AFLATOXIN AND FUMONISIN CONTAMINATION OF MAIZE AND BEANS ALONG THE FOOD AND FEED VALUE CHAIN IN BABATI DISTRICT, TANZANIA

CHACHA JOSEPH NYANGI

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EXTENDED ABSTRACT

The natural occurrence of aflatoxins and fumonisins in maize and beans at harvest, during storage and along the value chain, including processed, feed and marketed products in three villages of Long, Sabilo and Seloto in Babati District, Manyara region, Tanzania, was investigated in the year 2013/14. The villages were chosen as they represents three different climatic zone. Total aflatoxins and fumonisins contamination in 440 at harvest maize samples had levels up to 26.2 µg/kg and 46.2 mg/kg, respectively. Aflatoxins contamination in 38 common beans samples had levels up to 3 µg/kg. The aflatoxin and fumonisin contamination in all beans samples were within the maximum tolerable limit (MTL) of 10 µg/kg and 2 mg/kg respectively, by East African Commission standards (EAC, 2011b). Parameter estimates from the generalised linear model (GENMOD) indicated that medium altitude low rain zone that lies between 1500 and 1850 metre above sea level (m.a.s.l) and representing Sabilo village (0.26) was the major factor pre-disposing maize to aflatoxin contamination, while early planting (-0.22), hand hoe tillage (−0.59) and ox tillage (-0.55) were the major factor reducing the aflatoxin contamination. High altitude high rainzone (Long village) that lies between 2150 and 2450 m.a.s.l was the most important factor reducing fumonisin contamination in maize with a parameter estimate of -2.93. Total aflatoxin and fumonisin levels were also determined in 574 maize and 106 bean samples stored by 60 farmers over a period of 180 days from august, 2013 to March, 2014. Maize samples from Seloto village were more contaminated (mean value of 3.24 µg/kg) than those from Sabilo village (mean value of 3.12 µg/kg). Factors associated with higher aflatoxin contamination were storage for 0 to 80 days and storage with other crops, while for fumonisin most influential factor was storage of maize in granaries comparing to polypropylene and improved bags. The storage technique or facility that had a higher risk of aflatoxin development was polypropylene bags without

any insecticides treatment (control) with a mean contamination value of 3.57 µg/kg and polypropylene bags with insecticides and pesticides treatment (normally used by most of farmers) with a mean value of 3.30 µg/kg. Lower aflatoxin levels were related to the use of traditional storage insecticides, sorting, and storage in improved bags. Among the maize and beans samples collected from the market (vendors) and from processors (small-scale mills) were whole maize grains, maize flour, feed (maize bran and bad-sorted maize not fit for human consumption but normally fed to animals) produced locally from the three villages. Maize bran had highest levels of aflatoxin with a mean value of 2.38 µg/kg and bad sorted portion with fumonisins mean value of 7.42 mg/kg, followed by whole maize with a mean aflatoxin value of 1.73 µg/kg and maize bran with a fumonisin mean value 1.02 mg/kg, while, dehulled maize was least contaminated with fumonisin. During milling mycotoxin become concentrated in bran that most commonly become animal feed. This would reduce the mycotoxins levels in the fraction that is normally used for food (maize flour and dehulled maize). All animal feed grade grain materials had levels lower than MTL of 20 µg/kg for total aflatoxin and a range of 5 to 100 mg/kg for total fumonisin (FAO, 2004; FDA, 2001). The observations made in this study call for use of best practices along the commodity value chain that can reduce contamination in order to improve food and feed safety.

DECLARATION

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

_____ _____

Chacha J. Nyangi Date

(MSc. Food Science)

The above declaration is confirmed;

_____ _____

Prof. J. K. Mugula Date

(Supervisor)

_____ _____

Dr. Fen Beed Date

(Supervisor)

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DEDICATION

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LIST OF ABBREVIATIONS AND ACRONYMS

AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
EAC	East Africa Community
FB_1	Fumonisin B_1
CIAT	International Centre for Tropical Agriculture
CRM	Certified Reference Materials
DAICO	District Agriculture, Irrigation and Cooperative Officer
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FDA	Food and Drugs Authority
FAO	Food and Agriculture Organization
GPS	Global Positioning System
GSP	Good Storage Practices
HCC	Hepatocellular Carcinoma
iAGRI	Innovative Agricultural Research Initiative
IARC	International Agency for Research on Cancer
IITA	International Institute of Tropical Agriculture
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometer
LOD	Limit of Detection
LSMEANS	Least Square Means
MATI	Ministry of Agriculture Training Institute

µg/Kg	Microgram per Kilogram (ppm)
Mg/Kg	Milligram per Kilogram (ppb)
MTL	Maximum Tolerable Limits
MT	Metric Tones
NM-AIST	Nelson Mandela African Institute of Science and Technology
NTD	Neural Tube Defect
RPM	Revoultion per minutes
SAS	Statistical Analysis System
SE	Standard Error
SUA	Sokoine University of Agriculture
UK	United Kingdom
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

CHAPTER ONE

1.0 INTRODUCTION

Mycotoxins are toxic secondary metabolites produced in agricultural crops and food and feed products, by moulds belonging to various genera such as *Aspergillus, Penicillium, Fusarium* and *Byssochlamys* (Aziz *et al*., 2012; Bosco and Mollea, 2012). They are low molecular weight toxic metabolites, that when ingested, inhaled or absorbed through the skin have the potential to seriously affect human and animal health by acute and chronic effects such as the induction of hepatocellular carcinoma (HCC), liver cancer or sudden death due to acute toxicity in the case of aflatoxins (Lewis *et al*., 2005). This also reduces the efficiency of immunological system and retards growth and development of children (Bandyopadhyay, 2010; Kimanya *et al*., 2010). Fumonisins produced by *Fusarium* spp. cause oesophageal cancer and neural tube defect leading to abortion (Marasas *et al*., 2008; Bhat and Miller, 2010; Mahuku and Silla, 2011). Mycotoxicosis has been defined as diseases or physiological abnormalities resulting from exposure to mycotoxins (Piñeiro, 2008). It is estimated that mycotoxins contaminate 25% of agricultural crops worldwide (Zain, 2011).

Exposure of mycotoxins to human can be either through direct consumption of plant products contaminated with the toxins, or indirect through the consumption of animal products containing residual amounts of the mycotoxin ingested by the food-producing animals, through contact of skin with mould infected substrates and inhalation of spore-borne toxins (Boutrif and Bessy, 2001; Bennet and Klich, 2003). Animals' exposure can be through the consumption of feedstuffs contaminated with mycotoxins, contact of skin with mould infected substrates and through inhalation of spore-borne toxins (Bennet and Klich, 2003).

Mycotoxigenic moulds also cause direct economic losses by spoiling grain, which can result in lowered export earnings by most of the developing countries that cannot comply with the stricter lucrative markets' regulations (Hell *et al*., 2005). Commodities contaminated with aflatoxins have a lower market value and often are consumed locally, since they cannot be exported. At the farm level, animals fed with aflatoxin-contaminated grains have lower productivity and slower growth resulting in serious economic problems (Erick, 2003; Ting, 2010). Levels of mycotoxins acceptable in foods in developed countries have been lowered with the maximum limit for aflatoxin B_1 in the European Union as 5 µg/kg and 10 µg/kg for sum of Aflatoxin B_1, B_2, G_1 and G_2 in food (European Commission, 2010), while the limit set for total aflatoxin by United States Food and Drug Administration is 20 µg/kg (FDA, 2001) and for East African Commission the limit is 10 µg/kg (EAC, 2011a, b). Maximum tolerated levels for total fumonisins in food intended for direct human consumption in East African Community is 2 mg/kg (EAC, 2011a,b), while for European Union the limit is 1mg/kg (European commission, 2010) and, for USA the limit is 2 mg/kg for maize products and 4 mg/kg for maize grain food (FDA, 2001).

1.1 Aflatoxins

Aflatoxins are acute and chronic toxicity, immunosuppressive, mutagenic, teratogenic, genotoxic and carcinogenic compounds produced by two major *Aspergillus* species; *A. flavus* which produces aflatoxin B_1 and B_2, and *Aspergillus parasiticus* which produce aflatoxin G_1, G_2, B_1 and B_2 (Filazi and Sireli, 2013; Omar, 2013). The "B" and "G" refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography plates, and the subscript numbers 1 and 2 indicate major and minor compounds respectively (Dhanasekaran *et al*., 2011).

The source of aflatoxins are from agricultural commodities like oil seeds such as groundnut, soybean, sunflower and cotton; tree nuts such as almonds, pistachio, walnuts and coconut; cereals like maize, sorghum, pearl millet, rice and wheat; spices like cumin, cinnamon, clove, black pepper, cardamom, ginger, and coriander; vegetables, milk, meat and dried fruits (Marasas *et al*., 2008; Wild and Gong, 2010).

Aspergillus contamination can attack crops while in the field and that high moisture, high temperatures, drought and heat stress and low soil moisture content in field usually favour the growth of this fungus and toxin production. (Okoth and Kola, 2012; Milani, 2013), the infection and contamination continue during harvest, transport, processing and storage especially when the storage environment is humid and warm (Okoth and Kola, 2012).

The negative effects of aflatoxins on human health can well be classified as either acute toxicity which results in rapid onset of an adverse effect from a single exposure or chronic toxicity which is the slow or delayed onset of an adverse effect, usually from long-term exposures, depending on the kind of toxin and the amount ingested (Omar, 2013). The order of acute and chronic toxicity is AFB_1>AFG_1> AFB_2> AFG_2 (Filazi and Sireli, 2013). Aflatoxins also are responsible for the malabsorption of various nutrients, modification of micronutrients, and uptake of vitamin A and D leading to nutritional deficiencies, immunosuppressive, malnutrition and stunted growth and hence the development of kwashiorkor and marasmus in infants (Bandyopadhyay, 2010; Bbosa *et al*., 2013). Lizárraga-Paulín *et al*. (2011) reported that aflatoxins impair growth in animals and are immunosuppressive, with B aflatoxin found to induce liver and kidney tumors in rodents. Aflatoxins can affect almost all the different body systems and hence the health of the affected individuals as it is the case in poor developing nations of south East Asia and Sub-Saharan Africa (Lizárraga-Paulín *et al*., 2011), where there is poor pre- and post-

harvest practices like poor harvesting, processing and storage which encourage the growth of mold and mycotoxins production (Bbosa *et al*., 2013).

Among all aflatoxins, the Aflatoxin B_1 (AFB_1) is the most potent carcinogen in human and animals, and may interfere with normal process of protein synthesis as well as inhibition of several metabolic systems thus causing damages to various organs especially the liver, kidney and heart (Mohammed and Metwally, 2009; Zain, 2011). Most of aflatoxin B_1 and B_2 ingested by mammals are eliminated through urine and faeces, however a fraction is bio transformed in the liver and excreted together with milk in the form of aflatoxin M_1 and aflatoxin M_2, respectively (Sadeghi *et al*., 2009; Mulunda *et al*., 2013). Aflatoxins have been assessed and classified as class 1 human carcinogen, a highly poisonous toxic substances by the International Agency for Research on Cancer (IARC, 1993).

1.2 Fumonisins

Fumonisins are well-known mycotoxins first described in 1988 by Gelderblom *et al*. being produced by *Fusarium* spp. of the *Gibberella fujikuroi* complex *by Fusarium verticillioides* and *Fusarium proliferatum*. But could also be produced by *F. anthophilum, F. becomiforme, F. dlamini, F. Globosum, F. napiforme, F. nygamai. F. oxysporum, F. polyphialidicum, F. subglutinans* and *F. thapsinum* (Sydenham *et al*., 1997). Fumonisins B_1 and B_2 are of toxicological significance, while the others (B_3, B_4, A_1 and A_2) occur in very low concentrations and are less toxic (Perraica, 1999). Fumonisins occur in maize and infrequently in foodstuffs such as sorghum, asparagus, rice, beans and beer (Creppy, 2002; Zain, 2011), and animal feed from maize sources (Morgensen *et al*., 2010).

The effect of fumonisin on humans has not been fully established, but much evidence suggests a role in human oesophageal cancer from consumption of fumonisin-contaminated maize and maize based products in South Africa, specifically to regions where maize was their staple food (Marasas *et al*., 2008), this was also the case in northern Italy (Franceschi *et al*., 1990) and also cause liver cancer in addition to oesophageal cancer in China (Ueno *et al*., 1997). Fumonisin were also found to be associated with stunting and underweight in Tanzania (Kimanya *et al*., 2010). It was also reported that Fumonisin B_1 causes cranial neural tube defects (NTD) a defect of the brain and spinal cord in the embryo that results from failure of the neural tube to close (Blom *et al*., 2006). In 1990 and 1991, NTD outbreak was reported to occur along Texas-Mexico border and it was suggested that the outbreak might have been caused by high levels of fumonisin B_1 that had been reported in corn in the previous season (Missmer *et al*., 2006). A similar outbreak was also reported in China and South Africa in regions with high corn consumption (Stockmann-Juvala and Savolaine, 2008).

1.3 Maize and Bean Production and Consumption in Tanzania

Maize is the second agricultural commodity in terms of production after cassava in Tanzania and production was 4 700 000 MT, with a yield of 1 175 Kg/ha and a total of about four million hectares harvested annually and accounting for 511 KCal/Day of dietary calories to Tanzanian (FAOSTAT, 2013). The crop is mainly cultivated during two rain seasons, short-duration rains (*vuli*") and long-duration rains (*masika)* and grown almost in all regions in the country, though the productivity is more in the high rainfall areas of Tanzania, such as, southern highlands, the Lake Victoria zone, and the northern zone (Temu *et al*., 2010).

Despite the importance of maize as a staple food to most Tanzanians, it is mostly produced by small-scale farmers approximately 80% most of them own farm of up to 10 ha per household and account for about 85% of the maize produced in the country (Amani, 2004). In the study area, maize is commonly used to prepare typical meals such as *uji* (porridge), *ugali* (stiff porridge), *kande* (boiled maize grits and beans mixture).

The common bean (*Phaseolus vulgaris* L.) is the most important food legume in the world (CIAT, 2001). Production levels in Tanzania was 1 150 000 MT, with a yield of 8846 Kg/ha and a total of about 1 300 000 hectares harvested annually (FAOSTAT, 2013). Tanzania is a major common bean producing country in East Africa (Fivawo and Msola, 2011), with a quarter to one third of the households sell their beans and retain the remaining portion for household consumption, and in some of the beans producing regions like Iringa, Kilimanjaro and Arusha, commercial bean production for export is taking place, due to suitable climate and access to an international airport (Ronner and Giller, 2012).

1.4 Current Status of Mycotoxins in Africa

1.4.1 Maize

Among crops used as food and feed, maize is a good substrate for the growth of aflatoxins and fumonisins producing moulds above others, while groundnuts is an excellent substrate for aflatoxin contamination (Bankole and Adebanjo, 2003). Maize is a suitable substrate as it acts as a good source of energy in the form of carbohydrates, water activity (available moisture) for growth and toxin production (Atanda *et al*., 2013), which are potentially dangerous to both humans and animals (Kpodo *et al*., 2000).

From Tanzania the incidence and extent of fumonisins contamination of home-stored maize for human consumption was reported with levels up to 11.048 mg/kg (Kimanya *et al.,* 2008), also fumonisin levels of up to 3.201 mg/kg in complementary foods (Kimanya *et al.,* 2010) and levels up to 2.283 mg/kg in maize based complementary food (Kimanya *et al.,* 2013). In Uganda Atukwase *et al.* (2009) reported an overall contamination levels for total fumonisin in pre-harvest maize from different climatic zone to be between 0.27 and 10 mg/kg. Similar results were also reported in Uganda by Kaaya *et al.* (2006). In Benin Sétamou *et al.* (1997) reported an overall contamination levels for total aflatoxin in pre-harvest maize to be between 5-2500 µg/kg and 5-2200 µg/kg in 1994 and 1995 respectively. Warth *et al.* (2012) found maize from Mozambique to be contaminated by aflatoxin with a mean value of 2.4 µg/kg (n = 168). Ncube *et al.* (2011) reported fumonisins contamination of maize to be between 0 to 21.8 mg/kg in South Africa.

In Kenya, one of the largest known aflatoxicosis outbreaks occurred in 2004, with 317 reported cases and 125 deaths (Lewis *et al*., 2005; Probst *et al*., 2010). Food samples collected from households in the affected areas contained high levels of aflatoxin B_1 (20 to > 1000 µg/kg). The outbreak resulted from aflatoxin contamination of locally grown maize that was stored under damp conditions (Okioma, 2005). Bii *et al.* (2012) conducted a study in eastern province of Kenya on 86 stored maize samples and found mean fumonisin contamination in maize samples ranging from 0.912 mg/kg in Kitui to 1.17 mg/kg in Makueni. Another study was conducted by Lewis *et al*. (2005) where maize samples (n = 350) were collected from markets and vendors in the four most affected districts as identified by the 2004 outbreak. Mahuku and Sila (2011) conducted a study in 2010 and found the level of aflatoxin in maize stored by farmers in Kenya to be 1776 µg/kg while in the markets the concentration was 1632 µg/kg. These higher levels are likely to cause acute toxicity when contaminated products are consumed.

1.4.2 Beans

There are very few reports on beans contamination with mycotoxin producing moulds in Africa. A study conducted by Aiat (2006) in Egypt found the levels of total aflatoxins to be 1463 μg/kg. Another study was conducted in Rwanda by Nyinawabali (2013) and reported the levels of aflatoxins to range between 0.2 – 154.9 μg/kg with a mean value of 28.1 μg/kg, while the level of fumonisin ranged between 0.4 - 7.1 mg/kg with a mean value of 3.0 mg/kg. The contamination of the beans samples with aflatoxin and fumonisin has also been reported by Tseng *et al*. (1995)

1.4.3 Animal feeds

McDonald *et al*. (1995) defined animal feeds as any material provided to an animal as part of its daily ration and when ingested is capable of being digested, absorbed and utilised by the body of the animal to meet its nutritional need. Aflatoxin and fumonisin occur in many animal feed concentrates including cereal grains, soybeans products (soybeans meal), oil cakes (from groundnuts, cottonseed, sunflower, palm and copra), and fishmeal; Brewers grains, a by-product from the production of cereal-based alcoholic drinks (Grace, 2013)

The most susceptible animals to aflatoxin contamination are rabbits, turkeys, chickens, pigs, cows and goats (Lizárraga-Paulín *et al.*, 2011), and for fumonisins the most susceptible animals are horses, pigs and rats (Voss *et al.,* 1995; Smith *et al*., 1996; Segvic and Pepeljnjak, 2001). Grace (2013) reported that the effects of aflatoxins to animals depend on various factors such as genetic (species and breed strain), physiological (age, nutrition, and exercise) and environmental (climatic and husbandry). Foetuses are very susceptible to even low levels and young and fast growing animals are more affected than adults, Males are more susceptible than females, while old ruminants with a well-

functioning rumen are very resistant (Grace, 2013). It was also reported that livestock in intensive systems are at higher risk of dietary exposure than animals in more extensive systems. With high and increasing proportion of dairy cattle, poultry, and swine being kept in intensive systems, aflatoxins are thus likely to be an increasing problem (Grace, 2013). The total permissible aflatoxin levels in animal feeds range from 0 to 50 μg/kg with an average of 20 μg/kg (FAO 2004), and for fumonisin the range is 5 to 100 mg/kg in animal feeds (FDA, 2001), especially to countries where regulations exists.

1.5 Factors Affecting the Incidence of Mycotoxigenic Fungi and Mycotoxins

1.5.1 Climatic conditions

Temperature and humidity influence which fungi infect damaged crops. Aflatoxin producers are favoured by warm conditions; thus, global warming, particularly in currently temperate climates, poses a potential problem is this regard (Milani, 2013). Optimum conditions for aflatoxin production is a temperature of 33°C and water activity of 0.99 while that for growth is 35°C and water activity of 0.95 (Milani, 2013). Therefore, *Aspergillus flavus* and aflatoxin are more likely in corn and crops grown in the heat and drought stress associated with warmer climates (Milani, 2013).

Fumonisin are usually found in temperate zones (Atanda *et al*., 2013), hence maize grown in temperate regions is an appropriate substrate for *F. verticillioides* colonization and production of fumonisins. Climatic conditions during the growing season, is among the determinant factors for *F. verticillioides* infection and fumonisin accumulation in maize in the field. Higher temperatures during kernel maturation, and more rainfall before harvest are the factors that increase ear rot levels and fumonisin content at harvest (Fandohan *et al*., 2003). *Fusarium* is favoured by a temperature of 15 - 30^{o}C and water activity of 0.9 - 0.995 as an optimum condition for toxin secretion (Sanchis and Magan,

2004), and usually produce fumonisins in the field and, if the crop is harvested at high moisture content, conducive to fungal growth and mycotoxin production. Fumonisins can also affect storage crops especially when the storage conditions of water activity and temperature become favourable (Fandohan *et al*., 2003; Omar, 2013).

1.5.2 Soil conditions and nutrients availability

Soil is a natural factor that exerts a powerful influence on the incidence of fungi. Crops grown in different soil types may have significantly different levels of mycotoxin contamination (Atanda *et al*., 2013). This was the case with peanuts grown in light sandy soils which was found to support rapid growth of the fungi, particularly under dry conditions, while heavier soils result in less contamination of peanuts due to their high water holding capacity which helps the plant to prevent drought stress. The toxigenic (toxin producing) strains require enough nutrients for fungal growth and mycotoxin production (Atanda *et al*., 2013). Fungi also require a source of energy in the form of carbohydrates or vegetable oils in addition to a source of nitrogen either organic or inorganic, they also require trace elements and water activity for growth and toxin production (Atanda *et al*., 2013).

1.5.3 Tillage method

Tillage method is one of the major management practices affecting soil physical parameters (Janusauskaite *et al.*, 2013). Several studies have been conducted to study the effect of tillage on soil microbial population by comparing microbial numbers, the soil microbial community, enzyme activities and microbial biomass (Gil-Sortes *et al*., 2005). Helgason *et al.* (2009) found that both bacteria and fungi were more abundant under no tillage than conventional tillage. This indicates that there is a high possibility of fungal growth and mycotoxins production in no tillage than in conventional tillage.

1.5.4 Pest infestation

Insect feeding activity has been found to be associated with fungal infection of maize grain and the subsequent production of mycotoxins (Beti *et al*., 1995; Hell *et al*., 2000; Avantaggiato *et al*., 2003; Fandohan *et al*., 2005). Both *A. flavus* and *F. moniliforme* are known to be facilitated in their infection process of maize grain by insect feeding (Beti *et al*., 1995). Setamou *et al*. (1997) reported maize ears with less than 2% insect feeding damage had a mean aflatoxin contamination level considerably lower in both 1994 and 1995 than ears with more than 10% damage

Insect infestation in the field and in storage causes deterioration of grains as it predisposes them to fungal infection through increasing ease of access for infection through wounds (Alakonya and Monda, 2013), especially when loose-husked maize hybrids are used (Hell and Mutegi, 2011).

1.6 Pre Harvest Practices to Mitigate Aflatoxin and Fumonisin Contamination

1.6.1 Time of Planting

Time of planting has shown to have direct influence on the contamination of grain by aflatoxins, fumonisins and other mycotoxins. A study conducted by Abbas *et al.* (2007) found that maize planted in mid-April resulted in lower aflatoxin and fumonisin contamination and in significantly less frequent contamination above a regulatory action level than did the early-May planting date. Jones *et al*. (1981) reported that lower levels of aflatoxin B_1 contamination occurred in maize grain produced by April plantings as compared to May plantings. In another study it was found that maize planted in June has higher incidence of aflatoxin B_1 compared to maize planted in April and May (Lillehoj *et al*., 1978).

Parsons and Munkvold (2012) reported that earlier planting consistently resulted in lower ear rot severity, low fumonisin B_1 levels and less insect damage. Early planting was also reported to shifts the period between when the flower is fully open and functional (anthesis) and dough development in maize to a time frame in growing season when maize are less susceptible to drought and heat stress as compared to late plantings (Zuber and Lillehoj, 1979).

1.6.2 Time of harvesting

Harvest is the first stage in the production chain where moisture content becomes the most important parameter in terms of the management and protection of the crop (Bruns, 2003). Optimal harvest time is necessary as it ensures that crops are not left in the field exposed to environmental factors that predispose crops to pathogen infection. The general recommendation is to harvest maize grain after they attain physiological maturity and then artificially dried to a moisture content of below 13% for safe storage (Bruns, 2003), this is recommended since aflatoxin level can increase with delayed harvest interval (Kahaya and Kyamuhangire, 2006).

Bankole and Adebanjo (2003) found that early harvesting reduces fungal infection of crops in the field and consequent contamination of harvested produce. Kaaya *et al.* (2006) observed that aflatoxin levels increased by about four times by the third week and more than seven times when maize harvest was delayed for four weeks. However, if products are harvested early, they have to be dried to safe moisture levels (10-13%) to stop fungal growth.

1.6.3 Crop rotation

From his study Atukwase *et al.* (2009) found that crop rotation is significantly associated with fumonisin production in maize. There was a report that produce harvested from land on which groundnuts has been planted the previous year were infested more by *Aspergillus flavus* and contained more aflatoxin than crops grown on land previously planted with rye, oats, melon or potatoes indicating that crop rotation influences mycotoxigenic mould growth, and hence care must be taken to avoid rotation of crops that can influence contamination (Alakonya and Monda, 2013; Atanda *et al*., 2013).

1.6.4 Bio-control

One of the promising and potential strategies to mitigate mycotoxin contamination is biological control. A biological control technique greatly reduced aflatoxins in all the susceptible crops in a cost-effective manner and over a broad geographic area (Bandyopadhyay, 2010). Native strains of *A. flavus* that do not produce aflatoxins ("atoxigenic strains") are used to competitively exclude aflatoxin-producing strains from the crop environment, this has been successfully implemented in the Southern US, Northern Mexico, Nigeria and West Africa under the commercial names AF36™, Aflasafe™ and AflaGuard™ to reduce aflatoxin contamination in various crops such as cotton, maize and groundnut (Donner *et al*., 2010). Competition occurs when two or more micro-organisms require the same resources in excess of their supply, these resources can include space, nutrients, and oxygen (Bandyopadhyay, 2010).

1.6.5 Use of resistant varieties

The use of resistant hybrids like AO901-25 a yellow maize varieties with high yield of 7115 kg/ha, good resistance to *Aspergillus* and low aflatoxin level could be very promising, but commercial hybrids are not always available (Abbas *et al.*, 2009). Menkir

et al. (2008) reported the registered six tropical maize (*Zea mays* L.) germplasm lines with resistance to aflatoxin contamination developed by the International Institute of Tropical Agriculture (IITA) through a collaborative breeding project with Southern Regional Research Center of the USDA-ARS. Field tests of the six lines under artificial inoculation with an African strain of *Aspergillus flavus* in Nigeria revealed that these lines had lower levels of aflatoxin compared with elite tropical commercial inbred lines used as control.

1.7 Post-harvest Practices to Mitigate Aflatoxin and Fumonisin Contamination

The post-harvest practices are those practices following harvest and leading up to primary processing such as milling, they include; rapid drying on platforms to avoid direct contact with soil, proper shelling methods to reduce grain damage and fumonisin level in maize by 56 – 68% (Fandohan *et al*., 2005). Dehulling of maize prior to milling was also found to remove significant amounts of aflatoxins and fumonisins in maize and maize products, with a reduction of 92% aflatoxins (Fandohan *et al.,* 2005; Siwela *et al*., 2005), sorting to remove bad/moulded grain from the lot, use of clean and aerated storage structures, controlling insect damage, good transportation practices and avoiding long storage periods, 8- 10 months (Hell *et al*., 2005).

1.7.1 Drying conditions

Fungal growth and mycotoxin production can take place in a matter of days if maize grain is not properly dried and cooled before storage (Setamou *et al*., 1997). Rapid drying of agricultural products to safe moisture levels of 10-13% for cereals is very critical as it creates less favourable conditions for fungal growth, proliferation, and insect infestation (Hell *et al*., 2005). It helps keep products longer, since the free water required for their development is not available (Lanyasunya *et al*., 2005). Aflatoxin contamination was

found to increase 10 fold in a three day period, especially when field harvested maize is stored with high moisture content (Hell *et al*., 2005).

Atukwase *et al*. (2009) reported that drying maize on bare ground was found to be positively associated with fumonisin contamination, this may be attributed to drying harvested maize without husks. This practice brings maize grains into direct contact with soil which is a primary source of *Fusarium* (Odogora and Henricksson, 1991). In addition, drying maize on bare ground may cause an increase in water activity of the grains due to absorption of moisture from the soil and re-wetting by rain (Kaaya *et al*., 2006). Maize cobs which are dried on bare ground are therefore vulnerable to fungal infection and subsequent contamination with mycotoxins (Atukwase *et al*., 2009).

1.7.2 Storage factors

Storage is a critical stage where infection and mycotoxin accumulation occur. Care must be taken to store grains that are wholesome and apparently healthy. It is well known that aflatoxins contamination of foods increase with storage period (Hell *et al*., 2000), while Fandohan *et al*. (2003) found that fumonisin level overall, was decreasing over the storage period. Storage prior and during marketing has to be done in appropriate bagging, preferably sisal bags, as this kind of material facilitates aeration especially during transit and bagged commodities should be stored on pallets (Lizárraga-Paulín *et al.*, 2013). Many farmers store their grains in bags, especially polypropylene which are not airtight, with evidence that this method facilitates fungal contamination and aflatoxin development (Udoh *et al*., 2000; Hell and Mutegi, 2011). The use of traditional storage facilities made from plant materials (wood, bamboo, thatch) or mud placed on raised platforms and covered with thatch or metal roofing sheet is another way to prevent contamination. The stores should be constructed to exclude fungal growth, should include dry and well-

ventilated structures, provide protection from rain, drainage of ground water, prevent entry of rodents and birds, should allow minimum temperature fluctuations, and prevent moisture from getting into the grains (Lizárraga-Paulín *et al.*, 2013).

1.7.3 Physical separation and hygiene

Sorting out physically damaged and infected grains (based on their coloration, odd shapes, shriveled and reduced size) from the intact commodity can reduce aflatoxin levels by 40-80% (Park, 2002). Okioma (2005) reported that moldy and discoloured (sorted) grain was fed to livestock or sold at a price that is about half that for the clean grain. But during a severe famine in Kenya, sorted grain was in some cases mixed with clean grain and cooked for consumption by the family. The highest concentrations of aflatoxin and fumonisins usually are found on heavily molded and/or damaged kernels (Park, 2002; Afolabi *et al*., 2006). Shetty and Bhat (1999) found that broken maize kernels contain nearly 10 times higher levels of fumonisins.

Physical method can also involve basic sanitation measures such as removal and destruction of debris from previous harvest both in the field and store which would help in minimizing infection and infestation of produce both in the field and in storage (Hell *et al*., 2005).

1.7.4 Lack of awareness about mycotoxins

Awareness creation and sensitisation on the dangers posed by mycotoxin contamination of produce can be the most practical and fundamental intervention strategy to mitigate mycotoxins. Majority of the farmers are not aware of the major factor responsible for high incidence of mycotoxins in their areas. Also majority of farmers, foodhandlers and processors are illiterate with virtually no knowledge of the implications of toxigenic

mould growth. Most of the stake holders in Nigeria for example believe that the powdery substance can be easily dusted off or rinsed with water before the food material is eaten or processed for consumption with no associated risks (Alakonya and Monda, 2013). Hell and Mutegi (2011) recommended that farmers should be educated on proper use of low-energy technologies for food preservation, proper food handling and storage methods.

1.8 Problem Statement and Justification

Mycotoxin contamination of various foodstuffs and agricultural commodities is a major problem in the tropics and sub-tropics, due to lack of regulations on mycotoxins such as testing and setting of limit in some countries, and where climatic conditions, agricultural and storage practices are conducive to fungal growth and toxin production (Wagacha and Muthoni, 2008). Considering that maize is a staple food for the majority of Tanzanians, it is necessary to estimate the magnitude of the mycotoxin contamination (Kimanya *et al*., 2008). Tanzania is also a major common bean producing country in East Africa (Fivawo and Msola, 2011), with commercial bean production for export taking place in major beans producing regions like Iringa, Kilimanjaro and Arusha (Ronner and Giller, 2012). A study conducted by Nyinawabali (2013) in Rwanda found beans to be contaminated by both aflatoxins and fumonisins. Ghosia and Arsha (2012) conducted a study in Pakistan and observed that 10% of red kidney beans were contaminated above the suggested limit (4µg/kg) set by European Union regulations for total aflatoxins (European Commission, 2010). Tseng *et al*. (1995) from Taiwan and Ontario Canada reported fumonisin B_1 levels of 1.8 mg/kg. These findings indicated the presence of mycotoxins in maize and common beans and a need for surveillance on contamination with mycotoxins especially, aflatoxins and fumonisins in Tanzania.

The aim of this study was to establish the levels of aflatoxins and fumonisins contamination in naturally contaminated maize and beans along the commodity value chain, including at harvest, during storage in processed and marketed products in Tanzania by using Babati district, Manyara region as a case study.

1.8.1 Objectives

1.8.1.1 Main objective

To establish the prevalence of aflatoxins and fumonisins in maize and beans along the food and feed value chains in Babati District.

1.8.1.2 Specific objectives

(i) To quantify total aflatoxins and fumonisins in pre-harvest maize and beans.

(ii) To quantify total aflatoxins and fumonisins in maize and beans during storage.

(iii) To quantify total aflatoxins and fumonisins in processed and marketed maize and beans products including those used in animal feed formulation.

(iv) To identify pre- and post-harvest management practices, that influence aflatoxin and fumonisin contamination of maize and beans along food and feed value chains.

1.8.1.3 List of manuscripts

i. Aflatoxin and Fumonisin Contamination at harvest Maize and Beans in three villages in Babati District, Tanzania.

ii. The Influence of Storage Practices on Aflatoxin and Fumonisin Contamination of Maize and Beans in Babati District Tanzania.

iii. Aflatoxin and Fumonisin Contamination of Maize and Common Bean-Based Market, Processed and Feeds in Babati District, Tanzania.

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CHAPTER TWO

2.0 Aflatoxin and Fumonisin Contamination at harvest Maize and Beans in three villages in Babati District, Tanzania

Chacha J. Nyangi[1, 2*], Jovin K. Mugula[1], Fen Beed[2], Simon Boniphace[2], Koyano Emmanuel[2]

[1]*Department of Food Science and Technology, Sokoine University of Agriculture, P.O.Box 3006, Morogoro, Tanzania*

[2]*International Institute of Tropical Agriculture, P.O.Box 34441, Dar es salaam, Tanzania.*

2.1 Abstract

A survey was conducted on the natural occurrence of aflatoxins and fumonisins in 440 maize and 38 beans samples from three villages in Babati District. Quantification for total aflatoxin and fumonisin was done using ELISA (Reveal AccuScan®Neogen, USA), and the results were confirmed by using LC-MS/MS. Maize samples from Sabilo village had aflatoxin levels up to 26.2 µg/kg (mean, 3.32 µg/kg), while maize from Long and Seloto village had maximum contamination below the maximum tolerable limits (MTL) of 10 µg/kg. For fumonisin contamination, Seloto village had levels up to 46 mg/kg (mean, 6.6 mg/kg), followed by Long village with levels up to 14 mg/kg (mean, 6.75 mg/kg) and Sabilo village 14 mg/kg (mean, 3.17 mg/kg). Beans samples had aflatoxin and fumonisin contamination levels below MTL. The incidence of aflatoxin and fumonisins in the subsistence farming systems of Babati district call for intervention strategies including awareness creation programmes in order to enhance food and feed safety.

Keywords*: aflatoxins, fumonisins, pre-harvest, maize, beans mycotoxins, contamination, food safety*

**Corresponding author. Tel: +255-764-775727. Email dress: nyangichacha@yahoo.com*

2.2 Introduction

Mycotoxins are chemically and biologically active by-products of mould growth occurring naturally in a range of plant products. Mycotoxins have received considerable attention due to their significance in agricultural loss (because of lack of acceptance post harvest of contaminated foods and feeds), negative impacts on livestock and human health. It is estimated that mycotoxins contaminate 25% of agricultural crops worldwide (Zain, 2011), with 4.5 billion people living in developing countries exposed to chronic toxicity (Williams *et al.,* 2004). There are two important mycotoxigenic moulds associated with maize and beans. These are *Aspergillus flavus* that produce aflatoxins (Wagacha and Muthoni, 2008; Okoth *et al.,* 2012) and *Fusarium verticillioides* (previously known as *F. moniliforme*), which produces fumonisins (Omar, 2013; Nyinawabali, 2013).

Aflatoxins can cause acute and chronic toxicity depending on the concentrations present, are immunosuppressive, mutagenic, teratogenic, genotoxic and carcinogenic compounds produced mainly by *Aspergillus flavus* (Filazi and Sireli, 2013; Omar, 2013). The source of aflatoxins are from agricultural commodities like oil seeds such as groundnut, soybean, sunflower and cotton; tree nuts such as almonds, pistachio, walnuts and coconut; cereals like maize, sorghum, pearl millet, rice and wheat; spices like cumin, cinnamon, clove, black pepper, cardamom, ginger, and coriander; vegetables, milk, meat and dried fruits (Marasas *et al*., 2008; Wild and Gong, 2010).

Aflatoxin B_1 (AFB_1) is the most potent carcinogen in humans and animals, and has been classified as Class 1 human carcinogen, a highly poisonous toxic substance (IARC, 1993). The accepted threshold for total aflatoxin in foodstuffs intended for direct human consumption is 10µg/kg by European Union and East Afican Community standards

(European Commission, 2010; EAC, 2011a,b) and 20 μg/kg for the USA standards (FDA, 2001). Optimum conditions for aflatoxin production is a temperature of 33°C and water activity of 0.99 while that for growth is 35°C and water activity of 0.95 (Milani, 2013). Therefore, *Aspergillus flavus* and aflatoxin are more likely in corn and crops grown in the heat and drought stress associated with warmer climates (Milani, 2013).

Fumonisins is produced by *Fusarium* spp. mostly by *Fusarium verticillioides* (previously known as *F. moniliforme*), (Omar, 2013; Nyinawabali, 2013) and occur in maize and in frequently in other foodstuffs such as sorghum, asparagus, rice, beans and beers (Creppy, 2002; Zain, 2011), as well as in feeds especially those formulated using maize (Morgensen *et al*., 2010). Evidence suggests a role of fumonisin in human oesophageal cancer in South Africa (Marasas *et al*., 2008), Northern Italy (Franceschi *et al*., 1990), and China (Ueno *et al*., 1997). Fumonisins were also associated with liver cancer in certain endemic areas of the People's Republic of China (Ueno *et al*., 1997), poor child growth in Tanzania (Kimanya *et al*., 2010) and, cranial neural tube defects along the Texas-Mexico border, China and South Africa (Missmer *et al*., 2006; Stockmann-Juvala and Savolaine, 2008). The International Agency for Research on Cancer (IARC) has classified fumonisin as a group 2B toxin, considered as possibly carcinogenic to humans (IARC, 1993). MTL for fumonisins in food intended for direct human consumption is 2 mg/kg by the USA and East African standards (FDA, 2001; EAC, 2011a,b) and 1 mg/kg by the EU standards (European Commission, 2010). Very little has been reported on the relationship between production, handling practices and the occurrence of aflatoxins and fumonisins in maize and beans in Tanzania. The aim of this study was to investigate the effect of pre harvest field management (agronomy) practices and climatic zones on aflatoxin and fumonisin contamination of maize and beans in Tanzania.

2.3 Materials and Methods

2.3.1 Study area

The study was conducted in three villages namely, Long, Sabilo and Seloto, representing different climatic zones in the year 2013. The high altitude high rain zone representing Long village, that lies between 2150 and 2450 metres above sea levels (m.a.s.l) with a relatively high annual rainfall of 1200 mm. The mid altitude low rainfall zone representing Sabilo village,that lies between 1500 and 1850 m.a.s.l and has a production season characterised by relatively low rainfall of 900 – 1100 mm, and the mid altitude high rain zone represented by Seloto village that lies between 1850 – 2150 m.a.s.l and has the production season; characterised by relatively annual rainfall of 1100 – 1200mm. The villages were selected as they fall under USAID's Feed the Future priority research area, where the Africa RISING Eastern and Southern Africa project on sustainable intensification of farming systems is being implemented in collaboration with International Institute of tropical Agriculture (IITA). Maize and beans are also the major staple food in the study site and Tanzania as a whole.

2.3.2 Selection of farmers

The farmers who participated in the study were randomly selected using a list provided by the respective village leaders and extension officers. A total of 450 farmers, 150 farmers from each village, were randomly selected for maize sampling and, a total of 38 farmers for beans sampling, based on prior evidence that beans were not considered to be as much at risk as maize so a smaller sub-set was selected and if shown to be contaminated then a larger number of samples would be collected. The maize and beans sample from the same farmers was followed up to the post harvest but with only 20 farmers per village.

2.3.3 Sample collection

A total of 450 physiologically mature maize samples were collected at harvest in the year 2013 from the three villages. The 150 randomly sampled farmers per village were interviewed using a semi-structured questionnaire (Appendix 1). Responses were elicited on farmers' planted variety, previous crops, pest problems in the field, planted and harvested date, tillage method, planting pattern (flat, on ridges, on mounds), harvested condition (wet or dry), condition of harvested crop (clean or spoiled) and intended use of the harvested crops. Global Positioning System (GPS) coordinates and basic demographic details of farmers/producer were also collected. Responses from the farmers were used to evaluate farming practices and handling techniques.

The samples in the field were taken by walking in two diagonal directions and stopping at regular intervals to pick a sample so as to have as representative sample as possible. A total of five stops were chosen in each field and five maize cobs or beans pods were randomly taken from each stop making a total of 25 cobs/pods per field, these were then hand shelled, well mixed and approximate 1kg sample was randomly selected. The collected samples were packaged in a clean A4 envelope and transported to the Plant Pathology Laboratory at International Institute of Tropical Agriculture (IITA), Tanzania. The samples were then dried at 65^{o}C/ 72 h in a cabinet drier to < 13% moisture content.

2.3.4 Quantification of total aflatoxin and fumonisin

The samples were ground using a Bunn grinder (Man: Bunn-O-Matic Corporation Springfield, Illinois, U.S.A), homogenized, and sub divided to obtain a representative sub-sample for analysis (https://www.extension.iastate.edu/NR/rdonlyres/52E2F1B9-AC0C-4AE5-8096-25B9921348AB/0/USDAAflatoxinHandbook.pdf). A 50g sub-sample was taken from each of the ground samples and extracted with 250 mL mixture of

ethanol/water (65:35, v/v) and shaken vigorously at 150 revolution per minute (rpm) for 3 minutes using a laboratory shaker (IKA® Werke, Germany). Extracts were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). Then total aflatoxin (µg/kg) and fumonisin (mg/kg) were quantified following the manufacturer's protocol using Reveal AccuScan® III reader (Neogen, USA), a quantitative ELISA-based analytical test kits designed specifically for either aflatoxins or fumonisin (http://www.gipsa.usda.gov/lawsandregs/bulletins/pn12-03.pdf). The detection limit for total aflatoxin was 2 µg/Kg with a quantitation range of 2 - 150 µg/Kg and that for total fumonisin was 0.3 mg/Kg with a quantitation range of 0.3 - 6 mg/Kg. The analytical quality of the ELISA methods was assured by the use of certified reference material (CRM), a naturally contaminated maize sample with certified total aflatoxin content of 18.1 ± 3.6 µg/kg and total fumonisin content of 4.2 ± 0.6 mg/kg supplied by Neogen, USA. For the purpose of data analysis, non-detect levels were based on the detection limits (LOD) of the test method for each toxin.

Confirmatory test was done using Liquid chromatography tandem mass spectrometry (LC-MS/MS) at the Interuniversity Department of Agro biotechnology (IFA-Tullin, Austria), on 60 highly contaminated samples previously analysed using Reveal AccuScan® III reader (Neogen, USA) at the plant pathology laboratory of IITA-Tanzania. The results indicated a correlation between the two methods for both total aflatoxin and total fumonisin.

2.3.5 Statistical analysis

Data were analysed using StatisticalAnalysis System (SAS® Version 9.4, SAS Institute Incorporation, USA). A generalized linear model (GENMOD) was run to identify the factors that significantly affect contamination of maize and beans with aflatoxin and

fumonisins. The differences between means were detected using least square means (LSMEANS) to establish differences in mean total aflatoxin and fumonisin amongst the climatic zones and agricultural practices. Aflatoxin and fumonisin levels were transformed using the natural log to normalise the data before analysis.

2.4 Results

2.4.1 Characteristics of the farmers and their farming systems.

Seventy two percent of the sampled farmers in the study area were male and 28% female. Seventy seven percent completed primary education, while 15% either did not have any formal education, or did not complete primary education. Sixty two percent of the farmers were aware of mycotoxins, and 2%, all from one village, Sabilo had experienced health problems associated with consuming food that they believed to be contaminated either with mycotoxins or other food poisoning agents (Table 2.1).

Table 2.1: Demographic characteristics of farmers across three villages

Characteristics	**Surveyed villages**			
	Total samples (n = 442) (%)	**Long (n=154) (%)**	**Sabilo (n=145) (%)**	**Seloto (n=143) (%)**
Sex				
Male	318 (72)	134 (87)	71 (49)	113 (79)
Female	124 (28)	20 (12)	74 (51)	30 (21)
Education				
Primary	340 (77)	125 (81)	108 (74)	107 (75)
Secondary	26 (6)	6 (4)	6 (4)	14 (10)
Tertiary	7 (2)	2 (1)	0 (0)	5 (3)
None	69 (15)	21 (14)	31 (22)	17 (12)
Awareness				
Yes	276 (62)	55 (36)	112 (77)	109 (76)
No	166 (38)	99 (64)	33 (23)	34 (24)
Health problem				
Yes	10 (2)	0 (0)	10 (7)	0 (0)
No	432 (98)	154 (100)	135 (94)	143 (100)

n = number of farmers visited which is equal to the number of samples collected.
(%) = percentage of farmers responded

2.4.2 Agronomic practices used by farmers villages

Four tillage methods were identified in the study area, as well as two planting dates (categorised as early planting for those who planted maize in November or December and late planting for those who planted in January or February). Farmers planted both improved (purchased) and local (saved) maize varieties. The various agronomic practices in the study area are shown in Table 2.2.

Table 2.2: Agronomic practices used by farmers across three villages

Practices	Total samples (n=442) (%)	Surveyed villages		
		Long (n= 154) (%)	Sabilo (n= 145 (%)	Seloto (n= 143) (%)
Planting date				
Early planting	329 (74)	149 (97)	59 (41)	121 (85)
Late planting	113 (26)	5(3)	86 (59)	22 (15)
Tillage method				
Hand hoe	28 (6)	4 (3)	5 (3)	19 (13)
Ox	346 (78)	144 (94)	95 (66)	107 (75)
Hand hoe, ox	57 (13)	4 (3)	42 (29)	11 (8)
Hand hoe, ox, tractor	11 (2)	2 (1)	3 (2)	6 (4)
Variety				
Improved seed	432 (98)	152 (99)	139 (96)	141 (99)
Local seed	10 (2)	2 (1)	6 (4)	2 (1)

n = number of farmers visited which is equal to the number of samples collected.
(%) = percentage of farmers responded positively to each of the agronomic practices listed.

2.4.3 Total aflatoxin and fumonisin content in maize and beans

Nineteen percent and 35% of the maize sampleswere contaminated with aflatoxin and fumonisin, respectively. Eighteen percent of the bean samples were contaminated with aflatoxin (Table 2.3).

Table 2.3: Incidence and mean of total aflatoxin and fumonisin in maize and bean samples across three villages

Maize	N	Positive samples (%)	Maximum concentration	Mean ±SE
Aflatoxin (µg/kg)	440	84 (19)	26.2	2.94± 0.28
Fumonisin (mg/kg)	440	153 (35)	46.0	5.15± 0.63
Beans				
Aflatoxin (µg/kg)	38	7 (18)	3.0	2.49± 0.11
Fumonisin (mg/kg)	38	n.d	n.d	n.d

- Values are means of total aflatoxin and fumonisin levels of maize and beans for positive samples across three villages.
- Positive samples are all analysed samples with value > Limit of detection (LOD)
- n is the total number of analysed samples
- n.d means fumonisin levels were below LOD

The highest aflatoxin mean value for maize samples was found in Sabilo village and for fumonisin the highest mean value was found in Long village, while for beans the aflatoxin and fumonisin contamination was found only in Long village, with aflatoxin and fumonisin level below LOD in Sabilo and Seloto village (Table 2.4).

Table 2.4: Incidence and mean of total aflatoxin and fumonisin contamination in maize and bean samples in each villages

Village	n	Aflatoxin (µg/kg) Positive sample (%)	Range	Mean ± SE	Fumonisin (mg/kg) Positive sample (%)	Range	Mean ± SE
Maize							
Long	153	26 (17)	2.10 - 3.6	2.58^{a}± 0.08	6(4)	0.90 - 14.00	6.75^{a}± 2.41
Sabilo	144	40 (28)	2.20 - 26.2	3.32^{b} ± 0.59	65(45)	0.40 - 14.00	3.17^{b}± 0.43
Seloto	143	18 (13)	2.10 - 4.0	2.62^{a} ± 0.11	82 (57)	0.40 - 46.00	6.60^{a} ± 1.08
Beans							
Long	13	12 (92)	2.4	1.53 ± 0.15	10 (77)	0.1	0.1 ± 0.00
Sabilo	13	n.d	n.d	n.d	n.d	n.d	n.d
Seloto	12	n.d	n.d	n.d	n.d	n.d	n.d

- Values are means of total aflatoxin and fumonisin levels of positive maize and beans samples from each village.
- Positive samples are all analysed samples with value > Limit of detection (LOD)
- n is the total number of analysed samples
- Means with different letters (by column)are significantly different ($P<0.05$)
- n.d means aflatoxin and fumonisin levels were below LOD

2.4.4 Agronomic practices associated with aflatoxin and fumonisin levels in maize and beans

The occurrence of aflatoxin in maize was significantly associated with four practices; namely, early planting (Nov- December), medium altitude low rain zone, hand hoe tillage and ox tillage (Table 2.5). Results of parameter estimate from the regression model indicated that medium altitude low rain zone (Sabilo village) was positively associated with aflatoxin contamination (0.26), whereas early planting (-0.22), hand hoe tillage (-0.59) and ox tillage (-0.55) were negatively associated with aflatoxin contamination.

Occurrence of fumonisin in maize was negatively associated with three factors; high altitude high rain zone (Long village), medium elevation low rain (Sabilo village), hand hoe and ox tillage. Parameter estimates from the regression model indicate that the high altitude high rain zone was the major factor reducing fumonisin contamination (-2.93) followed by medium altitude low rainfall (-1.69) and hand hoe and ox tillage (-0.79) (Table 2.6). For aflatoxin and fumonisin contamination in beans, the results indicated that no climatic zone or agronomic practice was statistically significant for effects on aflatoxin or fumonisin levels.

Table 2.5: Agronomic practices significantly associated with aflatoxin contamination in maize (Y) tested across three villages

Practices	Estimates (standard error)	P value
Intercept	1.678(0.261)	0.0001*
X_1	-0.216(0.108)	0.0457*
X_2	0.260(0.115)	0.0243*
X_3	-0.587(0.288)	0.0417*
X_4	-0.548(0.252)	0.0297*

For Aflatoxin Y = 1.68 - 0.22 X_1+ 0.26 X_2- 0.59 X_3 -0.55 X_4; where X_1 represents early planting; X_2 represents medium attitude low rain zone (Sabilo); X_3 represents hand hoe tillage; X_4represents ox tillage.
* = Statistically significant at $P < 0.05$

Table 2.6: Agronomic practices significantly associated with fumonisin contamination in maize (Y) tested across threevillages

Practices	Estimates (standard error)	P value
Intercept	3.8592(1.5375)	0.0121*
X_1	-2.9297(0.5664)	0.0001*
X_2	-1.6876(0.5910)	0.0043*
X_3	-0.7946(1.5773)	0.0031*

For Fumonisin $Y = 3.86 - 2.93\ X_1 - 1.69\ X_2 - 0.79\ X_3$; where X_1 represents high altitude (Long); X_2 represents medium altitude (Sabilo) and X_3 represents hand hoe and ox tillage.
* = Statistically significant at $P < 0.05$

2. 5 Discussion

2.5.1 General aflatoxin and fumonisin contamination in maize

The maximum concentration of 26.2 µg/kg total aflatoxin (Table 2.3) was higher than MTL of 10 µg/kg by East African standards (EAC, 2011a). The same concentration was lower than 158 µg/kg reported by Kimanya *et al.* (2008) from other areas of Tanzania. It was also lower than the overall levels of 136.8 and 139.8 µg/kg reported in 1994 and 1995 respectively by Setamou *et al.* (1997) from Benin and 138 µg/kg reported by (Bankole *et al*., 2003) from Nigeria. The higher aflatoxin concentration observed in samples from high altitude high rain zone (Long village) to mid altitude high rain zone (Seloto village) could be due to environmental characteristics of the different climatic zones that affect mycotoxin contamination (Section 2.3.1 and Section 2.5.3.1). Relatively low levels of aflatoxins in this study may be due to high fertiliser appplications, particularly sufficient levels of nitrogen (N), which are known to be important in reducing the risks of fungal infection and the development of mycotoxins in the field crops by reducing plant stress and improving immunity against fungal infection and mycotoxin production (Bruns, 2003).

The maximum concentration of 46 mg/kg total fumonisin (Table 2.3) was higher than the maximum tolerable limit in East African Community of 2 mg/kg (EAC, 2011a) and also higher than 11.048 mg/kg reported by Kimanya *et al.* (2008) from other areas of Tanzania

and 21.8 mg/kg reported by Ncube *et al*. (2011) in South Africa, 1.78 mg/kg reported by (Bankole *et al.*, 2003) in Nigeria and 10 mg/kg by Atukwase *et al*. (2009) in Uganda. Thus, 35% (153/440) of the samples were not fit for human consumption because they contained fumonisin above the MTL. The high levels of contamination of the samples with fumonisin might also be attributed to environmental conditions of low temperature as previously recorded temperature in the study area was found to range from 12^{o}C to 25^{o}C, this low tempereature was reported to favour growth of *F. verticillioides* (Fandohan *et al*., 2003).

2.5.2 General aflatoxin and fumonisin contamination in beans

The maximum concentration of 3 µg/kg total aflatoxin (Table 2.3) was lower than MTL of 10 µg/kg set by East African Community for dry beans (EAC, 2011b) and 4 µg/kg set by European Union regulations, this maximum concentration was also lower than 1463 μg/kg one of the highest levels recorded and reported by Aiat (2006) from Egypt, and 154.9 μg/kg reported by Nyinawabali (2013) from Rwanda. It was higher than 0.02 µg/kg reported by Tseng *et al.* (1995) from Taiwan and Ontario, Canada. Ghosia and Arsha (2012) from Pakistan observed that 10% of red kidney beans were contaminated above the suggested limit set by European Union regulations for total aflatoxins (European Commission, 2010).

The fumonisin concentration was below the LOD of 0.3 mg/kg (Table 2.3), this value was much lower than MTL of 2 mg/kg by East African Standards (EAC, 2011b), also lower than 7.1 mg/kg reported in Rwanda by Nyinawabali (2013) and 1.8 mg/kg for FB_1 reported by Tseng *et al.* (1995) from Taiwan and Ontario Canada. All samples were considered fit for human consumption because they contained aflatoxin and fumonisin below the permissible levels.

The low aflatoxin and fumonisins levels could be attributed to environmental characteristics of the different climatic zones and different agricultural practices which have shown to have an impact on aflatoxin development (Section 2.3.1 and Section 2.5.3.1). Stössel (1986) reported that soy bean seed coat and integrity acts as a barrier against fungal attack and hence mycotoxins contamination, other factors being constant, this might be the reason for low levels of aflatoxin and fumonisin reported in beans samples from this study.

2.5.3 Effect of Agriculture practices and climatic zones on contamination of maize and beans with aflatoxin and fumonisin.

2.5.3.1 Climatic zone

High altitude high rain zone was identified as the most important factor reducing fumonisin contamination in maize with a parameter estimate of -2.93, this means that per each one unit decrease in the predictor/independent variable (in this case high altitude high rain zone) there was a decrease in response/dependent variable (in this case fumonisin levels) by -2.93. This was followed by mid altitude low rain zone with a parameter estimate of -1.69 and hand hoe and ox tillage with a parameter estimate of -0.79 (Table 2.6). Maize from the high altitude high rain zone had significantly lower aflatoxin and higher fumonisin content. The findings from this study are comparable to those reported by Kaaya *et al.* (2006) that maize from mid altitude moist zone had highest mean aflatoxin levels of 9.7 µg/kg, followed by mid altitude dry zone with a mean level of 7.7 µg/kg and high altitude zone with a mean level of 3.9 µg/kg, the findings were also comparable to the findings reported by Atukwase *et al*. (2009) with high mean fumonisin levels of 4.93 mg/kg from the high altitude and 4.53 mg/kg from mid altitude moist zone.

All maize samples were found to have aflatoxin levels below the maximum permissible levels of 10 µg/kg (European commission, 2010; EAC, 2011a), while 35% (153/440) of the maize samples had fumonisin levels above the maximum permissible levels of 2 mg/kg (FDA, 2001; EAC, 2011a). These levels of aflatoxins and fumonisins in maize obtained from the study area could be due to the prevailing environmental conditions during the production period (as per climatic data obtained from Babati District office), which consisted of relatively high rainfall, high altitude and relatively low temperatures favourable for the growth of *Fusarium* and production of fumonisin and unfavourable for the growth of *Aspergillus* and production of aflatoxin. Optimum conditions for aflatoxin production is a temperature of 33 °C and water activity of 0.99 (Milani, 2013), while for fumonisins production is a temperature of 15-30^{o}C and water activity of 0.9 - 0.995. The previously recorded temperature in the study area was found to range from 12^{o}C in Long village to 25^{o}C in Seloto village. Magan *et al*. (2014) showed that high temperatures favoured the proliferation of *A. flavus* and the elaboration of aflatoxin in maize prior to harvest.

2.5.3.2 Planting time

Time of planting had direct influence on the contamination of grain by aflatoxin, fumonisin and other mycotoxins. Maize planted at the end of November to December (early planting) had low levels of aflatoxin contamination with a parameter estimate of -0.22 (Table 2.5) compared to the maize planted in early January to February (late planting). A study conducted by Abbas *et al.* (2007) in Arkansas USA found that maize planted in mid-April resulted in lower aflatoxin and fumonisin contamination than did the early-May planting date, with the average temperature and rainfall during early planting of 33.0^{o}C and 680 mm respectively, while for late planting the average was 30.5^{o}C and 500 mm respectively. Several other studies on the effect of planting date

reported the same trend (Jones *et al*., 1981; Lillehoj*et al*., 1978). This is due to the fact that early planting reduce the levels of aflatoxin and fumonisin contamination by shifting the period between when the flower is fully open and functional (anthesis) and dough-development in maize to a time frame in growing season when maize are less susceptible to drought and heat stress as compared to late plantings (Zuber and Lillehoj, 1979).

2.5.3.3 Land tillage method

Tillage methods especially a hand hoe and ox was found to reduce the levels of aflatoxin contamination in maize compared to combination of hand hoe and tractor tillage, practiced by very few farmers. Parameter estimates from the regression model indicated that hand hoe tillage (−0.59) and ox tillage (-0.55) (Table 2.5) were the major factors reducing the aflatoxin contamination of maize, this means that for each one unit change in the predictor/independent variable (hand hoe tillage and ox tillage) there was a decrease in response/dependent variable (aflatoxin) by -0.59 and -0.55 respectively.While a combination of hand hoe and ox was found to reduce fumonisin levels in comparison to combination of hand hoe and tractor (Table 2.6). This is due to the fact that soil quality highly depends on factors such as soil structure, natural productivity and human influence, and tillage method is one of the major management practices affecting soil physical parameters (Janusauskaite *et al.,* 2013), as *A. flavus* sits on soil surface and jumps up to maize ears due to rain splash or wind, if this population is submereged due to tillage it is not able to contaminate the crop. The same also applies to Fusarium inoculum which is pushed deeper in soil and cannot contaminate grains or pods as cannot reach soil surface. Helgason *et al*. (2009) found that both bacteria and fungi were more abundant under no tillage than conventional tillage. This indicates that there is a high possibility of fungal growth and mycotoxins production in a poorly administered tillage or under no tillage than in a conventional tillage. Steinekellner and Langer (2004) observed that the

deeper the tillage the lower was the number of the isolated *Fusarium* spp. They also reported higher diversity of *Fusarium* spp in conservation tillage than in moldboard plough-based tillage system.

2. 6 Conclusion

Results indicated that some of the production practices used by farmers as well as environmental conditions prevailing in the production area predisposed maize and beans to aflatoxin and fumonisin contamination. Since it is not easy to control the environmental conditions, maize and bean farmers should adopt good agricultural practices that can reduce fungal colonization and mycotoxins contamination; these include timely planting, proper land tillage, fertilizer applications, weeding, pest and disease control practices, use of aflatoxin biocontrol, and removal of any visible unhealthy crops to protect the remaining healthy ones and good crop residue management.

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CHAPTER THREE

3.0 The Influence of Storage Practices on Aflatoxin and Fumonisin Contamination of Maize and Beans in Babati District Tanzania

Chacha J. Nyangi, [1, 2*] Jovin K. Mugula[1], Fen Beed[2], Simon Boniphace[2], Koyano Emmanuel[2]

[1]*Department of Food Science and Technology, Sokoine University of Agriculture, P.O.Box 3006, Morogoro, Tanzania*

[2]*International Institute of Tropical Agriculture, P.O.Box 34441, Dar es salaam, Tanzania.*

3.1 Abstract

Aflatoxin and fumonisin levels were determined in a total of 574 maize and 106 beans samples collected from 60 farmers' stores in three villages over a period of 180 days in Babati district of Tanzania. Quantification for total aflatoxin and fumonisin was done using ELISA (Reveal AccuScan®Neogen, USA), and the results were confirmed by LC-MS/MS. Maize samples from mid altitude high rain zone (Seloto village) had the highest aflatoxin mean value of 3.24 µg/kg. Factors associated with higher aflatoxin and fumonisin levels were storage duration and storage facilities. Polypropylene bags without any storage treatment had higher risk of aflatoxin development with a mean value of 3.57 µg/kg, while the use of improved storage bags lowered aflatoxin levels. Aflatoxins levels were below the acceptable limit for human consumption, while 11% of the maize samples had fumonisin levels above the acceptable limit, implying a risk to consumers' health.

Key words: *aflatoxins, fumonisins, maize, beans storage practices*

**Corresponding author. Tel: +255-764-775727. Email address:nyangichacha@yahoo.com*

3.2 Introduction

Mycotoxins are secondary metabolites elaborated by moulds in a range of plant products. In study area maize is generally harvested late and is stored in grain form in wooden granaries, mud silos, or in polypropylene bags. Most of these systems create inadequate storage conditions unfavourable for good drying of maize, particularly in humid and semi-humid zones, consequently this promote fungal infection and subsequent production of mycotoxins. Most important mycotoxigenic fungi mostly found associated with stored maize and other products are *Aspergillus flavus* that produces aflatoxins (Wagacha and Muthoni, 2008; Okoth *et al.,* 2012), and *Fusarium verticillioides* (previously known as *F. moniliforme*), which produces fumonisins (Omar, 2013; Nyinawabali, 2013).

Mycotoxins production depends on climate, plant and storage-associated problems, the bio-availability of micronutrients, insect damage, and other attack from other pests, as well as storage length, type of storage structure, hygiene and insect infestation. All these factors interact and influence fungal infection and mycotoxin contamination hat are in turn determined by climatic conditions (Orsi *et al.,* 2000; Fandohan *et al*., 2005; Milani, 2013).

At the post-harvest stage, proliferation of aflatoxin and fumonisin can be exacerbated in susceptible commodities under storage conditions such as hot and humid storage environment (Omar, 2013). Hell *et al*. (2000) found higher aflatoxin levels when maize was stored under or on top of the roof of farmers' houses, than in ventilated granaries. Aflatoxin contamination during storage has been related to insect infestation (Udoh *et al.*, 2000), long-term storage (Orsi *et al.*, 2000; Egal *et al*., 2005), high temperature and drought conditions (Kaaya and Kyamuhangire, 2006). Hell *et al*. (2010) found the

improved bags to control mycotoxins levels and insect infestation without the use of chemicals.

The aim of this study was to establish the effect of storage facilities, storage condition and post-harvest handling practices on the occurrence of aflatoxins and fumonisins in maize and beans cultivation and consumption areas in a bid to recommend storage facilities and practices that can be adopted by subsistence farmers to reduce aflatoxin and fumonisin and enhance food safety.

3.3 Materials and Methods

3.3.1 Study area

The study was conducted in three villages namely Long, Sabilo and Seloto in Babati District, Manyara Region, Tanzania. The high altitude high rain zone representing Long village lies between 2150 and 2450 metres above sea levels (m.a.s.l) and characterised by relatively high annual rainfall of 1200 mm. The mid altitude low rainfall zone representing Sabilo village lies between 1500 and 1850 m.a.s.l characterised by relatively low rainfall of 900 – 1100 mm, while the mid altitude high rain zone representing Seloto village lies between 1850 – 2150 m.a.s.l and characterised by relatively annual rainfall of 1100 – 1200 mm. The villages were purposively selected as they represented different climatic zones and also they fall under USAID's Feed the Future priority research area, where the Africa RISING Eastern and Southern Africa project on sustainable intensification of farming systems is being implemented in collaboration with International Institute of Tropical agriculture (IITA). Maize and beans are also the major staple food in the study sites and Tanzania as a whole.

3.3.2 Selection of farmers

Twenty farmers were randomly selected from a list of 150 farmers generated by respective village's extension officers and previously used in collecting at harvest maize and beans samples, this ensured that the same samples were followed to the post harvest stage (storage steps). The selected farmers were supposed to provide 350 kg of maize to be stored in their household for at least 6 months. They were also required to store beans. Each farmer provided three maize samples per survey as each had three different storage facilities to collect samples from, these were; improved bags (Super grain safe bags), traditional storage (cribs/polypropylene bags) and control facilities (polypropylene bags) in which no treatment was applied. Each farmer also provide one bean sample from own storage facilities.

3.3.3 Sample collection

Samples were collected from farmers' traditional storage facilities (i.e., farmers' own storage facilities, either granary or polypropylene bags), improved storage facilities (promoted by International Institute of Tropical Agriculture, IITA-Tanzania) and control (polypropylene bags in which no any storage treatment was applied). Samples were collected at an interval of 0, 40, 80 and 180 days. The 20 randomly sampled farmers per village were interviewed using a semi-structured questionnaire (Appendix 1). Responses were elicited on farmers' storage practices, storage structures, pest problems in storage, storage treatment, storage form, length of storage, sorting, source of samples and farmers' solutions to these problems. Global Positioning System (GPS) coordinates and basic demographic details of farmers/producer were also collected. Responses from the farmers were used to evaluate storage practices and handling techniques.

One sub-sample was drawn from each storage facility, if there was more than one package of the same lot as explained by the interviewee, then the sub-samples were mixed to have approximately 1kg of each sample that will be a good representative of samples. Farmers with two lots of crop, say a good lot for human consumption as food and another lot of especially bad/sorted for livestock or other uses, two separate samples were taken. The samples were then placed in a clean paper bag (A4 envelope) provided, this was then well sealed, labelled and immediately transported to plant pathology laboratory of IITA-Dar es salaam, Tanzania. The collected samples were then dried at 65^{o}C for 72 hours in a cabinet drier to attain a moisture content of less than 13%.

3.3.4 Quantification of total aflatoxin and fumonisin

The samples were ground using a Bunn grinder (Man: Bunn-O-Matic Corporation Springfield, Illinois, U.S.A), homogenized, and sub divided to obtain a representative sub-sample for analysis (https://www.extension.iastate.edu/NR/rdonlyres/52E2F1B9-AC0C-4AE5-8096-25B9921348AB/0/USDAAflatoxinHandbook.pdf). A 50g sub-sample was taken from each of the ground samples and extracted with 250 mL mixture of ethanol/water (65:35, v/v) and shaken vigorously at 150 revolution per minute (rpm) for 3 minutes using a laboratory shaker (IKA® Werke, Germany). Extracts were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). Then total aflatoxin (µg/kg) and fumonisin (mg/kg) were quantified following the manufacturer's protocol using Reveal AccuScan® III reader (Neogen, USA), a quantitative ELISA-based analytical test kits designed specifically for either aflatoxins or fumonisin (http://www.gipsa.usda.gov/lawsandregs/bulletins/pn12-03.pdf). The detection limit for total aflatoxin was 2 µg/Kg with a quantitation range of 2 - 150 µg/Kg and that for total fumonisin was 0.3 mg/Kg with a quantitation range of 0.3 - 6 mg/Kg. The analytical quality of the ELISA methods was assured by the use of certified reference

material (CRM), a naturally contaminated maize sample with certified total aflatoxin content of 18.1 ± 3.6 μg/kg and total fumonisin content of 4.2 ± 0.6 mg/kg supplied by Neogen, USA. For the purpose of data analysis, non-detect levels were based on the detection limits (LOD) of the test method for each toxin

Confirmatory test was done using Liquid chromatography tandem mass spectrometry (LC-MS/MS) at the Interuniversity Department of Agro biotechnology (IFA-Tullin, Austria), on 60 highly contaminated samples previously analysed using Reveal AccuScan® III reader (Neogen, USA) at the plant pathology laboratory of IITA-Tanzania. The results indicated a correlation between the two methods for both total aflatoxin and total fumonisin.

3.3.5 Statistical analysis

Data were analysed using Statistical Analysis System (SAS® Version 9.4, SAS Institute Incorporation, USA). A generalized linear model (GENMOD) was run to identify the factors that significantly affect contamination of maize and beans with aflatoxin and fumonisins. The differences between means were detected using least square means (LSMEANS) to establish differences in mean total aflatoxin and fumonisin amongst the villages, storage facilities and agricultural practices. Aflatoxin and fumonisin levels were transformed using the natural log to normalise the data before analysis.

3.4 Results

3.4.1 Characteristics of farmers

Overall 94% and 6% of the farmers interviewed were males and females respectively. Eighty percent completed primary education, 8% had secondary education, and five percent had tertiary education, with 7% having no formal education. Results for demographic data of all respondents are presented in Table 3.1.

Table 3.1: Demographic characteristics of farmersacross three villages

Characteristics	Total samples (n = 576) (%)	Surveyed villages Long (n = 196) (%)	Sabilo (n = 194) (%)	Seloto (n = 186) (%)
Sex				
Male	544 (94)	164 (84)	194 (100)	186 (100)
Female	32 (6)	32(16)	0 (0)	0 (0)
Education				
Primary	457 (80)	159 (81)	176 (91)	122 (66)
Secondary	48 (8)	10 (5)	8 (4)	30 (16)
Tertiary	27 (5)	17 (9)	0 (0)	10 (5)
None	44 (7)	10 (5)	10 (5)	24(13)
Awareness				
Yes	517 (90)	196 (100)	135 (70)	186 (100)
No	59 (10)	0 (0)	59 (30)	0 (0)
Health problem				
Yes	0 (0)	0 (0)	0 (0)	0 (0)
No	576 (100)	196 (100)	194 (100)	186 (100)

n = Number of samples collected.
(%) = percentage of farmers responded

3.4.2 Storage practices used by farmers in the study area

3.4.2.1 Storage structures

Traditional storage structures in all three villages were almost similar, the commonly used being locally made granaries known as ‘Vihenge’ in Kiswahili. It is made of wooden and woven with twigs or bamboo from surrounding forests and covered with thatch grass or iron sheets and sometimes kept inside a house, and also polypropylene bags. The improved storage facilities known as Super grain safe bag is a penetration-resistant model and gas-tight storage solution for a vast range of dry agricultural commodities was also used (Table 3.2).

3.4.2.2 Sorting

Sorting was another postharvest handling practiced by farmers in all three villages. Sorting was done manually by removing physically damaged and infected grains based mainly on coloration, physical damage and those infected with mold from the intact

commodity. Most of the famers sorted their maize before shelling and after they were properly dried. The sorted bad portion was mainly used as animal feeds and few farmers were using the portion for food after they dehull and mill the bad sorted portion mixed with sorghum to obtain flour (Table 3.2).

3.4.2.3 Drying

Drying of maize and beans was mainly done on the bare ground or on a raised platform. The raised platform was constructed with medium sized pieces of trees and at a height of approximately one metre above the ground, and constructed outside and well protected against animals. Ninety nine percent of farmers dried their maize on bare ground and 1% dry the maize on platform (Table 3.2).

3.4.2.4 Stores treatment

Twenty three percent of the farmers in the study area treated their stores against insects' infestation before introducing crops to be stored. Store treatment was done by using either chemical pesticides or natural protectants. Chemical pesticides were sprayed in the store especially on walls, floor and ceiling before introducing crops to be stored, this was done by 16% of the responded farmers. The common pesticides used were Actelic (pirimiphos-methyl) and Bami force (permethrin and malathion). An alternative treatment involved the use of natural protectants to smear the granaries/cribs prior to introduction of crops, and this comprised a mixture of dried, ground plant leaves combined with burnt cowdung and sometimes ashes, this was found to be practiced by 7% of the farmers (Table 3.2).

3.4.2.5 Grain treatment

Grains ready to be stored, whether maize or beans, were treated before being introduced into the storage facilities. Seventeen percent of the farmers used chemical pesticides which were specific formulation for stored grains such as Super Shumba (pirimiphos-

methyl and permethrin), Actellic (pirimiphos-methyl), Bami force (permethrin and malathion) or zinc phosphate, in most cases chemical pesticides were applied once during the storage period and in few cases it was applied twice depending on the length of storage and the extent of insects infestation. Seven percent of the farmers applied traditional protectants (Table 3.2).

3.4.2.6 Storage pests

There was no insect infestation in the maize stored in improved bags (Super grain safe bags) during the entire storage period, while the control storage facility had the highest levels of insect infestation as no insecticides or other storage treatment like regular sundrying was applied. In all three study villages the common pest infesting maize was identified as *Sitophillus zeamaiz* (Table 3.2).

3.4.2.7 Storage with other crops

The most common crops that are usually stored alongside maize were beans and few farmers stored maize with wheat, sunflower and pigeon pea. All farmers in the three villages usually cleaned their stores and removed all previous crop residues from the store before introducing new harvest (Table 3.2).

Table 3.2: Storage practices practiced by farmers across three villages

Practices	Total samples (n = 576) (%)	Surveyed villages: Long (n = 196) (%)	Sabilo (n = 194) (%)	Seloto (n = 186) (%)
Storage structures				
Improved bags	179 (31)	60 (31)	60 (31)	59 (32)
Polypropylene bags	167 (29)	71 (36)	43 (22)	53 (28)
Cribs/granaries	54 (9)	6 (3)	32 (17)	16 (9)
Control	176 (31)	59 (30)	59 (30)	58 (31)
Storage pests				
Insects	92 (16)	36 (18)	26 (13)	30 (16)
Insects and rodents	79 (14)	14 (7)	37 (19)	28 (15)
No pests	405 (70)	146 (75)	131 (68)	128 (69)
Remove crop residue				
Yes	573 (99)	196 (100)	191 (98)	186 (100)
No	3 (1)	0 (0)	3 (2)	0 (0)
Storage with other crops				
Yes	270 (47)	76 (39)	89 (46)	104 (56)
No	306 (53)	119 (61)	105 (54)	82 (44)
Stores treatment				
Chemical spray	93 (16)	32 (16)	11 (6)	50 (27)
Traditional pedticides	40 (7)	7 (4)	24 (13)	9 (5)
Not treating stores	443 (77)	157 (80)	159 (81)	127 (68)
Grain treatment				
Chemical pesticides	74 (13)	43 (22)	15 (8)	16 (9)
Traditional pesticides	39 (7)	6 (3)	26 (13)	7 (4)
No use of pesticides	463 (80)	147 (75)	153 (79)	163 (87)
Drying method				
On bare ground	568 (99)	188 (96)	194 (100)	186 (100)
On platform	8 (1)	8 (4)	0 (100)	0 (0)
Sorting				
Yes	501 (87)	188 (96)	136 (70)	177 (95)
No	75 (13)	8 (4)	58 (30)	9 (5)

n = Number of samples collected.
(%) = percentage of farmers responded.

3.4.3 Total aflatoxin and fumonisin content in maize and beans

Results obtained from farmers storage structures during the storage period indicated that 27% and 45% of maize samples were contaminated with aflatoxin and fumonisin, while for beans it was 34% and 7% respectively. The range of aflatoxins and fumonisins

concentrations for maize and beans is reported in Table 3.3. The highest aflatoxin mean value of 3.24 µg/kg was found in Seloto village and for fumonisin the highest mean value of 3.11 mg/kg was found in Sabilo village while for beans the highest aflatoxin mean value of 3.74 µg/kg was found in Sabilo village and for fumonisin the highest mean value of 9 mg/kg was found in Long village (Table 3.4).

Table 3.3: Occurrence/prevalence of aflatoxin and fumonisin in maize and beans across three villages

Maize	**n**	**Positive sample (%)**	**Range**	**Mean ±SE**
Aflatoxin (µg/kg)	574	155 (27)	2.1 – 10.1	3.12 ± 0.09
Fumonisin (mg/kg)	574	257 (45)	2.1 – 90	0.68 ± 0.20
Beans				
Aflatoxin (µg/kg)	106	36 (34)	2.1 – 14.2	3.34 ± 0.34
Fumonisin (mg/kg)	106	7 (7)	0.4 – 9.00	3.81 ± 1.47

- Values are means of total aflatoxin and fumonisin levels for positive maize and beans samples stored in different storage structures.
- n = total number of samples analysed
- Means values are for all analysed samples.
- Positive samples are all analysed samples with values > Limit of detection (LOD)

Table 3.4: Prevalence, range and mean total aflatoxin and fumonisin content in maize and beans in each village

Village		**Aflatoxin (µg/kg)**			**Fumonisin (mg/kg)**		
	n	**Positive sample (%)**	**Range**	**Means ± SE**	**Positive sample (%)**	**Range**	**Means ± SE**
Long	196	75 (38)	2.1 - 4.9	3.04^{a}± 0.09	10 (0.05)	0.10 - 3.30	1.02^{a} ± 0.27
Sabilo	193	28 (15)	2.1 - 4.6	3.12^{a} ± 0.17	97(50)	0.4 - 90.00	3.11^{b}± 1.16
Seloto	185	52 (28)	2.1 – 10.1	3.24^{a} ± 0.20	49 (26)	0.4 – 5.20	1.01^{a}± 0.13
Beans							
Long	36	36 (100	0.4 – 4.6	2.22^{a} ± 0.21	1(3)	0.90 – 9.00	9.0^{a} ± 0.25
Sabilo	37	10 (27)	2.10- 3.00	2.64^{a} ± 0.09	27 (57)	0.00 – 0.2	0.08^{a} ± 0.01
Seloto	33	11 (33)	2.10 – 14.2	3.74^{b} ± 1.07	6 (18)	0.40 – 7.90	2.95^{a} ± 0.31

- Values are means of total aflatoxin and fumonisin levels for positive maize and beans samples from each Village.
- Means with different letters (by column) are significantly different ($P<0.05$).
- Positive samples are all analysed samples with value > Limit of detection (LoD)
- n is the total number of analysed samples

Maize samples collected from polypropylene bags used as control storage facility had the highest levels of aflatoxins with a mean value of 3.57 µg/kg while those collected from Super grain safe bags (Improved) had the lowest mean levels of 2.38 µg/kg. For Fumonisins, samples collected from traditional cribs/granaries (*Vihenge*) had the highest mean value 5.71 mg/kg while those collected from polypropylene bags used by farmers had the lowest levels of 1.19 mg/kg. There were statistical significant differences in aflatoxins and fumonisins levels from maize samples collected in different storage facilities as shown in Table 3.5.

Table 3.5: Prevalenve, range and mean total aflatoxin and fumonisin content in maize and beans stored in different storage structures across three villages

		Aflatoxin (µg/kg)			Fumonisin (mg/kg)		
Maize Storage structure	**n**	**Positive sample (%)**	**Range**	**Means ± SE**	**Positive sample (%)**	**Range**	**Means ± SE**
Improved	178	41 (23)	2.1 - 4.7	2.38^{a}± 0.11	46 (26)	0.40 - 11.00	1.42^{a}± 0.25
POP bags	166	41 (25)	2.2 - 10.1	3.30^{b} ± 0.22	43 (25)	0.40 - 5.20	1.19^{a}± 0.17
Granaries	54	11 (20)	2.10– 4.7	2.76^{a} ± 0.24	23 (43)	0.40 – 90.00	5.71^{b}± 3.88
Control	176	62 (35)	2.10 – 6.7	3.57^{b}± 0.13	45 (26)	0.40 – 70.00	2.53^{c}± 1.54
Beans							
POP bags	106	36 (34)	2.10 – 14.2	3.34 ± 0.34	7 (7)	0.40 – 9.00	3.81 ± 1.47

- Values are means of total aflatoxin and fumonisin levels for positive maize and beans samples stored in different storage structures.
- Means with the different letters (by column) are significantly different (P<0.05).
- Positive samples are all analysed samples with value >Limit of detection (LOD)
- POP represents polypropylene bags commonly used as a storage facility.
- n is the total number of analysed samples

The results from the storage duration for maize indicated that the mean aflatoxin levels increased from day 0 to day 180. The observed increase was statistically significant in day 180 from the rest of the storage period (P< 0.05). The mean fumonisins level decreases during the entire storage period (day 0 to day 180) and the decrease was statistically significant (Table 3.6).

Table 3.6: Prevalence, range and mean total aflatoxin and fumonisin content in maize and beans during storage across three villages

		Aflatoxin (µg/kg)			Fumonisin (mg/kg)		
Maize storage in days	**n**	**Positive sample (%)**	**Range**	**Means ± SE**	**Positive sample (%)**	**Range**	**Means ± SE**
Day 0	60	9 (15)	2.1 - 4.4	2.69^{a}± 0.25	28(46)	0.40 - 90.00	4.42^{a}± 3.17
Day 40	178	60 (33)	2.1 - 4.7	2.92^{a}± 0.09	51(29)	0.40 - 70.00	3.03^{b}± 1.36
Day 80	176	46 (26)	2.1 – 4.9	2.92^{a} ± 0.12	48 (27)	0.40 – 14.00	1.24^{c}± 0.29
Day 180	174	19 (11)	2.1 – 10.1	3.89^{b} ± 0.46	35 (20)	0.40 – 3.10	0.80^{d} ± 0.09
Beans							
Day 120	55	7 (13)	2.1–4.50	2.73^{a}± 0.32	4(7)	0.40 – 7.90	3.93^{a}± 1.99
Day 160	51	29 (57)	2.1–14.2	3.49^{b}± 0.41	3(6)	0.90 – 9.00	3.67^{a}± 2.67

- Values are means of total aflatoxin and fumonisin levels for positive maize and beans samples
- Means with different letters (by column) are significantly different ($P<0.05$).
- Positive samples are all analysed samples with value >Limit of detection (LOD)
- n represents total number of all analysed samples

Effect of sorting of bad or moulded maize in the stored lot indicated that the bad portion had higher levels for both aflatoxins and fumonisins with means of 3.23 µg/kg and 14.45 mg/kg, respectively, compared with the good portion which had a mean aflatoxin and fumonisins levels of 2.69 µg/kg and 5.30 mg/kg respectively (Table 3.7).

Table 3.7: Mean total aflatoxin and fumonisin content in sorted maize across three villages

		Aflatoxin (µg/kg)			Fumonisin (mg/kg)		
Sorting	**n**	**Positive sample (%)**	**Range**	**Means ± SE**	**Positive sample (%)**	**Range**	**Means ± SE**
Good portion	52	9 (17)	2.1 - 4.4	2.69^{a}± 0.25	22 (42)	0.40 - 90.00	5.30^{a}± 4.04
Bad portion	40	11 (28)	2.1 - 4.7	3.23^{a} ± 0.31	21 (52)	0.20 - 62.00	14.45^{b} ± 3.69

- Values are means of total aflatoxin and fumonisin levels for positive maize samples
- Means with different letters (by column) are significantly different ($P<0.05$).
- Positive samples are all analysed samples with value >Limit of detection (LOD)
- n represents total number of all analysed samples

3.4.4 Storage practices significantly associated with aflatoxin and fumonisin in maize and beans

Occurrence of aflatoxin in maize during storage was correlated with only four practices/factors that were storage at day 0, day 40, day 80 and storage with other crops. Sorting, storage of maize in improved storage bags, and the use of traditional protectant as pesticides were the practices/factors negatively associated with aflatoxin contamination. Parameter estimates from the regression model indicated that sorting (-0.24) was the major factor reducing the contamination of maize to aflatoxin (Table 3.8).

Occurrence of fumonisin in maize was associated with three practices/factors; sorting, use of chemical pesticides, storage in cribs/granaries and storage of maize with other crops in the same store (Table 3.9). Parameter estimates from the regression model indicated that sorting (-0.53) was the major factors reducing fumonisins levels. For aflatoxins and fumonisins contamination in beans, the results indicated that there was no any predictors from the regression model that was statistically significant.

Table 3.8: Storage practices/factors significantly associated with aflatoxin contaminationin maize (Y) across three villages

Practices/variables	Estimates (standard error)	P value
Intercept	0.16 (0.08)	0.0339*
X_1	-0.24 (0.11)	0.0310*
X_2	-0.11 (0.16)	<0.0001*
X_3	0.17 (0.03)	<0.0001*
X_4	0.20 (0.02)	< 0.0001*
X_5	0.25(0.13)	< 0.0001*
X_6	-0.08 (0.04)	0.0288*
X_7	0.04 (0.02)	0.0153*

For Aflatoxin $Y = 0.16 - 0.24\ X_1 - 0.11\ X_2 + 0.17\ X_3 + 0.20\ X_4 + 0.25\ X_5 - 0.08X_6 + 0.04X_7$; where X_1 represents sorting; X_2 represents storage in improved facilities; X_3, $X_4$and X_5represents storage days at 0, 40, and 80 respectively; X_6 represents traditional pesticides; X_7 represents storage with other crops.
* Statistically significant at $P < 0.05$

Table 3.9: Storage practices/factors significantly associated with fumonisins in Maize (Y) across three villages

Practices/variables	Estimates (standard error)	P value
Intercept	0.0026(0.08)	0.9728
X_1	-0.5322(0.2682)	0.0472*
X_2	0.1128 (0.0431)	0.0088*
X_3	-0.0902 (0.0283)	0.0015*
X_4	-0.0391 (0.0163)	0.0166*

For Fumonisin $Y = 0.0026 – 0.532X_1 + 0.1128X_2 – 0.0902\ X_3 – 0.0391X_4$; where X_1 represents sorting; X_2 represents storage in granaries; X_3 represents the use of chemical pesticides; X_4 represents storage of maize with other crops.
* Statistically significant at $P < 0.05$

3.5 Discussion

3.5.1 Aflatoxin and fumonisin contamination in maize

Total aflatoxins levels in maize ranged from 2.1 to 10.1 µg/kg with a mean of 3.12 µg/kg. Thus, only one sample, according to the East African Community standards was not fit for human consumption because the measured aflatoxin concentration was above the MTL of 10 µg/kg. This maximum concentration of 10.1 µg/kg aflatoxins was, however, lower than 221 µg/kg reported by Hell (1997) and 355 µg/kg reported by Kpodo *et al*. (1996) in Ghana.

The maximum concentration of total fumonisin in maize was 90 mg/kg. It was observed that 1% (8/574) of the maize samples were not fit for human consumption according to the USA and EAC standards of 2mg/kg (FDA, 2001; EAC, 2011a). This maximum concentration of fumonisin in maize was higher than the 6.54 mg/kg reported by Queiroz *et al*. (2012) in Brazil; 2.4 mg/kg reported by Fandohan *et al*. (2005) in Benin; 49.31 mg/kg reported by Orsi *et al*. (2000) in the State of São Paulo, Brazil; in 86 stored maize samples in the eastern province of Kenya Bii *et al*. (2012), and exceeded the maximum permitted level of 2 mg/kg (EAC, 2011b). The observed low levels of aflatoxin and higher fumonisin levels could be attributed to environmental and climatic

conditions of low temeperature and high rain from the different climatic zones and different storage practices in the three villages.

3.5.2 Aflatoxin and fumonisin contamination in beans

Total aflatoxin and fumonisin was quantified in 106 beans samples. The highest concentration for total aflatoxin was 14.2 µg/kg. Only one sample had aflatoxin levels above permitted levels by USA and EAC standards of 10 µg/kg (FDA, 2001; EAC, 2011b). Thus about 99.1% of the samples were fit for human consumption according to the EAC and US standards. However, the highest level observed in this study was lower than 21.48 µg/kg reported by Tseng *et al.* (1995) in Ontario, Canada and Taiwan; 154.9 μg/kg reported by Nyinawabali (2013) in Rwanda and 0.02 µg/kg reported by Aiat (2006) in Egypt.

The observed maximum concentration of total fumonisins in beans was 9 mg/kg, this was higher than the limit of 2 mg/kg set by EAC standards (EAC, 2011b). Thus, about 3% (3/106) of samples were not fit for human consumption according to the EAC and USA standards. The observed maximum fumonisin level was higher than 1.8 mg/kg of fumonisin B_1 reported by Tseng *et al.* (1995) in Ontario, Canada and Taiwan; and 7.1 mg/kg reported by Nyinawabali (2013) from Rwanda.

The low aflatoxin levels could be attributed to environmental characteristics of low temeperature and high rain which do not favour aflatoxin formation, but favourable to fumonisin. Also different agricultural practices have shown to have an influence on aflatoxin and fumonisin development (Milani, 2013). Stössel (1986) reported that soy bean seed coat and integrity acts as a barrier against fungal attack and hence mycotoxins

contamination, other factors being constant, this might be the reason for low levels of aflatoxin and fumonisin reported in beans samples from this study.

3.5.3 Storage factors significantly associated with aflatoxin and fumonisin contamination in stored Maize

3.5.3.1 Sorting

Parameter estimates (coefficient) from the regression model used indicated that sorting was one of the practices that reduced aflatoxins (-0.2411) and fumonisins (-0.5322) levels in maize (Table 3.8, 3.9), this means that for each one unit change in the predictor/independent variable (sorting) there was a decrease in response/dependent variable (aflatoxin and fumonisin levels) by -0.2411 and -0.5322 respectively. This observation was comparable to that made by Park (2002) and Afolabi *et al*. (2006) who reported that sorting reduced aflatoxin levels by 40-80%. Kedera *et al.* (1999) reported that poor quality maize grains were correlated with higher levels of fumonisins. Hell and Mutegi (2011) also reported that sorting reduced toxin concentrations to safe levels without the production of toxin degradation products or any reduction in the nutritional value of food. Almost all farmers in the study area cleaned their stores and removed residue from the previous harvest before loading new harvest. This might also help in the management of mycotoxins. Hell *et al*. (2000) observed that cleaning of stores before loading new produce reduced aflatoxins concentration in Benin.

3.5.3.2 Storage structure

The parameter estimate (coefficient) from the regression model (-0.11) indicated that the improved bags were associated with reduction of aflatoxin levels (Table 3.8). These observations were similar to those reported by Hell *et al.* (2010) in Senegal that the improved bags controlled insect infestation and mycotoxins levels without using

chemicals. The Parameter estimate (regression coefficient) from the regression model (0.11) indicated that storage in cribs or granaries was associated with the increase in fumonisin levels (Table 3.9), this means that for each one unit change in the predictor/independent variable (storage in granaries) there was an increase/change in response/dependent variable (fumonisin levels) by 0.11. However, the results are contrary to those reported by Fandohan *et al.* (2005) in Benin who observed a significant decrease in fumonisins in granaries. This might be due the poor ventilation in the structures as some farmers covered the granary with cow dung mixed with mud.

3.5.3.3 Storage length

The parameter estimate from the regression model indicated that short term storage at day 0 (0.17, P<0.0001), day 40 (0.20, P<0.0001) and day 80 (0.25, P<0.0001) predisposed maize to aflatoxin contamination (Table 3.8). These findings were similar to those reported by Hell *et al*. (2000) in Benin that higher aflatoxins levels were associated with short storage period of 3 – 5 months and lower level with longer storage duration of 8 - 10 months.The results of this study were contrary to findings by Liu *et al.* (2006) in China who reported a significant increase in aflatoxins with storage duration, from 0.84 µg/kg in 12 months to 1.17 µg/kg in 24 months. Fandohan *et al*. (2005) reported an increase in aflatoxins levels in all storage systems throughout the storage period (8 months) in Benin. Hell *et al*. (2003) also reported that higher incidence of aflatoxins contamination was observed in maize stored for 6 months compared to the fresh harvested maize at 0 month of storage. Egal *et al.* (2005) reported that aflatoxin contamination was facilitated by long-term storage under unhygienic and non-ventilated conditions in Benin and Togo. Ninety nine percent of farmers in the study area removed residue from the previous crops in the store and 100% % of farmers cleaned the stores before introducing new harvest.

These practices improve hygiene and, in combination with environmental condition may be among the reasons for low levels of aflatoxins and fumonisins during storage.

3.5.3.4 Grain treatment

From the regression model, it was found that local plants mixed with burnt cow dung decreases aflatoxins levels (parameter estimate of -0.08 and P value of 0.0288) (Table 3.8). These results are contrary to those reported by Hell *et al*. (2000) who observed that the mixing of plant substances with stored cobs may increase the risk of aflatoxin development instead of controlling it. Farmers in the study area were using plant materials in a powder form, as natural protectant. Some of the plant parts might prevent mould growth and mycotoxin elaboration. Application of chemical/commercial pesticides such as Super Shumba (pirimiphos-methyl and permethrin), Actellic (pirimiphos-methyl), Bami force (permethrin and malathion) or zinc phosphate reduced fumonisins levels (parameter estimate of -0.0902 and P value of 0.0015).

3.6 Conclusion

Mycotoxin contamination of maize and beans increased with storage duration. Several factors that might facilitate reduction of aflatoxin and fumonisins levels in stored maize and beans in the study area were identified. These included control of storage insects and mycotoxins levels (aflatoxins and fumonisins) through the removal of damaged cobs,sorting, the use of appropriate storage insecticides and use of storage structures (improved bags) which were found to control the levels of aflatoxin, also avoiding long term storage.

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CHAPTER FOUR

4.0 Aflatoxin and Fumonisin Contamination of Maize and Common Bean-Based Market, Processed and Feeds in Babati District, Tanzania

Chacha J. Nyangi, [1, 2 *], Jovin K. Mugula[1], Fen Beed[2], Simon Boniphace[2], Koyano Emmanuel[2]

[1]*Department of Food Science and Technology, Sokoine University of Agriculture, P.O.Box 3006, Morogoro, Tanzania*

[2]*International Institute of Tropical Agriculture, P.O.Box 34441, Dar es salaam, Tanzania.*

4.1 Abstract

Aflatoxin and fumonisin contamination in maize and common bean-based market, processed and feeds was investigated in three different villages in Babati District, Tanzania. Quantification for total aflatoxin and fumonisin was done using ELISA (Reveal AccuScan®Neogen, USA), and the results were confirmed by using LC-MS/MSMaize bran hadhighestlevels of aflatoxins (2.38 µg/kg) and sorted bad portion for animal feeds had the highest fumonisin mean value of 7.42 mg/kg, followed by maizegrains from mills with a mean value of 1.73 µg/kg aflatoxins and maize from market with a mean value of 0.34 mg/kg fumonisins, maize flour had a mean value of 1.42 µg/kg aflatoxins and maize from mills with a mean value 0.3 mg/kg fumonisins. Dehulled maize and maize flour were less contaminated with the mycotoxins. All animal feeds were found to have levels less than the total permissible levels for animal feeds.

Key words: *aflatoxins, fumonisins, market, processors, feed, maize, flour, Tanzania*

**Corresponding author. Tel: +255-764-775727. Email address:nyangichacha@yahoo.com*

4.2 Introduction

Mycotoxins are toxic secondary metabolites produced by various molds frequently contaminate food and feed worldwide (Warth *et al*., 2012). Their incidence depends on various factors, such as the commodity, climatic conditions, agricultural practices, storage conditions, and seasonal variances (Warth *et al*., 2012; Milani, 2013). Two most important mycotoxigenic fungi mostly found associated with stored maize and other products are *Aspergillus flavus* that produces aflatoxins and *Fusarium verticillioides*, which produces fumonisins (Fandohan *et al*., 2003; Wagacha and Muthoni, 2008; Okoth *et al*., 2012; Omar, 2013; Nyinawabali, 2013).

Aflatoxins are acute and chronic toxicity, immunosuppressive, mutagenic, teratogenic, genotoxic and carcinogenic compounds (Filazi and Sireli, 2013; Omar, 2013) with potential to seriously affect human health by induction of hepatocellular carcinoma (HCC) or sudden death (Lewis *et al*., 2005), the source of aflatoxins are from agricultural commodities like cereals such as maize, sorghum, pearl millet, rice and wheat; oil seeds such as groundnut, soybean, sunflower and cotton, milk, meat and dried fruits (Marasas *et al*., 2008; Wild and Gong, 2010). Fumonisins have been linked with esophageal cancer in South Africa (Marasas, *et al*., 2008), stunting and underweight in Tanzania (Kimanya *et al*., 2010), cranial neural tube defects (NTD) a defect of the brain and spinal cord in the embryo that results from failure of the neural tube to close (Blom *et al*., 2006). Fumonisins occur in maize and infrequently in foodstuffs such as sorghum, asparagus, rice, beans and beers (Creppy, 2002; Zain, 2011), as well as feeds especially those formulated using maize (Morgensen *et al*., 2010).

It is estimated that mycotoxins contaminate 25% of agricultural crops worldwide (Zain, 2011), with 4.5 billion people living in developing countries exposed to chronic

toxicity (Williams *et al.,* 2004). This higher exposure is because the population is often consume affected crops as a staple diet and because crops in tropical and subtropical regions are more susceptible to contamination due to favorable climatic conditions (Bankole and Adebanjo, 2003).

Aflatoxins and fumonisins are not uniformly distributed in maize kernels and higher concentrations tend to be found in germ and bran fractions in dry milling due to the presence of the pericarp. The pericarp is the first part of the kernel colonized by moulds because of its peripheral location and also the part to which kernel dusts adhere (Katta *et al*., 1997; Park, 2002; Brera *et al*., 2004). Katta *et al*. (1997) reported that during the dry milling of corn, fumonisin B_1 was found in highest amounts in the bran fraction that is used as animal feed, followed by the germ fraction, which may be used as animal feed or for oil extraction. The same results were also reported by Vanara *et al.* (2009).

The maximum tlorelable limit (MTL) for total aflatoxin levels in animal feeds range from 0 to 50 μg/kg with an average of 20 μg/kg (FAO, 2004), while MTL for total aflatoxins in foodstuffs intended for direct human consumption in East African Commission and the European Union is 10 µg/kg (European Commission, 2010; EAC, 2011a, b) and for USA is 20 µg/kg (FDA, 2001). While for total fumonisin by East Africa standards is 2 mg/kg (EAC, 2011a,b), and for European Union standards is 1 mg/kg (European Commission, 2010), in the USA the limit for total fumonisin is 2 mg/kg for maize products and 4 mg/kg for maize grain food. And for animal feeds the range is from 5 to 100 mg/kg (FDA, 2001).

Very little is known in Tanzania on the levels of contamination of maize and beans with aflatoxins and fumonisins on the market, processed or in animal feeds. The aim of this

study was to investigate the level of contamination in these products The findings of this study might contribute to interventions to be adopted by subsistence farmers and processors to reduce aflatoxin and fumonisin contamination in the products in order to improve food safety of the products.

4.3 Materials and Methods

4.3.1 Study area

The study was conducted in three villages namely, Long, Sabilo and Seloto in Babati District, Manyara Region, Tanzania. The high altitude high rain zone representing Long village lies between 2150 and 2450 metres above sea levels (m.a.s.l) and characterised by relatively high annual rainfall of 1200 mm. The mid altitude low rainfall zone representing Sabilo village lies between 1500 and 1850 m.a.s.l characterised by relatively low rainfall of 900 – 1100 mm, while the mid altitude high rain zone representing Seloto village lies between 1850 – 2150 m.a.s.l and characterised by annual rainfall of 1100 – 1200 mm. The villages were purposively selected as they represented different climatic zones and also they fall under USAID's Feed the Future priority research area, where the Africa RISING Eastern and Southern Africa project on sustainable intensification of farming systems is being implemented in collaboration with International Institute of Tropical agriculture (IITA). Maize and beans are also the major staple food in the study sites and Tanzania as a whole.

4.3.2 Selection of farmers/vendors

The vendors and small scale mill from which samples were collected were selected from two villages of Long and Seloto, as Sabilo village shared the same market and small scale mill with Seloto village. Five vendors were randomly selected from each of the two

market one from Long and one from Seloto village. Samples were also collected from one small scale mill from each of the two villages of Long and Seloto.

4.3.3 Samples collection

Maize and beans samples were randomly collected from the markets, farmers' stores (bad sorted maize) and small scale mills in the three villages. Sampling was carried out in a way that ensured the analytical sample effectively represented the product. Maize grain samples (44) were obtained from the market, sorted/bad portion for animal feed (41) from farmers' households. From the small scale mill the following samples were collected; maize grain (29), maize flour (24), maize bran (20) and dehulled maize (3) making a total of 161 maize based samples. A total of 10 bean samples were also collected from the two villages of Long and Seloto. Animal feed was obtained from sub-samples of bad/sorted portion from farmer's household and maize bran from small-scale mills. Multiple samples were taken from different parts of one bag or several bags belonging to one vendor and combined to produce a 1-kg sample for analysis, using the respective vendor's sampling tools (i.e., scoops). Samples were then placed in a clean paper bag (A4 envelope) provided, this was then well sealed, labelled and transported to IITA plant pathology laboratory, Dar es salaam, where samples were then dried in a cabinet drier at 65^{o}C/ 72 h to < 13% moisture content.

4.3.4 Quantification of total aflatoxin and fumonisin

The samples were ground using a Bunn grinder (Man: Bunn-O-Matic Corporation Springfield, Illinois, U.S.A), homogenized, and sub divided to obtain a representative sub-sample for analysis (https://www.extension.iastate.edu/NR/rdonlyres/52E2F1B9-AC0C-4AE5-8096-25B9921348AB/0/USDAAflatoxinHandbook.pdf). A 50 g sub-sample was taken from each of the ground samples and extracted with 250 mL mixture of

ethanol/water (65:35, v/v) and shaken vigorously at 150 revolutions per minute (rpm) for 3 min using a laboratory shaker (IKA® Werke, Germany). Extracts were filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). Then total aflatoxin (µg/kg) and fumonisin (mg/kg) were quantified following the manufacturer's protocol using Reveal AccuScan® III reader (Neogen, USA), a quantitative ELISA-based analytical test kits designed specifically for either aflatoxin or fumonisin (http://www.gipsa.usda.gov/lawsandregs/bulletins/pn12-03.pdf). The detection limit for total aflatoxins was 2 µg/Kg with a quantitation range of 2 - 150 µg/Kg and that for total fumonisins was 0.3 mg/Kg with a quantitation range of 0.3 - 6 mg/Kg. The analytical quality of the ELISA methods was assured by the use of certified reference material (CRM), a naturally contaminated maize sample with certified total aflatoxin content of 18.1 ± 3.6 µg/kg and total fumonisin content of 4.2 ± 0.6 mg/kg supplied by Neogen, USA. For the purpose of data analysis, non-detect levels were based on the detection limits (LOD) of the test method for each toxin

Confirmatory test was done using Liquid chromatography tandem mass spectrometry (LC-MS/MS) at the Interuniversity Department of Agro biotechnology (IFA-Tullin, Austria), on 60 highly contaminated samples previously analysed using Reveal AccuScan® III reader (Neogen, USA) at the plant pathology laboratory of IITA-Tanzania. The results indicated a correlation between the two methods for both total aflatoxin and total fumonisin.

4.3.5 Statistical analysis

Data were analysed using Statistical analysis System (SAS® Version 9.4, SAS Institute Incorporation, USA). A generalized linear model (GENMOD) was used. The differences between means were detected using least square means (LSMEANS) to establish

differences in mean total aflatoxin and fumonisin amongst the climatic zones (villages), market and small scale mill as well as animal feed. Aflatoxin and fumonisin levels were transformed using natural log to normalise the data before analysis.

4.4 Results

4.4.1 Descriptive results

Two markets, one each in Long and Seloto village and within each market, a total of 5 vendors were interviewed and a total of 43 maize and 10 beans samples were collected. For the samples collected from the small scale mills (processors), all 76 samples (whole maize-29, maize bran - 20, maize flour - 24 and dehulled maize – 3) had a total aflatoxins levels below the maximum tolerable levels (MTL) for East African Community (EAC) and European Union of 10 µg/kg, and fumonisin levels below MTL of 2 mg/kg. Maize bran samples had a mean fumonisin concentration of 1.02 mg/kg, a level lower than the MTL for animal feeds of 20 mg/kg (FAO, 2004). Whole maize grains collected from the small-scale mills, 10% (3/29) of the samples had fumonisins levels above the MTL of 1 mg/kg by EU standards (European Commission, 2010) and 4% (1/29) of the samples had fumonisins level above the MTL of 2 mg/kg for US and EAC standards (FDA, 2001; EAC, 2011a) as indicated in Fig.4.1.

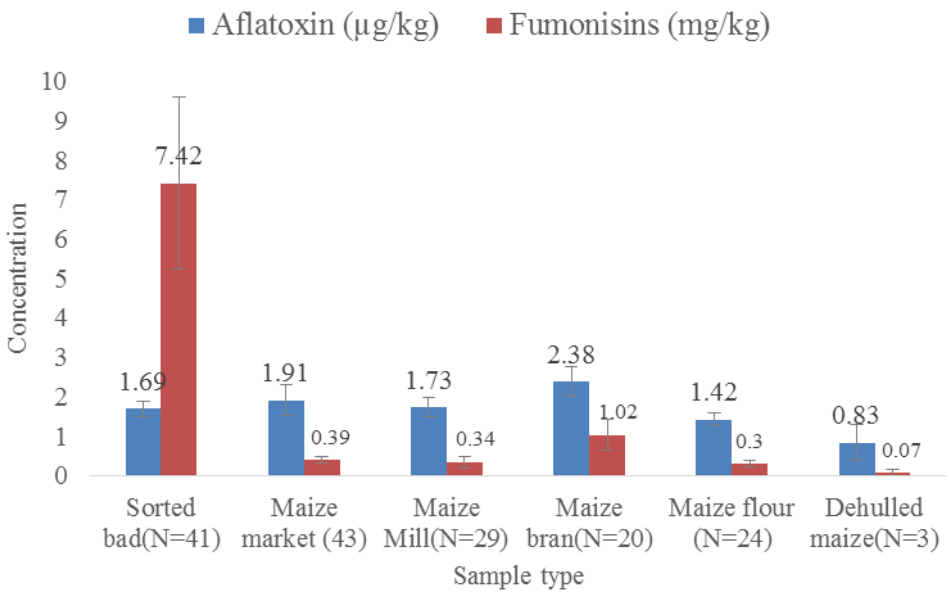

Figure4.1: Mean value for maize-based samples from market, processors and animal feed

Only 2% (1/43) of market maize samples had a total aflatoxin greater than the MTL of 10 µg/kg (European commission, 2010; EAC, 2011a), while all samples had aflatoxin levels below the MTL of 20 µg/kgby US standards (FDA, 2001). About 5% (2/43) of the samples had levels above the MTL of 2 mg/kg (EAC, 2011a). All of the 41 (100%) sorted bad portion maize samples collected from farmers household and 20 (100%) maize bran samples from posho mill, both as animal feed had levels below the MTL for aflatoxin in animal feeds which range from 0 to 50 µg/kg, and average of 20 µg/kg (FAO 2004) and, also had levels below the MTL for fumonisins in animal feeds which range from 5 to 100 mg/kg (FDA, 2001).

4.4.2 Prevalence and mean value for aflatoxin and fumonisin in maize and beans

The overall contamination with total aflatoxins was 32% and 39% for total fumonisins in maize and maize products collected in all three villages, while for beans it was 10% for both total aflatoxin and total fumonisin respectively (Table. 4.1). Maize and maize

product samples collected from Seloto village had the highest mean aflatoxin level of 4.15 µg/kg and for fumonisin the highest mean levels of 31.43 mg/kg was found in Sabilo village (Table. 4.2). Significant differences were found in the mean aflatoxin levels of maize and maize products samples between Long and Seloto village as well as between Sabilo and Seloto village. For fumonisins there was significant differences among the samples from all three villages (Table 4.2).

Table 4.1: Overall occurrence/prevalence of aflatoxin and fumonisin in maize, maize products and beans across three villages

Maize	**n**	**Positive sample (%)**	**Range**	**Mean ±SE**
Aflatoxin (µg/kg)	160	51(32)	2.10 – 16.20	3.40 ± 0.30
Fumonisin (mg/kg)	160	62 (39)	0.40 – 62.00	5.66 ± 1.48
Beans				
Aflatoxin (µg/kg)	10	1 (10)	2.60 – 2.60	2.60
Fumonisin (mg/kg)	10	1 (10)	12.0 – 12.0	12.00

- Values are means of total aflatoxin and fumonisin levels in all analysed positive samples
- Positive samples are all analysed samples with value > Limit of detection (LOD)
- n is total number of all analysed samples

Table 4.2: Overall mean total aflatoxin and fumonisin content in maize and maize by products in each village.

		Aflatoxin (µg/kg)			**Fumonisin (mg/kg)**		
Village	**n**	**Positive sample (%)**	**Range**	**Means ± SE**	**Positive sample (%)**	**Range**	**Means ± SE**
Long	85	22 (26)	2.10– 4.70	2.66^{a}± 0.12	19 (22)	0.4 – 2.7	0.86^{a}± 0.14
Sabilo	8	4 (50)	2.10 – 3.90	2.75^{a}± 0.40	7 (88)	10.0 – 62	31.43^{b}± 7.67
Seloto	67	25(37)	2.30 – 16.2	4.15^{b}± 0.28	36 (54)	0.4 – 14	3.18^{c}± 0.61

- Values are means of total aflatoxin and fumonisin levels in all analysed samples
- Positive samples are all analysed samples with value > Limit of detection (LOD)
- n represents total number of samples analysed

Maize bran samples obtained from the small-scale mills across all three villages had the highest aflatoxin contamination (Table 4.3). For fumonisin, the highest levels was found from the sorted bad portion for animal feed from Sabilo village (Table 4.4). Significant differences were found in the mean aflatoxin levels of maize bran compared with maize flour from the samples collected across all three villages and from Long village,

significant differences were found for samples collected in maize from mills compared with maize bran, maize bran compared with maize from market, as well as maize bran compared with dehulled maize. Total aflatoxin and fumonisin levels in all samples collected in each village and across all villages with their significant differences are shown in Table 4.3 and 4.4.

Table 4.3: Total aflatoxin contamination in maize and maize products across three villages

		Concentration (µg/kg) Mean ±S.E			
Sample type	**n**	**Overall***	**Long**	**Sabilo**	**Seloto**
Sorted Bad	41	1.69 ± 0.19^{a}	1.38 ± 0.21^{a}	1.85 ± 0.41	1.89 ± 0.37^{a}
Maize mills	29	1.73 ± 0.24^{a}	1.18 ± 0.21^{ab}	-	2.69 ± 0.42^{a}
Maize bran	20	2.38 ± 0.38^{ab}	2.18 ± 0.27^{acd}	-	2.62 ± 0.81^{a}
Maize flour	24	1.42 ± 0.17^{ac}	1.08 ± 0.16^{abeh}	-	1.82 ± 0.27^{a}
Maize market	43	1.91 ± 0.38^{a}	1.45 ± 0.23^{abfh}	-	2.45 ± 0.78^{a}
Dehulled	3	0.83 ± 0.45^{a}	0.83 ± 0.45^{abgh}	-	-

- Values are means of total aflatoxin levels for all analysed samples across three villages
- Means with different letters (by column) are significantly different ($P<0.05$)
- n is the total number of samples analysed
- * represents samples from all three villages.

Table 4.4: Total fumonisin contamination in maize and maize by-products across three villages

		Concentration (mg/kg) Mean ±S.E			
Sample type	**n**	**Overall***	**Long**	**Sabilo**	**Seloto**
Sorted Bad portion	41	7.42 ± 2.19a	0.09 ± 0.07^{a}	27.50 ± 7.71	5.16 ± 1.12^{a}
Maize from mills	29	0.34 ± 0.15ba	0.17 ± 0.09^{ac}	-	0.65 ± 0.36^{bf}
Maize bran	20	1.02 ± 0.39a	0.39 ± 0.09^{bde}	-	1.79 ± 0.81^{cfg}
Maize flour	24	0.3 ± 0.07b	0.16 ± 0.04^{acef}	-	0.45 ± 0.13^{dfgi}
Maize from market	43	0.39 ± 0.08b	0.41 ± 0.14^{bdefg}	-	0.37 ± 0.08^{efhi}
Dehulled Maize	3	0.07 ± 0.07ab	0.07 ± 0.07^{acefg}	-	-

- Values are means of total fumonisin levels for all analysed samples across three villages
- Means with different letters (by column)are significantly different ($P<0.05$)
- n is the total number of samples analysed and (%) for positive samples
- * represents samples from all three villages

4.5 Discussion

Maize is the primary dietary staple in the study area. Aflatoxin and fumonisin contamination were found in maize and maize products from market, small scale mills and those intended for animal feed (bran and sorted bad portion) as well as beans from market, This is an important observation as far as food safety or public health is concerned. Several studies of aflatoxin poisoning in human have shown that low-level chronic intake may be more devastating than one-time high-level intake leading to hepatocellular carcinoma (McGlashan, 1982; Okoth and Kola, 2012). Williams *et al.* (2004) reported that 4.5 billion of people living in developing countries are exposed to chronic toxicity.

4.5.1 Maize and beans samples from market

The mean concentration of 1.91 µg/kg for total aflatoxins from the maize collected in market in all three villages was lower than the MTL of 10 µg/kg in East Africa (EAC, 2011a). It was also lower than 45 µg/kg reported by Saleemullah *et al.* (2006) on aflatoxin content of cereals (wheat, maize and rice) from local markets of North-West Frontier Province in Pakistan; 62 µg/kg as reported by Ahsan *et al.* (2010) in Pakistan; In Kenya, the very high level up to 46400 µg/kg reported by Lewis *et al*. (2005) where, was due to prolonged drought and food shortages that were followed by off season rains during harvest, which probably favoured the growth of aflatoxigenic aspergilli in household stored maize. The mean concentration of 0.39 mg/kg total fumonisins observed in this study was lower than MTL of 2 mg/kg by East African standards (EAC, 2011a). It was also lower than a mean value of 2.9 mg/kg reported by Nikiema *et al.* (2004) from Bukina Faso. The low levels for aflatoxin and fumonisin was due to low levels observed from harvested samples.

The results from the beans samples collected from the market, indicated a maximum aflatoxin value of 2.6 µg/kg, this value was below the MTL of 10 µg/kg by EAC standards (EAC, 2011b). While for fumonisin maximum contamination value was 12 mg/kg, this value was above MTL of 2 mg/kg by EAC standards (EAC, 2011b). These results were lower than those reported by Aiat (2006) in Egypt who found the levels of total aflatoxins to be 1463 μg/kg, and also lower than those reported from a study conducted in Rwanda by Nyinawabali (2013) and found the maximum levels of aflatoxins to be 154.9 μg/kg with a mean value of 28.1 μg/kg, while the maximum level for fumonisin was 7.1 mg/kg with a mean value of 3.0 mg/kg.

The low contamination of the beans samples with fumonisin may be attributed to the fact that beans contamination with mycotoxins has been reported to occur in low concentration as reported by various researchers (Tseng *et al.*, 1995; Aiat, 2006; Nyinawabali, 2013). Stössel (1986) reported that soy bean seed coat and integrity acts as a barrier against fungal attack and hence mycotoxins contamination, other factors being constant, this might be among the reasons for low levels of aflatoxin and fumonisin reported in beans samples from this study.

4.5.2 Aflatoxin and fumonisin occurrence in maize and maize by products from small-scale mills

The results from Table 4.3 and 4.4 underline the interaction between different maize products obtained from the small scale mill with aflatoxin and fumonisin content. The results from this study indicated that all whole maize, maize flour and dehulled maize samples had aflatoxin and fumonisin levels below the MTL of 10 µg/kg and 2 mg/kg respectively by East African standards (EAC, 2011a). Maize bran was found to have higher levels for both aflatoxin and fumonisins with a value of 2.38 µg/kg and 1.02 mg/kg

respectively (Table 4.3, 4.4), although the levels were still lower than total MTL for both aflatoxins and fumonisin in animal feed. The results were comparable to Katta *et al*. (1997) who reported that during the dry milling of corn, fumonisin B_1 was found in highest amounts in the bran fraction that is used as animal feed, followed by the germ fraction, which may be used as animal feed or for oil extraction. Vanara *et al*. (2009) reported the same results from the samples derived from maize kernel in a dry-milling and found high fumonisin concentration in germ and bran.

Similar results were also reported by Broggi *et al*. (2002), in a study in commercial dry-mill in Argentina, who found a three times higher fumonisin contamination level in germ and bran than in whole corn. The results from this study were also comparable to that by Brera *et al*. (2004) who reported highest amounts of aflatoxin and fumonisin in a fractions of the commodity that are less likely to be used for food production (germ and bran fractions), while fractions used for food production, including flaking grits and flour, had the least amount of contamination. The aflatoxin levels from whole maize grain and grain flour reported in this study are lower than those reported by Ramesh *et al*. (2013) who conducted a study in India and found that 68.18% of the food grains and grain flour samples were contaminated with aflatoxin B_1 with a mean concentration of 75.18 and 60.41 µg/kg respectively.

Aflatoxin and fumonisin are not uniformly distributed in maize kernels and the high content tend to be found in germ and bran fractions in dry milling due to the presence of the pericarp, the first part of the kernel colonized by fungi because of its peripheral location and also the part to which kernel dusts adhere (Katta *et al*., 1997; Park, 2002; Brera *et al*., 2004).

4.5.3 Aflatoxin and fumonisin occurrence in animal feeds

Feed samples collected during this study were not typical mixed-feed formulations, but consisted of bad sorted portion of maize which were obviously of bad quality and, therefore, intended for animal feeding and maize bran from the small scale mills intended for the same use. The bad sorted portion was a result of sorting of maize from the whole lot before storage. All of the bad sorted portion maize samples collected from farmers' household and maize bran samples from small scale mill (Table 4.3, 4.4), had levels less than the MTL for animal feeds which range from 0 to 50 µg/kg with an average of 20 µg/kg (FAO, 2004) and also had levels below the MTL for fumonisins in animal feeds which range from 5 to 100 mg/kg (FDA, 2001). The aflatoxin levels reported from this study were lower than those reported by Njobeh *et al.* (2012) who found that feed samples in South Africa were contaminated with aflatoxin (30% of samples) in the range of 0.2– 71.8 µg/kg. Oruç *et al*. (2012) reported 100% feed material contamination with aflatoxin B_1, with mean level of 8.29 µg/kg in Turkey.

Mean of 1.69 µg/kg aflatoxin level for bad sorted portion and 2.38µg/kg from maize bran both as animal feed reported from this study were higher than the maximum level of 0.61 µg/kg reported by Grajewski*et al.* (2012) from Poland. Rezaei *et al*. (2014) from Iran reported that among 40 samples collected from eight centres, the total aflatoxin ranged from 0.9 to 4.2 µg/kg which was comparable to the results of this study.

4.6 Conclusion

The results from this study showed that maize bran had highest levels for both aflatoxin and fumonisin compared to other maize products from the milling. This showed that during milling process mycotoxin contamination may be redistributed and concentrated in certain mill fractions, but there is no step or operation that destroys mycotoxins. Processes

that can reduce mycotoxins levels are; cleaning to removes broken and moldy grain kernels, milling process itself which include dehulling and that dilute and distribute mycotoxins into certain fractions that most commonly become animal feed. However, some toxins in animal feed fractions may have the potential to become residues in animal products (i.e. aflatoxins) and still enter the human food chain. The results from the samples collected as animal feed indicated that the levels for both aflatoxin and fumonisin are below the maximum tolerable limit. It is very important to undertake further research that will help small-scale farmers to meet international quality standards and continue to profitably market their crops, as well as to adopt to those practices that minimize risks to mycotoxin contamination.

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CHAPTER FIVE

5.0 Conclusions and Recommendations

5.1 Conclusions

The results of this study demonstrated that maize and beans produced and sold in the study area are contaminated with both aflatoxin and fumonisin. All analysed beans samples were found to be contaminated with both aflatoxin and fumonisin below the maximum permitted levels by East African Community, European Commmunity and United States of America standards.

Major agricultural practices found to reduce aflatoxin and fumonisin contaminations in maize in the field were; early planting, hand hoe tillage and ox tillage, environmental conditions of the production region especially those prevailing in Long village (high altitude high rain zone) and in Sabilo village (Mid altitude high rain zone). Since it is not easy to control the environmental conditions, maize and beans farmers should adopt production practices (good agricultural practices) that reduce contamination of maize with aflatoxin and fumonisin.

The study also investigated several storage factors that may help to reduce aflatoxin and fumonisins levels in stored maize and beans in the study area, the identified factors/practices were; control of storage insects and mycotoxins levels (aflatoxins and fumonisins) through the sorting out of damaged cobs, the use of appropriate storage insecticides and use of a storage structures that can help to control toxins levels. The results showed that improved storage structures (Super grain safe bags) were found to control the insect's infestation and the levels of aflatoxin and fumonisin contamination as compared to other storage structures in this study. To avoid mycotoxin contamination,

maize should be monitored regularly to assure safe storage conditions. As maize and beans contaminated by fungi and moulds not only render grains unfit for human consumption by discoloration, but can also lead to toxin production such as aflatoxins and fumonisins. Farmers should be encouraged to adopt Good Storage Practices (GSP) as recommended from this study.

From this study, it was also observed that marketed, processed (small scale mill) and feed samples were contaminated by aflatoxin and fumonisin to various extent. Processes that can reduce mycotoxins as reported from this study are therefore; cleaning/sorting to removes broken and moldy grain kernels and milling process which include dehulling and that dilute and distribute mycotoxins into certain fractions that most commonly become animal feed. There is no step or operation that destroys mycotoxins, however, some toxins in animal feed fractions may have the potential to become residues in animal products and still enter the human food chain.

Mycotoxin contaminated products cause significant economic and trade problems at almost every stage of production and marketing. Maize and maize products as well as beans are also affected by these mycotoxins, and standards are becoming progressively stricter. It is very important to undertake further research that will help small-scale farmers to meet international quality standards and continue to profitably market their crops, as well as to adopt to those practices that minimize risks to mycotoxin contamination.

5.2 Recommendations

The presence of aflatoxins and fumonisins cannot be completely eliminated in various food products. However, mycotoxins can be controlled in order to avoid ill-health effects

and economic loss. Preventive practices along the maize and beans value chain can help reduce the risks faced by farmers and consumers. The way forward with mitigation strategies is therefore:-

i. Applying proper agronomic and management practices to reduce the damage of the crops by insects and fungi which are the major source of infection and contamination. Toxigenic fungi often infect plants that are subject to stress, such as drought or pests, however several field practices can reduce fungal colonization and mycotoxins contamination; these include timely planting, proper crop rotation, adequate irrigation, proper tillage, fertilizer applications, weeding, pest and disease control practices and removal of any visible unhealthy crops to protect the remaining healthy one and good crop residue management.

ii. There should be extensive awareness programmes across all regions in the country. Awareness of aflatoxin and fumonisin problem and management strategies should be extended to inform farmers, traders, processors, extension officers, other agriculture research partners, private sector, government regulatory agencies and the Ministry of agriculture about the risk of toxin contamination.

iii. The International Institute of Tropical Agriculture- Tanzania have already identified local atoxigenic strains that can compete and exclude toxigenic strains in crops and the field test and development of a package for legal registration for use in aflatoxin management and develop capacity for manufacturing the strains is underway. The Government should provide incentives to resource poor farmers to access non-toxigenic strains that should be available in small packages, especially after it has been tested and verified.

iv. To avoid deterioration of maize in tropical and subtropical regions, maize should be dried to moisture contents below 13% immediately after harvest.

v. Hygiene and sanitation from harvest to storage are key factors in eliminating sources of infection and reducing levels of contamination; this includes sorting or separating foreign materials and broken corn kernels produced during harvesting from clean maize; removal of residues from the previous harvest or separate old grain from new grain to avoid contamination and transfer of pests from one lot to another as well as milling process itself which include dehulling and that dilute and distribute mycotoxins into certain fractions.

vi. Maize and other crops should be stored in a sealed, airtight container or structure, to reduce oxygen concentration, which will limit the presence of aerobic organisms (Improved storage facilities).

vii. Use of maize cultivars less susceptible to insects and reducing insect infestation in field and during storage are also very important recommendations for farmers.

viii. Frequent analytical surveillance program by food control agencies is highly recommended to control the incidence of mycotoxin contamination. This can be achieved by having a sensitive technique for routine assay of mycotoxins in foods, that can be cheaper and easy to use and can be well adapted by agricultural research institute that are working close with farmers. The application of immunoassays, especially Enzyme-Linked Immunosorbent assay (ELISA) such as Neogen accuscan is recommended.

ix. Further studies are also needed to understand the interaction between different agronomic and storage practices and the contamination of maize with aflatoxins and fumonisins, as well as the influence of storage practices and different processing methods on contamination of maize with aflatoxin and fumonisin.

APPENDICES

Appendix 1: Questionnaire administered to farmers, vendors and processorsin the three villages for maize and beans samples

FARM INFORMATION	
Interviewee (Farmhead) name: Sex: M ☐ F ☐ Telephone number:	Date: Interviewer name and institution:
Sample code:	GPS latitude:
Region: District:	GPS longitude:
Village:	GPS altitude:
Education: primary ☐ secondary ☐ tertiary ☐ none ☐	Money received:
Wealth status: bicycle☐ motorcycle ☐ car☐ tractor ☐truck ☐ oxen ☐ donkey☐ power tiller ☐ none☐	Signature:
Have you heard of mycotoxins? yes ☐ no ☐	Have you ever been sick after eating food made from the crop you gave us? yes☐ no ☐
Comments, if any:	

MAIZE AND BEAN	
Sample type (MAIZE): pre-harvest☐ post-harvest ☐	Sample type (BEANS): pre-harvest☐ post-harvest ☐

cob ☐ entire grain ☐ flour ☐ polished grainBran ☐	with pods ☐ grains ☐
	Spoiled beans for livestock: ☐ grains with pods ☐
Variety:	
Previously grown crop(s):	Planting date:
Harvested date (post-harvest):	Harvest date (expected if pre-harvest):
Tillage method: none ☐ hand/hoe ☐ ox ☐ Other:	Planting pattern: flat ☐ on ridges ☐ on mounds ☐
Pre-harvest treatment (name(s) and quantity):	Post-harvest treatment (name(s) and quantity):
Harvested wet or dry? wet ☐ dry ☐ do not know ☐	
Drying methods: mats ☐ roof ☐ floor ☐ smoke ☐ Others	
Storage (MAIZE): on cob ☐ as grains ☐ in sacks ☐ not in ☐ sacksopen crib ☐ mud house ☐ brick sheltered ☐ Other:	Storage (BEANS): with pods ☐ grains ☐ in sacks ☐ not in ☐ sacksopen crib ☐ mud house ☐ brick sheltered ☐ Other:
Intended use for human Maize, Bean: household ☐ market ☐	Intended use for livestock Maize, Bean:household ☐ market ☐
Condition of Maize: clean ☐ spoiled ☐ If spoiled, intended use: brewing ☐	Condition Bean: clean ☐ spoiled ☐ If spoiled, intended use: livestock ☐

livestock ☐	
Others:	Others:

Storage information

- Storage type; Traditional ☐ Improved ☐ Specify (list)
- Source of grain; Within the village ☐ Outside ☐ If outside give name _____ or approximate distance (in form of <5km ☐ 5-10km ☐ >10km ☐
- Any sorting before storage; Yes ☐ No ☐

  If yes How do you sort: Manually (Hand picking) ☐ Other_____
- What criteria do you use when sorting: Colour ☐ size ☐ shape ☐ insect infested

  Physical ☐ damaged ☐ mould ☐ other (specify)_____
- The use of defective Maize/ Beans: animal feed ☐ brews ☐ human food ☐

If it is for livestock, which animal(s):

cattle ☐ goat ☐ sheep ☐ pig ☐ chicken ☐ Rabbit ☐ duck ☐ dog ☐ cat ☐

- For how many seasons have you used the store? _____
- Do you store maize/beans in the store every season? No ☐ why? _____

  Yes ☐ Why? _____
- Do you store other products in the store, together with maize? No ☐ Yes ☐

  If yes list? _____
- To whom are you selling your products; Local residents ☐ retail traders ☐

  Small scale millers ☐ other merchants specify_____
- Length of storage period_____
- % MC _____
- General storage condition: Good ☐ fair ☐ poor ☐ leaking roof ☐ ; poor

  Aeration ☐ good aeration ☐ Others_____

Sample from processors.

- Source of grain for processing; Within the village ☐ Outside ☐ If outside give name _____ or approximate distance (in form of <5km☐5-10km ☐ >10km☐
- Any sorting before processing; Yes ☐ No ☐

  If yes How do you sort: Manually (Hand picking) ☐ Other_____
- What criteria do you use when sorting: Colour☐ size ☐shape☐ insect infested☐

  Physical damaged ☐mould ☐other (specify)_____
- The use of defective Maize/ Beans: animal feed ☐ raw material for local brews☐

human food ☐

If it is for livestock, which animal(s):

cattle☐goat ☐ sheep ☐ pig☐ chicken ☐ Rabbit☐duck ☐dog ☐ cat ☐

- For how many years have you used the store? _____
- Do you store maize/beans in the store every season? No☐ why?

  _____Yes ☐ Why? _____
- Do you store other products in the store, together with maize? No☐ Yes ☐

  If yes list? _____
- To whom are you selling yourproducts; Local residents☐ retail traders ☐

other merchants specify_____

- Length of storage period_____
- % MC _____
- Level of processing: dehulling ☐ dehulling and milling ☐
- Type of processed products (e.g Flour, dehulled maize) _____
- How processed products are stored: packaging type: polyethylene bags ☐

plastic containers ☐metallic containers ☐ others ☐

_____

- General storage condition: Good ☐ fair ☐poor ☐leaking roof ☐ aeration; poor ☐ good☐Others_____

C: Sample from market

- Source of grain; Within the village ☐ Outside ☐ If outside give name _____ or approximate distance (in form of <5km ☐5-10km ☐>10km☐
- Any sorting before marketing; Yes ☐No ☐

  If yes How do you sort: Manually (Hand picking) ☐ Other_____
- What criteria do you use when sorting: Colour☐ size ☐shape☐ insect infested☐ Physical damaged ☐ mould☐ other (specify)_____
- The use of defective/sorted maize/ beans: animal feed ☐ for local brews ☐ human food ☐

  If it is for livestock, which animal(s):

  cattle☐goat☐ sheep ☐pig☐ chicken ☐ Rabbit ☐duck ☐dog ☐ cat ☐
- For how many years have you used the store? _____
- Do you store maize/beans in the store every season? No☐ why? _____Yes ☐ Why?_____
- Do you store other products in the store, together with maize? No☐ Yes ☐

  If yes list?_____
- Who purchase your maize; Local residents ☐ small scale millers☐other merchants; specify_____
- Length of storage period_____
- % MC _____
- General storage condition: Good☐ fair☐poor☐leaking roof ☐ aeration; poor☐ good☐ Others_____

Storage problems across storage structures, market and processors

- Do you have storage problems? Yes ☐ No ☐
- Which storage problem is the most important? Insects ☐ Birds ☐ Mould ☐ Rodents ☐ Others (specify)_____
- What did you do to solve this problem? List_____
- Does the grain germinate in storage? Yes ☐ No ☐
- Do you clean the storehouse before storage? Yes ☐ No ☐
- Do you remove old grains? Yes ☐ No ☐
- What else did you do to clean the store before storage? List_____
- If you treated the storehouse before storage, what methods did you use? Ash Insecticides (specify)_____Smoke ☐ Manure ☐ Others ☐ (specify)_____
- How did you store your maize? As grain ☐ In the husk ☐ Dehusked ☐ Other_____
- Do you use pesticides during storage No ☐ Yes ☐ If yes, give name_____ rate of application_____ and quantity_____
- Did you take any other precautions? List_____

Comments/Remarks

Appendix 2: Sampling Protocol for Maize and Beans from Field, Farmers Store, Processors and Market in Long, Seloto and Sabilo villages in Babati, Tanzania

Villages Survey Planning Meeting

The District Agriculture, Irrigation and Cooperative Officer (DAICO) was consulted to provide village map to facilitate planning for the villages to be visited. He/she also provided phone numbers of the local Extension Officers located in the villages to be visited. The sampling unit was randomly selected from the village register provided by the DAICO and extension officers.

Sampling methods and procedures

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

i. Samples were collected at harvest in the field, after physiological maturity (about a week before harvest).

ii. When sampling from storage structures, small numbers of samples were collected from different areas of a container and then mixed to produce a representative sample of approximately 1 kg.

iii. Samples were also collected from the processors (small scale posho millers) and vendors (market), at an interval similar to that of samples collected from farmers stores (0, 40, 80 and 180 days).

- Clear and detailed explanation was given to farmers about what is needed and for which purpose. This was intended to minimize farmers' suspicions.
- Both good and bad sorted portion were collected from farmers prior to storage

Sampling procedures

1. The interviewee was brief and clearly explained the intention and reason of collecting from him/her the sample of the crop in question.
2. The questionnaire provided was well filled with all information needed as indicated.
3. Coloured-printed photographs (fact sheet) of crops infected with mycotoxins was shown and given to the interviewee and ask if he/she has seen such symptoms in his/her crop. Fill in his/her answer on the appropriate space in the questionnaire.
4. For samples from the field: samples were taken following the two diagonals of the field and stop at regular intervals (5 stops) and five cobs/pods were picked at each stop to make a total of 25 cobs/pods These were then hand shelled, mixed and approximately 1kg was randomly picked.
5. For stored/market sample: Multiple samples were taken from different parts of one bag or several bags belonging to one farmer/vendor and combined to produce a 1-kg sample for analysis, for market samples the respective vendor's sampling tools (i.e., scoops) was used.
6. If the farmer had two lots of crop, say a good lot for human consumption as food and another lot of especially spoiled crop for livestock or other uses, two separate samples were taken. In this case, the sample code was the same for each sample except that the one for human food was marked "A" and the one for livestock was marked "B".
7. The collected samples were kept in the paper bag (envelope) provided and well labelled using pencil by copying the sample code already filled in on the questionnaire.

8. All samples were kept dry in the vehicle and all the time; avoid any moisture risk. Samples were well kept in a moisture-free environment while waiting for dispatch to IITA laboratory in Dar es salaam.