, waeleze jinsi wanza kuwapa watoto wao vyakula vigumu, laini kiasi na laini sana, kwa kutumia vifaa gani, namna mtoto anavyokalishwa wakati wa kulishwa, anavyolishwa kama hapendi kula, mara kwa mara wanatumia vyakula vya aina gani na vyanzo vyake.*

10. Ni pingamizi zipi na matendo yapi ambayo yanazuia kupungua kwaukungu katika mazao yanayotumika kutengeneza chakula cha mtoto kwenye jamii yenu?

- *Taja vitu vya asili/kimila vinavyopinga kupunguza uvundo au ukungu au fangasi (aflatoxins) katika eneo hili.*
- *Taja matendo ya asili/kimila yanayozuia kupunguza uvundo au ukungu au fangasi (aflatoxins) katika eneo hili. Muongozaji: Dodosa, kwa mfano maji, mambo ya kifedha, ukosefu wa vyakula (njaa), kupoteza muda n.k.*

Asanteni kwa muda wenu