PREVALENCE OF AFLATOXIN AND FUMONISINS ($B_1 + B_2$) IN MAIZE, RICE AND GROUND NUTS CONSUMED IN RURAL MALAWI.

By

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ABSTRACT

PREVALENCE OF AFLATOXIN AND FUMONISINS ($B_1 + B_2$) IN MAIZE, RICE AND GROUND NUTS CONSUMED IN RURAL MALAWI.

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The objective of this study was to assess levels of contamination of aflatoxins and fumonisins (B_1+B_2) in maize, ground nuts and rice produced, stored and consumed in rural households in Malawi. A total of 9 districts were selected across the country: 3 districts in North, Central and Southern regions respectively. Households were selected at random in each district where 10 maize samples were collected for laboratory analysis. A total of four districts were selected for rice and six districts for ground nuts which were sampled the same as for maize. Aflatoxins and fumonisins were analyzed using a single step lateral flow immunochromatographic assay based on a competitive immunoassay format. The detection limit for aflatoxins was 2 µg/Kg with a quantitation range of 2 - 150 µg/Kg and that for fumonisins was 1 mg/Kg with a quantitation range of 1 – 7 mg/Kg. It was found that samples in the Southern region were highly contaminated, with the Chikhwawa district having high levels of both aflatoxins and fumonisins in maize and rice. The Northern region had the least contamination. The maximum detected amounts of aflatoxins were 140, 210 and 18.5 µg/Kg in maize, rice and ground nuts, respectively. The maximum detected amounts of fumonisins were 7000, 7000 and 2600 µg/Kg in maize, rice and groundnuts respectively. About 20% of maize, 15% of ground nuts and 8% of rice samples exceeded the tolerable maximum limit for aflatoxins in Malawi. Aflatoxins and fumonisins were found to co-occur in maize and rice with contamination levels exceeding 100 µg/Kg for both aflatoxins and fumonisins.

I dedicate this paper to my deceased, loved brother Rodgers Ophaniel Mwalwayo whose untimely death in South Africa left me with no friend than he was. He would have done a similar thing if he was around, do something to help the underprivileged. I miss you dear; may your soul rest in peace.

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CHAPTER 1

1.0 INTRODUCTION

The presence of mycotoxins in food consumed by Africans is often undetected due to lack of public awareness concerning mycotoxin presence and toxicity. Lack of proper regulatory mechanisms and capacity of regulatory agencies thwart efforts to educate people about mycotoxin toxicity associated with consuming and handling contaminated foods. Most African countries are lagging behind industrialized countries in pre- and post-harvest practices that would minimize mycotoxin consumption. Donating ("dumping") mycotoxin contaminated food products and the introduction of contaminated commodities into the human food chain during acute and chronic food shortage due to drought, political and economic instability also contribute to the problem.

Most regulatory agencies in African governments concentrate on the sanitary and phytosanitary aspects of commodities meant for foreign trade, and little if anything is done related to food consumed by the local populations. As a result, the local populations are more prone to eating contaminated grain which impacts their health status.

The problem is compounded when you consider that most villagers eat the food that they grow and process at their home. Limited capacity in food production leads villagers to consume any crop that can be used as food, even if mold growth has changed the organoleptic quality of the food.

The consequences of consuming mycotoxin-contaminated food are well known. Aflatoxins are hepatocarcinogens in animals and humans. They are acutely toxic, immunosuppressive, mutagenic and carcinogenic to both humans and animals. The main target organ for toxicity and

carcinogenicity is the liver. Fumonisins specifically are known to cause esophageal cancer and suppress immune function. Mycotoxicoses often remain unrecognized by medical professionals, except when large numbers of people are involved. Several outbreaks of mycotoxicoses have occurred in tropical countries, mostly among adults in rural populations, with a poor level of nutrition, for whom maize is a staple food (Sibanda, et el., 1997; Wagacha and Muthomi, 2008).

Mycotoxicosis in sub-Saharan Africa is due mainly to aflatoxin contamination. About 250,000 hepatocellular carcinoma-related deaths occur annually in parts of sub-Saharan Africa due to aflatoxin ingestion alone. Up until the mid-1990's reports of acute aflatoxin poisonings, approximately 25% of which result in deaths has been reported.

No serious mycotoxicosis outbreak has been reported so far in Malawi, but the climatic conditions, outbreaks of mycotoxicosis in neighboring countries and knowledge of pre- and post-harvest practices strongly suggest that Malawians are consuming mycotoxin-contaminated foods. This research, therefore, is aimed at assessing the extent of mycotoxin contamination of foods widely consumed in Malawi – maize, rice and groundnuts.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 MYCOTOXINS

A central issue in the field of food and feed safety and quality in storage is the problem of mold spoilage. Foodborne illness, both in humans and animals, is of considerable public health concern as well as economic significance in view of the productivity loss and other monetary losses. Several environmental factors at the time of harvest and during storage, often lead to development of molds and subsequent formation of mycotoxins, (Bhat, et al., 2000). Fungal growth reduces nutritional value and may result in the production of mycotoxins.

Most mold species regularly associated with food and feed spoilage belong to the genera *Aspergillus, Penicillium, Fusarium, Mucor, Absidia, Monascus, Scopulariopsis* and *Trichoderma*, (El-Shanawany, et al., 2004). Growth of commonly occurring filamentous fungi in foods may result in production of toxins known as mycotoxins. Mycotoxins are secondary metabolites produced during the late stages (stationary phase) in the growth cycle of the fungi. They appear to have no role in the normal metabolism involving growth of the microorganism, but are generally considered to be a mechanism of overcoming stress by the microorganism (Pitt, 2000). Many are complex molecules, with structures ranging from single heterocyclic rings with molecular weights of around 50 Da, to groups of irregularly arranged 6 or 8 membered rings with total molecular weights greater than 500 Da, (Pitt, 2000). Only in the last 30 years has it become clear that commonly occurring fungi growing in foods and feeds may produce toxins. These toxins have caused major epidemics in man and animals throughout history. The most important ones being ergotism that killed hundreds of thousands of people in Europe in the last

millennium; alimentary toxic aleukia (ATA) that was responsible for the death of at least 100,000 Russian people between 1942 and 1948; stachybotryotoxicosis that killed tens of thousands of horses in the USSR in the 1930's; and aflatoxicosis that killed about 100 000 young turkeys in the UK in 1960, (Pitt, 2000). According to Wagacha and Muthomi (2008) more than 400 deaths of people have occurred in Kenya in the years between 1981 and 2005.

The term mycotoxin literally means poison from fungi. Out of the several thousand fungi species, only about 100 belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium* are known to produce toxins. There are more than 400 known toxins, but the most important ones are aflatoxins, ochratoxins, deoxynivalenol (DON or vomitoxin), zearalenone, fumonisins, T-2 toxin and T-2-like toxins (trichothecenes), (Wagacha and Muthomi, 2008). The toxic effects of a mycotoxin on animal and human health are referred to as mycotoxicosis. The severity of mycotoxicosis depends on the toxicity of the mycotoxin, extent of exposure, nutritional status and age of the exposed individual (Peraica, et al., 1999).

Specific mycotoxins are among the most potent mutagenic and carcinogenic substances known to date (Bhat & Vasanthi, 2003). Prolonged exposure through diet has been linked to cancer, kidney and liver failure, and a compromised immune system. Mycotoxicosis problems are most prevalent in the tropics where environmental conditions are prevalent that are favorable to mold growth. The diets in these areas consist mainly of the crops susceptible to mycotoxin contamination (Bhat & Vasanthi, 2003). Mycotoxins have four basic kinds of toxicity; acute, chronic, mutagenic and teratogenic. The most commonly described effect of acute mycotoxin poisoning is deterioration of liver or kidney function which in extreme cases may lead to death (Pitt, 2000). The symptoms of mycotoxicosis are almost as diverse as the chemical structures of the compounds themselves.

The history of mycotoxicosis dates back to ancient times. There is documentation that in the seventh and eighth centuries BC, the festival "Robigalia" was established to honor the god Robigus, who was believed to protect grain from attack by rust or mildew, (Peraica, et al., 1999). The earliest scientific reports of fungal toxicity are those associated with ingestion of the sclerotia of the ergot fungus *Claviceps purpurea.* However, the toxicity of higher fungi *Amanita phalloides (*death angel) has been known for many hundreds of years (Jarvis, 1971). Ergotism is the oldest mycotoxicosis identified in humans. It represents a group of producing fungi that grow on the heads of grasses such as wheat and rye. Ergot was responsible for a disease known as "St. Anthony's Fire" which broke out in Europe around 430 B.C. and continued as late as 870 A.D. Similar outbreaks of public health significance have occurred in more recent years in Russia (1924-1944), Ireland (1929), France (1953) and Ethiopia (1978). But ergotism is currently of less significance to the food industry because of food quality procedures that screen out ergot-infected grains (Patricia, et al., 2006).

General interest in mycotoxins rose in 1960 when a feed-related mycotoxicosis called turkey X disease, which was later proved to be caused by aflatoxins, appeared in farm animals in England (Peraica, et al., 1999). It was found then that aflatoxins are hepato-carcinogens in both animals and humans and this finding stimulated mycotoxin-related research. Aflatoxins and fumonisins have been shown to be directly responsible for several diseases in both humans and animals, sometimes causing illness and even death (Fandohan, et al., 2005). They occur either alone or together (Fandohan, et al., 2005).

Approximately 25% of the world's food crops are affected each year by mycotoxins, (Choudhary and Kumari, 2010) with aflatoxin and fumonisin contamination being of particular importance. Aflatoxin is a problem in many commodities, but, as far as grains are concerned, aflatoxin

contamination is primarily a problem associated with maize. Rice can also be contaminated with aflatoxins due to poor storage conditions in tropical and subtropical areas, (Miller, 1995).

2.2 AFLATOXINS

Aflatoxins are common contaminants of foods particularly in the staple diets of many developing countries. Aflatoxins are produced by the fungi *Aspergillus parasiticus* and *Aspergillus flavus* as secondary metabolites when the temperatures are between 24^0C and 35^0C. They form in many commodities in conditions of excess moisture during harvest and storage. Aflatoxins are considered by the United States Food and Drug administration (USFDA) to be unavoidable contaminants of foods.

Aflatoxins are a group of closely related compounds with small differences in chemical composition. There are four main aflatoxins – B_1, B_2, G_1 and G_2 – with aflatoxin B_1 being the most prevalent. Table 1 lists the different types of aflatoxins and their sources. Figures 1 – 5 depict the chemical structure of the aflatoxins listed in Table 1.

Table 1. Types and sources of aflatoxin

TYPE	SOURCE
Aflatoxin B_1 & B_2	*Aspergillus flavus* and *A. parasiticus*
Aflatoxin G_1 & G_2	*Aspergillus parasiticus*
Aflatoxin M_1	A metabolite of Aflatoxin B1 found primarily in milk of humans and animals

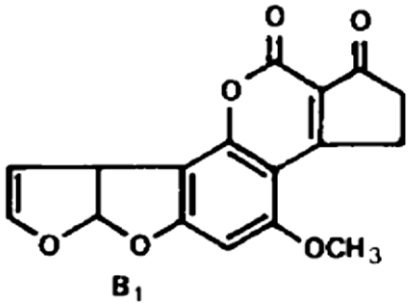

Figure 1. Structure of aflatoxin B_1

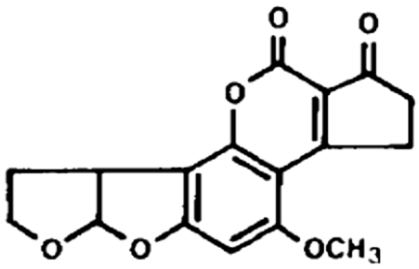

Figure 2. Structure of aflatoxin B_2

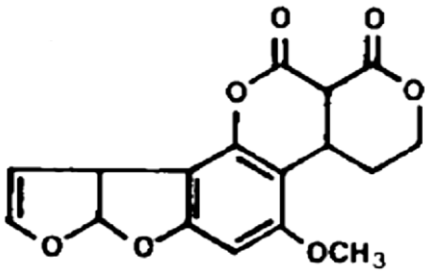

Figure 3. Structure of aflatoxin G_1

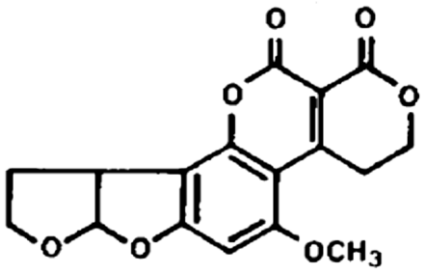

Figure 4. Structure of aflatoxin G_2

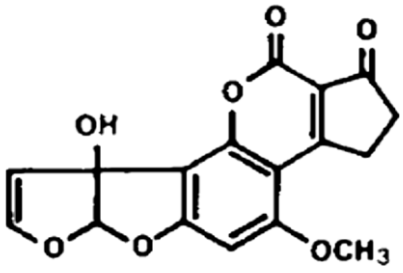

Figure 5. Structure of aflatoxin M_1

2.2.1 TOXICITY

Health hazards resulting from ingestion of aflatoxins are of worldwide concern. Even minute traces of these compounds in agricultural products are cause for alarm (Iyer, et al., 1994). Among the aflatoxins and their metabolites, only AFB_1, AFB_2, AFG_1 and AFG_2 have been found as natural contaminants in agricultural products. They cause mycotoxicosis in poultry and mammals. Acute aflatoxicoses have been reported in humans in Taiwan, Canada, Uganda, Germany, India and Kenya.

When animals or humans consume foods contaminated with aflatoxins, AFB_1 is metabolized in the liver leading to formation of highly reactive chemical intermediates. The binding of these intermediates to DNA results in the disruption of transcription and in abnormal cell proliferation, leading to mutagenesis and carcinogenesis (Guengerich, 2001; Imaoka, et al., 1992 and Sell, et al., 1998). The differences in susceptibility to aflatoxin across species and between persons is largely dependent on time and fraction of the dose that is directed into the various metabolic pathways, with the most deleterious effects resulting from formation of the AFB_1 8,9-epoxide and its reaction with protein and DNA (Williams, et al., 2004). The AFB_1-8, 9-epoxide, an

intermediate in AFB_1 metabolism, is postulated to be the active carcinogen (Degen, et al., 1981; Baertischi, et al., 1988 and Denissenko, et al., 1999). In 1993, the International Agency for Research on Cancer (IARC) classified AFB_1 as chemical carcinogen Group 1 (International Agency for Research in Carcinogenesis, 1993a). The key structural feature of aflatoxin B_1 related to its genotoxicity is the furofuran ring system. Aflatoxin B_1 undergoes epoxidation to form AFB exo-epoxide 2 which reacts rapidly with DNA to give high adduct yields (Iyer, et al., 1994).

AFB_1 ingestion by humans is becoming more and more important as new results from laboratory animal studies and epidemiological studies are reported. Chronic aflatoxicosis with high incidence of primary liver cancer has been reported in Uganda, Thailand, Kenya, Mozambique and China (Casado et al., 2001; Sorenson, 1993). Aflatoxin ingestion impaired child growth in Benin and Togo (Gong, et al, 2002). In spite of nearly 50 years of research, the extent of the global exposure to this carcinogen is still poorly documented, hampering estimation of the associated disease burden. Currently, the World Health Organization does not recognize mycotoxins as a disease burden. Using aflatoxin biomarkers, it has been shown that aflatoxins cross the placental barrier as revealed by the presence of aflatoxin albumin adducts in cord blood samples. In West Africa, this exposure has been shown to continue in infancy and once children are weaned, they have a similar high prevalence and level of exposure as observed in adults (Wild and Gong, 2010). This is a very serious issue, considering that maize/peanut porridge is the major weaning food in most local African communities, and much so in Malawian villages. Application of these biomarkers in a systematic manner to characterize regional exposure and risk around the world therefore would be of great value.

It has been estimated that more than 600,000 people die of liver cancer worldwide each year with the majority of cases occurring in China, South East Asia and sub-Saharan Africa. Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV), representing more than 350 million (5% of the world population) and 170 million people, respectively, worldwide, are major risk factors associated with exposure to aflatoxins and its relation to hepatocellular carcinomas (HCC). The fraction of HCC cases attributable to HBV and HCV has been estimated to be 23 and 20% in developed countries and 59 and 33% in developing countries (Wild and Gong, 2010).

The maximum tolerable limits for aflatoxin in foods vary from country to country. Codex Alimentarius sets general global safety standards and this commission set the maximum tolerable limit as 15 ppb total aflatoxin. The United States Food and Drug Administration (FDA) maximum tolerable limit is 20 ppb total aflatoxin. The European Union regulation is 2 ppb for aflatoxin B_1 and 4 ppb total aflatoxin in foods intended for direct human consumption. If maize is subject to sorting or any physical treatment prior to human consumption, The European Union regulation is 5 ppb aflatoxin B_1 and 10 ppb total aflatoxin. The standard applied in many African countries, Malawi included, is the same as recommended by Codex Alimentarius.

2.2.2 CLINICAL SIGNS OF AFLATOXICOSIS

With over 400 identified mycotoxins and more being added as new methods and techniques evolve, it is obvious why it is so difficult to link symptoms to a particular aflatoxin poisoning. Aflatoxin is a potent liver toxin causing hepatocarcinogenesis, hepatocellular hyperplasia, hepatic necrosis, cirrhosis, and biliary hyperplasia in animals. Other effects include mutagenic and teratogenic effects. Aflatoxins affect many species including humans, dogs, pigs, dairy cattle

and chickens. Trout, one of the early animal models for study of aflatoxicosis, is very sensitive to aflatoxin and these fish develop numerous clinical signs including hepatoma. On the other hand, swine at weaning and marketing stages are resistant to dietary levels of aflatoxin up to 300 μg/Kg. Clinical signs associated with aflatoxicosis in dairy cattle include reductions in feed intake, milk production, and weight gain, and evidence of liver damage (Sharma, 1993)

2.3 FUMONISINS

Fumonisins, like aflatoxins, are a group of toxic metabolites produced by the molds *Fusarium verticillioides, F. proliferatum* and *F. nygamai* with *Fusarium verticillioides* being the predominant contaminant in food and feeds, (Michael and Wyatt, 1993). Fumonisins were first isolated in 1988 and consist of a long hydroxylated hydrocarbon chain with added tricarboxylic acid, methyl, and amino groups. They are polyols with a long chain (20 carbons) esterified in the C14 and C15 with two groups of tricarboxylic acids. Fumonisin B_1 (FB_1), Fumonisin B_2 (FB_2) and Fumonisin B_3 (FB_3) are the major naturally occurring fumonisins. However, Fumonisin A_1 and A_2 (FA_1 & FA_2) also occur naturally (Segvic and Pepeljnjaks, 2001). In 1993, the IARC classified fumonisins as Group 2B compounds – "probably carcinogenic for humans" (International Agency for Research in Carcinogenesis, 1993b). Figure 6 shows the chemical structures of fumonisins

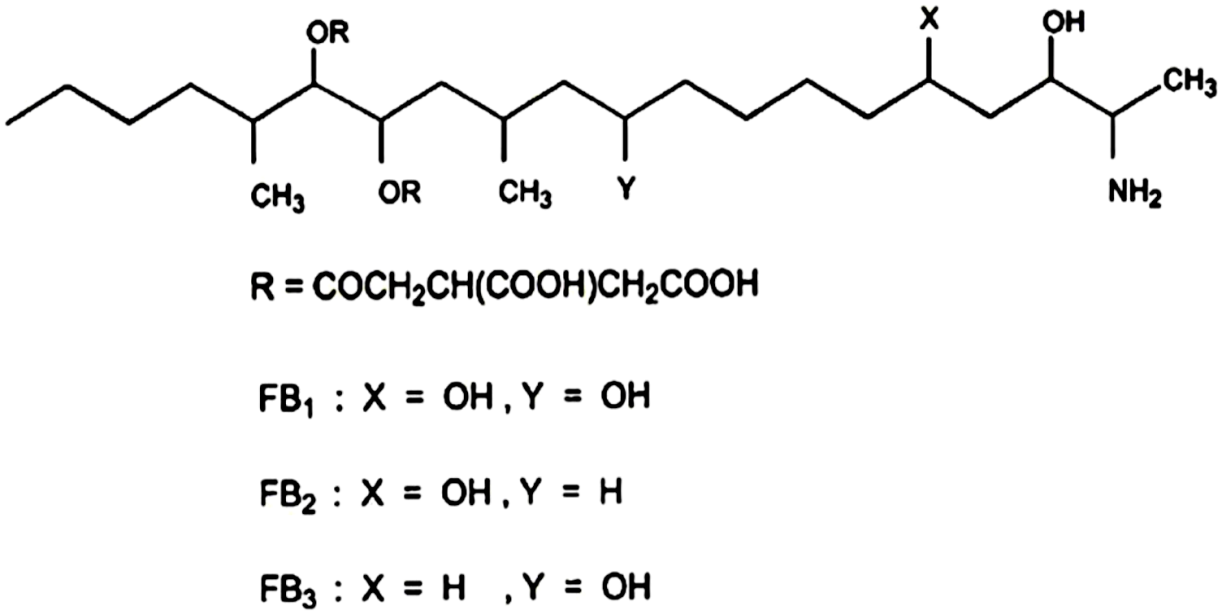

Figure 6. Structure of fumonisins

FB_1 is by far the most prevalent fumonisin in the human diet (Wild and Gong, 2010). Fumonisin contamination of maize occurs in many parts of the world with reported levels greater than 100, 000 µg/Kg (100 ppm) in some regions. Fumonisin contamination of agricultural produce is dependent on geographical region, season and the conditions under which the particular grain is grown, harvested and stored. Grain grown in tropical and subtropical regions is more prone to fumonisin contamination due to the relatively long and warm growing season (Michael and Wyatt, 1993). Contamination of corn with high levels of fumonisin has been reported in Tanzania, South Africa, United States and China. Fumonisin contamination results in economic losses to farmers and health hazards to both farm animals and humans.

2.3.1 TOXICITY

Fumonisin B_1 is considered the most prevalent and most toxic derivative within the group of fumonisins. Contamination of cereals with the fungus *Fusarium moniliforme*, a common contaminant of corn throughout the world, has been associated with several human and animal diseases. Consumption of moldy maize containing fumonisin B_1 has been associated with an outbreak of abdominal pain and diarrhea in India (Bhat, et al., 1997). Pathogenic effects due to fumonisin ingestion in animals include leukoencephalomalacia, pulmonary edema, hepatotoxicity, hepatocarcinogenicity and nephrotoxicity (Segvic and Pepeljnjaks, 2001). Consumption of contaminated maize has been associated with an elevated risk of human esophageal cancer in the Transkei region in South Africa and China (Williams, et al., 2010). It has been shown that culture material of *F. verticillioides* was hepatocarcinogenic in rats, exhibiting both initiating and promoting effects. FB_1 was subsequently shown to be a liver cancer promoter in a diethyl nitrosamine-initiated rat model. Fumonisins, in particularly FB_1 are prototypic inhibitors of cellular sphingosine (sphinganine) N-acetyltransferase. Inhibition of this enzyme is followed by an accumulation of sphinganine and sometimes also sphingosine and a depletion of complex sphingolipids in eukaryotic cells. The beginning and progression of diseases associated with FB_1 have a close relationship to the disruption of sphingolipid metabolism (Merrill, et al., 2001; Riley, et al., 2001 and Voss, et al., 2001). This leads to impairment of cell cycle regulation and cellular differentiation. It also results in oxidative stress as well as apoptosis and necrosis, (European Food Safety Authority, 2005 and Haschek, et al., 1992). Altered apoptosis and mitosis is thought to contribute to carcinogenesis through an altered balance of cell death and replication (Wild and Gong, 2010).

The regulatory limit for fumonisins in the United States is 2 – 4 mg/Kg (2 to 4 ppm) in foods meant for direct human consumption (United States Food and Drug Administration, 2001) and in the European Union it is 4 mg/Kg (4 ppm) in foods meant for further processing and 1 mg/Kg (1ppm) in foods meant for direct human consumption, (European Union 1881/2006). The maximum tolerable daily intake limit as set by FAO/WHO is 2 μg/kg body wt. /day for FB_1, FB_2 and FB_3 alone or combined.

The suggested regulatory limit by Codex Alimentarius, which is yet to be approved, is 5 mg/Kg (5 ppm) for fumonisins B_1 +B_2 in unprocessed corn, (Codex Committee on Contaminants in Food ,2012). No regulatory limits have been set in Malawi for fumonisins.

2.3.2 CLINICAL SIGNS

FB_1 and FB_2 pose great risk to humans as they have been shown to be statistically correlated with the prevalence of human esophageal cancer in some parts of the world and liver cancers, neural tube defects and cardiovascular problems in populations consuming relatively large amounts of food made with fumonisin-contaminated maize (Voss, et al., 2007; Thiel, et al., 1992 and EFSA, 2005). An increased sphinganine: sphingosine ratio in body fluids and tissues serves as a sensitive biomarker of exposure to fumonisins, (European Food Safety Authority, 2005). Thus far, there has been no assessment of fumonisin contamination of food in Malawi using either biomarkers or quantitation of fumonisin in foods.

2.4 SIGNIFICANCE

Malawi has a population of over 14 million people. Maize in Malawi is the most important staple food crop; it is grown by 97% of farming households and accounts for 60% of total food consumption. It is cultivated on more than 70% of the total arable land and contributes significantly to diets of more than 80% of the population (Matumba, et al., 2009). Rice is mostly a staple food for those people living along Lake Malawi, but it is also grown in other parts of the country in 'dambos' by means of irrigation. However, in most parts of the country rice is mainly grown as cash crop serving as source of income in most rural households, leaving maize as the most consumed grain by the population. Ground nuts are especially grown as a cash crop, and for local consumption in villages where they are used as weaning foods for children when milled together with corn and consumed as morning snacks when roasted or added as condiments to vegetables and dried fish. Over half of Malawi's farming households operate below subsistence. Because of low productivity and small farm size, only 20% of maize farmers produce surplus and sell their product (Denning et al., 2009). This means that about 80% of these farmers are not able to produce enough maize for their own home consumption. In addition most farmers will sell the best quality maize that they have. As a result what is left as food for consumption is grain of poor quality, most of which may be contaminated by mycotoxins, leaving this population at a health risk. Also due to food insufficiency, the populations in villages do not have a choice regarding the quality of food that they consume. Approximately 25% of the world's food crops are affected each year by mycotoxins (Choudhary and Kumari, 2010) with aflatoxin and fumonisin contamination being of particular importance. Aflatoxin is a problem in many commodities, but, as far as grains are concerned, aflatoxin contamination is primarily a problem associated with maize. Rice can also be contaminated with aflatoxins due to poor storage

conditions in tropical and subtropical areas (Miller, 1995). Mycotoxicosis problems are most prevalent in the tropics, where environmental conditions are prevalent that are favorable to mold growth. The diets in these areas consist mainly of the crops susceptible to mycotoxin contamination, (Bhat and Vasanthi, 2003). The population in Malawi is therefore at greater risk of exposure to mycotoxins due to consumption of maize flour, rice and ground nuts processed locally. It is, therefore, important to assess the extent of contamination of the food crops being consumed in these rural areas and determine the extent of exposure to these toxins in the areas most affected.

2.5 RATIONALE

Due to low maize production and food insufficiency most people in villages have no choice regarding the quality of food they eat. These people consume food that is locally processed in their home with no regard to quality checks and food safety. It is thought that maize, rice and ground nuts are usually contaminated with mycotoxins, especially aflatoxins. These populations are frequently not aware of the existence of these toxins in their food and their effects on health. People in rural Malawi are therefore at greater risk of exposure to these toxins. In Malawi, data on prevalence of these toxins is scanty. This research therefore is aimed at assessing the prevalence of these toxins in staple foods that are locally produced and processed in villages. Only when the problem is clearly defined can control measures be efficiently planned and implemented.

2.6 HYPOTHESIS

People in rural villages in Malawi are at risk of exposure to mycotoxins due to consumption of maize, rice and ground nuts that are locally produced and processed in their homes.

CHAPTER 3

3.0 PREVALENCE OF AFLATOXIN AND FUMONISINS (B_1+B_2) IN MAIZE, GROUND NUTS AND RICE IN MALAWI

3.1 INTRODUCTION

Malawi is a country in southern Africa with a population of about 14 million people. The backbone of Malawi's economy is agriculture, which employs about 90% of the population. Agriculture contributes more than 35% of the country's gross domestic product (GDP) and accounts for almost 85% of the export earnings, (Mkumbila, et al., 2007). Maize in Malawi is the most important staple food crop; it is grown by 97% of farming households and accounts for 60% of total food consumption. It is cultivated on more than 70% of the total arable land and contributes significantly to diets of more than 80% of the population, with per capita consumption of 182 Kg per year (Matumba, et al., 2009). Other than maize, cassava and rice complement maize as staple foods, with cassava being the second most important food crop. They both supply around 70% of the staple diet, (Rusike, et al., 2010). Cassava is grown in most parts of the country. It is a staple crop for about 30% of the 14 million people in Malawi especially those living along Lake Malawi, (Mkumbila, et al., 2007). Just like cassava, rice is mostly a staple food for those people living along Lake Malawi, but it is also grown in other parts of the country in 'dambos' by means of irrigation. However, in most parts of the country cassava and rice are mainly grown as cash crops, serving as a source of income in most rural households, leaving maize as the single-most consumed grain by the population.

Over half of Malawi's farming households operate below subsistence. Because of low productivity and small farm size, only 20% of maize farmers produce surplus and sell their product (Denning, et al., 2009). This is very critical in a country where agricultural produce is the main source of income. This means that about 80% of these farmers are not able to produce enough maize for their own home consumption. Most farmers will sell the best quality maize that they have. As a result what is left as food for family consumption is frequently grain of poor quality, some of which may be contaminated by mycotoxins, leaving this population at a health risk. Ground nuts on the other hand are especially grown as a cash crop and for local consumption in villages where they are often used as weaning foods for children when milled together with corn. They are consumed as morning snacks when roasted or added as condiments to vegetables and dried fish. In a previous study by ICRISAT and NASFAM, 30% of the nuts had aflatoxin levels exceeding the EU regulatory limit, 6.5% of which had contamination levels greater than 100ppb (Monyo et al., 2009).

All of the foods mentioned above are prone to mycotoxin contamination. Because the poorest quality foods are likely to be contaminated by molds and mycotoxins, the safety of food in most rural areas is compromised. This is compounded considering that most of food consumed is locally processed and hence there is no regulatory control over the quality of food as regards to its safety. Food safety with its relationship to food quality in the developing countries of Africa is an issue which frequently must be balanced by issues of food security with an emphasis on sufficiency of supply (Shephard, 2003). According to Akinnifesi, et al., 2006, food insecurity in most households in Malawi results from high costs and suboptimal use of chemical fertilizers, sporadic rainfall and frequent droughts as well as lack of farm input loan facilities.

Finally, the lack of an effective regulatory and enforcement framework coupled with a lack of consumer awareness and understanding of the role of molds and mycotoxins on human health combine to increase risk to human health. Currently in Malawi there are insufficient data on the extent of fumonisin prevalence and there is only limited data regarding aflatoxin contamination in maize and ground nuts. There is a regulatory safe limit for Aflatoxins available in product standards, but these only apply to maize or any farm produce meant for export or for those products meant for super markets. Fumonisins on the other hand are not being regulated currently in Malawi; however, there is as much risk associated with consuming maize contaminated by fumonisin as from aflatoxins. It has been shown in Tanzania that aflatoxins coexist with fumonisins in maize (Kimanya, et al., 2008), and there is also evidence suggesting that aflatoxins act synergistically with fumonisins, putting consumers at more risk from their combined effects. Only when the problem is clearly defined can control measures be efficiently planned and implemented. The objective of this study is to test the hypothesis that the health of rural consumers is at high risk from exposure to aflatoxins and fumonisins. To test this hypothesis, three dietary staple crops (maize, rice, and ground nuts) will be analyzed for prevalence and concentration of these mycotoxins. The information obtained from this study will be used to define the degree of risk of rural populations as well as the Malawi population in general. In addition, this information will provide an important basis from which to build an effective regulatory system to minimize exposure to these toxins.

3.2 MATERIALS AND METHODS

3.3 SAMPLING PLAN AND STATISTICAL DESIGN

3.3.1 SAMPLING PLAN FOR MAIZE

Maize is grown and consumed throughout Malawi and therefore a country-wide sampling plan was established. Malawi is divided politically into Northern, Central, and Southern Regions. Each region is divided into districts and three districts from each region were randomly chosen for sampling. The districts selected are shown in Table 2. In each district 10 households were randomly selected to provide samples. One Kg of maize from the bag of maize currently being consumed was purchased from the selected households. A total of 30 samples of maize were collected per region resulting in a total of 90 samples for the whole country.

Table 2. Sampling plan for maize

	REGION		
DISTRICT	North	Central	South
1	Karonga	Kasungu	Machinga
2	Nkhata-Bay	Lilongwe	Mulanje
3	Mzimba	Salima	Chikhwawa

3.3.2 SAMPLING PLAN FOR RICE

Rice samples were obtained from districts in the Northern and Southern regions. In the Northern and Central region rice is grown mainly along the lake and prevailing conditions along the rift valley are almost the same as compared to the Southern region where in most districts it is grown in 'dambos'. Two districts that produce rice in each region were randomly chosen for sampling. The districts selected are shown in Table 3. In each district 10 households were randomly selected to provide samples. One Kg of rice from the bag of rice currently being consumed was purchased from the selected households. A total of 20 samples were collected per region resulting in a total of 40 samples for the whole country.

Table 3. Sampling plan for rice

	REGION	
DISTRICT	North	South
1	Karonga	Machinga
2	Nkhata-Bay	Chikhwawa

3.3.3 SAMPLING PLAN FOR GROUNDNUTS

Groundnuts are grown in most parts of Malawi and samples were obtained from districts in the Northern, Central and Southern regions. Two districts in each region were randomly chosen for sampling. The districts selected are shown in Table 4. In each district 10 households were

randomly selected to provide samples. One Kg of groundnuts from the bag of nuts currently being consumed was purchased from the selected households. A total of 20 samples were collected per region resulting in a total of 60 samples for the whole country.

Table 4. Sampling plan for ground nuts

	REGION		
DISTRICT	North	Central	South
1	Karonga	Kasungu	Mulanje
2	Mzimba	Lilongwe	Chikhwawa

3.4 SAMPLE PREPARATION AND EXTRACTION

The aflatoxin and fumonisin contents of maize, rice and ground nut samples were determined according to the manufacturer's directions provided with Reveal Q^+ kits (Neogen®Corporation, Lansing, MI, USA). Briefly, the one Kg samples collected from rural households were thoroughly mixed and 500g was ground with a blender (OMNIBLEND V-Heavy duty professional blender, TM-800A, JTC-China). The ground samples were stored in plastic bags in a cool, dry place until analyzed. Ten g of a ground sample was weighed into a 250 ml round bottomed flask using a top-loading pan balance (METTLER PJ 300, METTLER instrument AG, CH-8606 Greifensee-Zurich Switzerland). Fifty ml of 65% ethanol was added to the flask and mycotoxins were extracted by shaking the mixture for 3 minutes. The mixture was filtered through fluted filter paper (Whatman No 1, WHATMAN International LTD, Mad stone, England) and both aflatoxin and fumonisin assays were performed on the 65% ethanol extract. The Reveal Q^+ kits for aflatoxin and fumonisin quantitation are single-step lateral flow immunochromatographic assays, based on a competitive immunoassay format. Lateral flow strips coated with antibodies interact with antigen (mycotoxin) molecules in the sample extract. The developed strip is removed and inserted into a Reveal Accuscan III Reader System (AS 5130, Neogen®Corporation, Lansing, MI, USA) and the reader displays the aflatoxin or fumonisin content of the sample. The Reveal Q^+ assay for aflatoxin is quantitative for total aflatoxins. The linear range of detection is 2-150 µg/Kg. The Reveal Q^+ assay for fumonisin is semi-quantitative for quantification of B_1 plus B_2. The linear range of detection for B_1 plus B_2 is

1 to 7 mg/Kg. Maize samples were analyzed in duplicate whereas single assays were performed on the rice and groundnut samples.

3.5 STATISTICAL ANALYSES

The differences in group means of the ranked scores for aflatoxin and fumonisin contamination in each district sampled were tested for significance ($\rho < 0.05$) by using the Kruskal-Wallis non-parametric multiple comparison test for all pairwise differences between means. Means and standard deviations for each district were calculated individually using Microsoft Excel 2010

3.6 RESULTS AND DISCUSSION

3.6.1 AFLATOXIN CONTAMINATION

Almost all maize samples contained detectable levels of aflatoxin. The Reveal Q^+ method is quantitative and the detection limit was 2 µg/Kg and the quantitation range was 2 – 150 µg/Kg for total aflatoxin. The maximum concentration of aflatoxin in maize was 140 µg/Kg (140 ppb) with an overall mean of 8.3 µg/Kg ± 8.2 (8.3 ppb) for maize collected from 90 households (Table 5).

Table 5. Aflatoxin concentrations in raw maize, groundnuts and rice in selected districts in Malawi

Parameter	Maize	Groundnuts	Rice
Overall mean ± SD (µg/Kg)	8.3 ± 8.2	3.3 ± 2.0	10.56 ± 15.0
Range, all samples (µg/Kg)	0.7 - 140	1.2 - 18.5	0.8 - 210
No. of Districts	9	6	4
No. of samples	90	60	42

Table 6. Samples meeting country-specific regulatory limits for aflatoxin in Malawi, the EU and USA

	Maize	Groundnuts	Rice
Maximum tolerable limits (µg/kg) for:			
Malawi (3) *	71	48	39
European Union (3- 4)* *	3	7	0
Codex Alimentarius (4-15) * **	7	5	0
USA (15-20)* * **	2	0	0
No. of samples containing > 20 µg/kg	7	0	3

*Number of samples meeting the Malawian regulation (3 **µg/kg**) for total aflatoxin
Number of samples exceeding the Malawian regulation but meeting the European Union regulation (4 **µg/kg)
***Number of samples exceeding the EU regulation but meeting the Codex regulatory limit (15**µg/kg**)
****Number of samples exceeding the Codex regulatory limit but meeting the United States regulatory limit.

The highest aflatoxin concentrations were observed in the Southern region in the Chikhwawa district with mean of 22.5 followed by Machinga, 18.5 and then Salima with 11.8µg/Kg. (Figure 7). All three districts sampled in the Northern region and two in Central region had mean aflatoxin levels below 3 µg/Kg. About 20% of the households were consuming maize that exceeded the regulatory limit for aflatoxins in Malawi (3 µg/Kg), (Table 6)

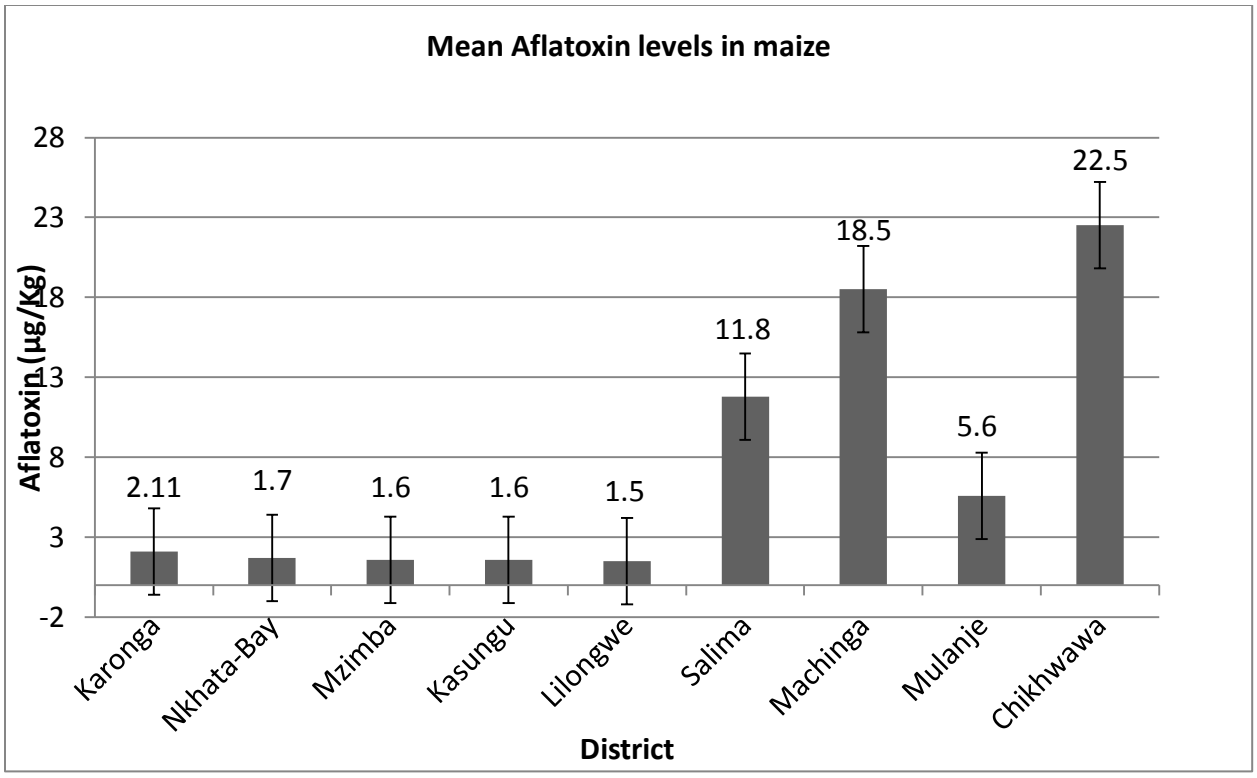

Figure 7. Average aflatoxin concentrations in maize in nine districts representing a cross section of Malawi

Table 7. Statistical comparison of aflatoxin contamination in maize in the Districts sampled

	District								
	MULANJE	NKHATA BAY	KASUNGU	LILONGWE	CHIKHWAWA	MZIMBA	SALIMA	MACHINGA	KARONGA
	47.9	1.3	1.6	1.3	69.8*	1.6	1.5	10.9	2.5
	0.8	1.3	1.4	1.4	140*	3.5	1.4	67.5*	2
	0.9	1.1	1.5	1.7	0.7	1.6	120.7*	1.1	1.4
	0.8	5.7	1.8	1.3	0.8	1.4	5.7	5.5	3.2
	0.8	1.6	1.6	1.6	1.4	1.6	1.8	0.9	1.9
	0.8	1.3	1.1	1.6	1.3	1.2	1.5	42.5*	2
	1.2	1.4	1.5	1.5	5.6	1.6	1.8	10.2	2.2
	1.3	1.1	1.2	1.6	1.2	1.2	1.4	44.5*	1.6
	0.9	1.6	1.2	1.4	2.7	1.3	1.9	1.2	2.2
	0.9	1.1	1.6	1.3	1.2	1.7	1.8	10.9	2.1
Ave (µg/Kg)	5.63	1.75	1.45	1.47	22.47	1.67	13.95	19.52	2.11
SD	14.85	1.40	0.22	0.15	46.51	0.67	37.53	23.35	0.49
Households with aflatoxin >3µg/Kg	1	1	0	0	3	1	2	7	1
	a**	ab	abc	abcd	bcd	bcd	bcd	cd	d

*Values are much greater than other households in same district leading to large standard deviations and skewed means.
** Districts with similar letters beneath them have similar aflatoxin concentrations.

Chikhwawa and Machinga had more households with aflatoxin contamination greater than 3 µg/Kg, the regulatory limit for aflatoxin in Malawi. Based on the group rank scores from the Kruskal –Wallis test, Machinga had the highest contamination and Mulanje had the lowest

(Table 7). There were significant differences in aflatoxin contamination between districts ($p \leq 0.05$).

The maximum aflatoxin concentration in rice was 210 µg/Kg and the mean aflatoxin concentration was 10.6 µg/kg ± 15.0 (Table 5.). The highest aflatoxin concentrations were observed in the Southern region.

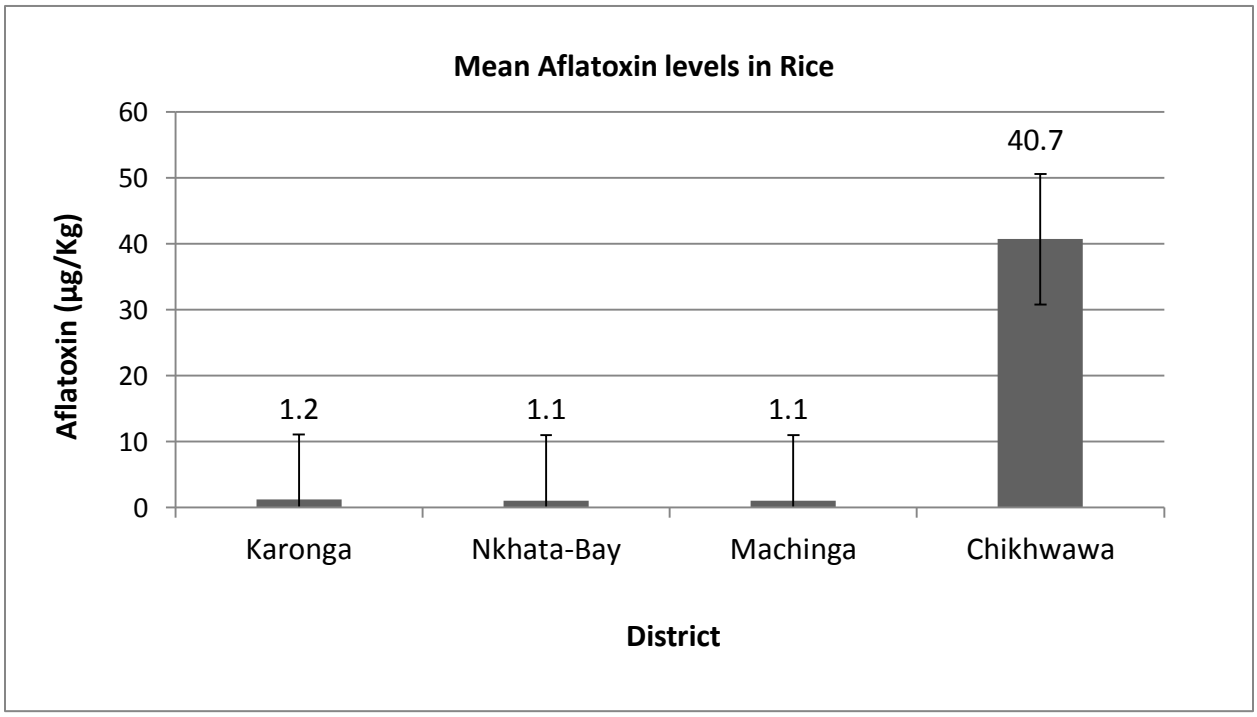

Figure 8. Average aflatoxin concentrations in rice in four districts

Similar to what was found for maize, the maximum total aflatoxin contamination was found in rice samples obtained from the Chikhwawa district. The average aflatoxin concentration in samples from the Chikhwawa district was 40.7 µg/Kg ± 71.25 while mean aflatoxin concentrations for other districts were less than 2 µg/Kg (Figure 8). About 8% of the rice samples exceeded the regulatory limit for aflatoxins in Malawi (Table 6).

Table 8. Statistical comparison of aflatoxin contamination in rice of the Districts sampled

	District			
	MACHINGA	NKHATA-BAY	KARONGA	CHIKHWAWA
	1.2	1.1	1.1	105.6*
	1	1.3	1.1	1.2
	1.4	0.9	1.3	1.1
	1.1	1	1.3	1.2
	1	1	1.2	1.3
	1	1.1	1.4	0.8
	0.9	1	1.1	1.1
	1.1	1.2	1	1.2
	1.3	1.3	1.3	210*
	1.2	1.3	1.2	83.7*
Ave (µg/Kg)	1.12	1.12	1.2	40.72
SD	0.15	0.15	0.12	71.25
Households with aflatoxin >3µg/Kg	0	0	0	3
	a**	a	a	a

*Values are much greater than other households in same district leading to large standard deviations and skewed means.
** Districts with similar letters beneath them have similar aflatoxin concentrations.

From the statistical comparison there was no significant difference ($p \leq 0.05$) in aflatoxin contamination in rice between districts, (Table 8). However, Chikhwawa district had three households with aflatoxin concentration greater than the regulatory limit in Malawi (3 µg/Kg).

In ground nuts the maximum concentration of aflatoxin was 18.5 µg/Kg (18.5 ppb) with a mean of 3.3 ± 2.0 µg/Kg (3.3 ppb), (Table 5).

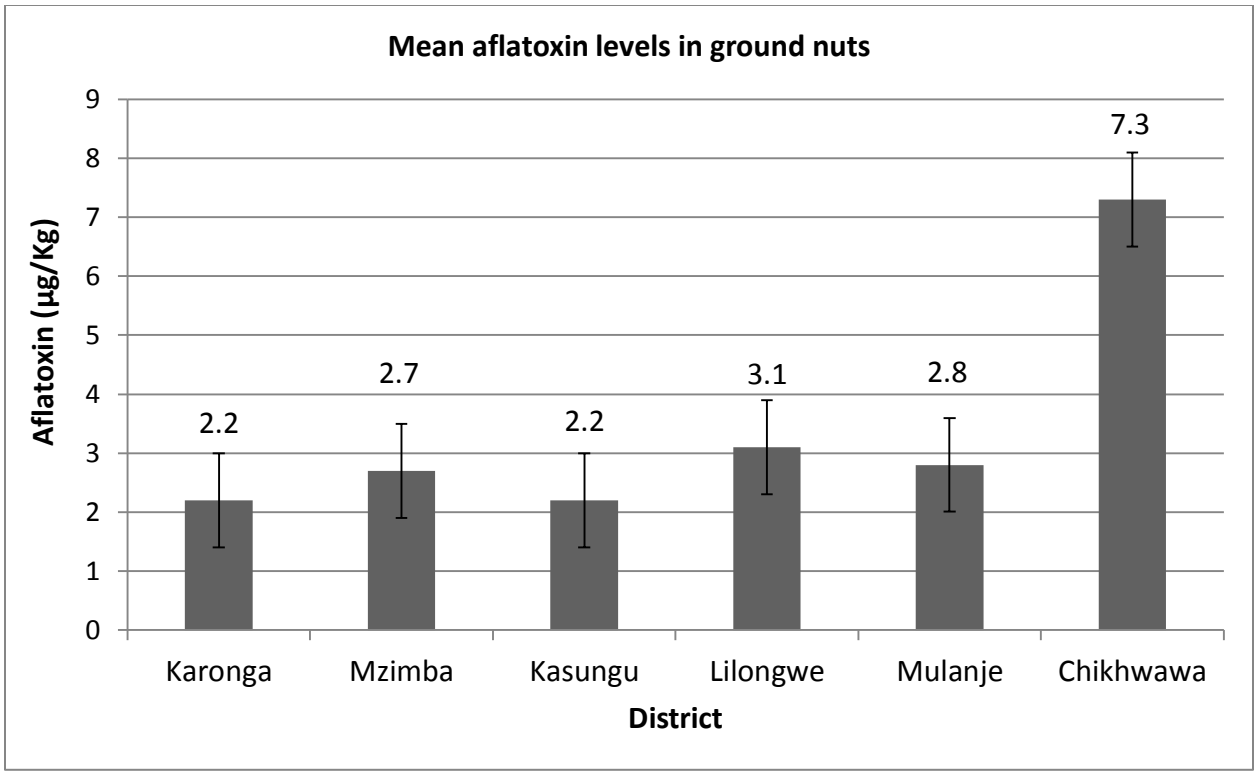

Figure 9. Average aflatoxin concentrations in groundnuts in six districts

The greatest aflatoxin contamination occurred in the Chikhwawa district (mean of 7.3 µg/kg ± 5.47) with six households having concentrations greater than 3.0 µg /kg, (Table 9). The other five districts had an overall mean less than 3.0 µg /kg (Figure 9). But three households in each of the districts; Mulanje, Lilongwe and Mzimba also had concentrations greater than the Malawi regulatory limit. There was a significant difference ($p \leq 0.05$) in aflatoxin contamination between the districts sampled, (Table 9). About 15% of ground nut samples exceeded the regulatory limit for aflatoxins in Malawi and 0% of ground nuts exceeded the regulatory limit for aflatoxins in the EU and US, (Table 6).

Table 9. Statistical comparison of aflatoxin contamination in ground nuts in the Districts sampled

	District					
	KARONGA	KASUNGU	MULANJE	LILONGWE	MZIMBA	CHIKHWAWA
	1.7	3.5	1.3	5.1	1.4	7.6
	1.6	3.4	3.4	9.4	1.8	1.4
	2	3	2.7	2.5	1.3	9.3
	1.6	1.6	2	3.3	1.6	9.8
	2.3	1.6	1.9	1.6	7.2	2.4
	1.9	1.8	0.4	1.9	2.5	12.7
	1.2	1.4	2.7	2.3	2.7	2.9
	1.4	1.2	1.3	1.2	2.6	2.3
	2	1.3	9.2	1	4.4	6.1
	2.1	1.3	3.4	2.7	5.1	18.5
Ave (µg/Kg)	1.78	2.01	2.83	3.1	3.06	7.3
SD	0.34	0.92	2.44	2.51	1.92	5.47
Households with aflatoxin >3µg/Kg	0	2	3	3	3	6
	a**	a	a	a	ab	b

*Values are much greater than other households in same district leading to large standard deviations and skewed means.
** Districts with similar letters beneath them have similar aflatoxin concentrations. Kasungu

had the lowest and Chikhwawa the greatest aflatoxin contamination of maize ($p \leq 0.05$).

Only about 21% of maize, 20% of ground nuts and 8% of rice samples exceeded the tolerable maximum limit for Malawi. These results compare very well with those reported by Sangare-Tigori, et al., (2006) in Ivory Coast, Madbouly, et al., (2012) in Egypt, and Kimanya, et al., (2008) in Tanzania. In a similar study in Malawi, ICRISAT and NASFAM reported very high levels of aflatoxin in maize and groundnuts, 1335 and 3871 µg/Kg, respectively (Monyo et al., 2009). However, in the previous and current study, the southern region in general and the

Chikhwawa district in particular had the greatest aflatoxin contamination. In the previous study this was attributed to high literacy levels in the North as compared to farmers in the Central and Southern regions respectively.

Figures 7 - 9 show mean aflatoxin concentrations for all districts sampled. Samples obtained from the Chikhwawa district had the highest aflatoxin concentrations for all three crops – maize, groundnuts and rice – with mean values of 22.5 μg/Kg ± 46.51 (22.5 ppb) 7.3 μg/Kg ± 5.47 (7.3 ppb) and 40.7 μg/Kg ±71.25 (40.7 ppb) for maize, groundnuts and rice respectively. The highest concentration was observed in rice. Mean values above maximum tolerable limit for Malawi was observed in Chikhwawa for both maize and rice and only in maize in Mulanje, Machinga and Salima. Machinga and Chikhwawa had more households with aflatoxin concentrations greater than the maximum regulatory limit in the country (3 μg/Kg).

In ground nuts all the mean values for the districts sampled were below the maximum limit allowed with a maximum mean value of 2.7 μg/Kg (2.7 ppb) except for Chikhwawa which again had a high mean aflatoxin value (7.3μg/Kg) compared to other districts. The relatively low levels of aflatoxins in groundnuts may be because a groundnut is mainly a cash crop and one of the main export crops in Malawi. As an export crop ground nuts have been subjected for some time now to measures aimed at reducing aflatoxin contamination. In 2009 ICRISAT and NASFAM reported that about 75% of groundnut farmers had knowledge of aflatoxins. They also reported their involvement in training farmers on management of aflatoxins in the crop. It is also interesting to note that districts sampled in the Northern region, Karonga, Nkhata-bay and Mzimba had mean contamination levels below the maximum allowed limit and only Lilongwe and Kasungu in the Central region.

3.6.2 FUMONISIN CONTAMINATION

Unlike aflatoxin almost all maize samples contained non-detectable levels of fumonisin. Seventy-six out of ninety samples (84%) of maize tested had fumonisin levels <1mg/Kg (1 ppm). The maximum concentration of fumonisin (B_1 + B_2) in maize was 7 mg/Kg (7 ppm) with an overall mean of 0.9/Kg ± 1.0 (0.9 ppm) for the maize collected in all the 90 households (Table 10).

Table 10. Fumonisin concentrations in raw maize, groundnuts and rice in selected districts in Malawi

Parameter	Maize	Groundnuts	Rice
Overall mean ± SD (mg/Kg)	0.9 ± 1.0	0.3 ± 0.3	0.4 ± 0.7
Range, all samples (mg/Kg)	0.1 - 7	0.1 – 2.6	0.1 - 7
No. of Districts	9	6	4
No. of samples	90	60	42

The highest fumonisin concentrations in maize were observed in the Southern region in the Chikhwawa district (Figure 10). About 60% of maize samples (6 households out of 10) analyzed in the Chikhwawa district had fumonisin levels > 4 mg/Kg, the maximum regulatory limit in the US, (Table 11).

Table 11. Samples meeting country specific regulatory limits for fumonisins in the EU and USA

	Maize	Groundnuts	Rice
Maximum tolerable limits (mg/kg) for:			
European Union (<1)*	76	53	40
USA (1 - 4)**	5	1	0
No. of samples containing > 4 mg/kg	9	0	2

*Number of samples meeting the regulatory limit in the European Union (1 mg/kg) for total fumonisin

**Number of samples exceeding the European Union regulatory limit but meeting the United States regulation (4 mg/kg)

There was a significant difference ($p \leq 0.05$) in fumonisin contamination between the districts with Chikhwawa having the greatest contamination and Kasungu the lowest. In spite of having more households with high fumonisin concentrations Chikhwawa was still not significantly different from districts; Nkhata Bay, Machinga, Karonga, Mulanje and Lilongwe, (Table 12).

Table 12. Statistical comparison of fumonisin contamination in maize in the Districts sampled

	District								
	KASUNGU	MZIMBA	SALIMA	LILONGWE	MULANJE	KARONGA	MACHINGA	NKHATA BAY	CHIKHWAWA
	0.1	0.1	0.1	0.1	0.2	0.6	0.9	0.8	0.4
	0.1	1.9	0.1	0.1	0.1	0.2	0.9	1	7*
	0.1	0.1	7*	0.1	0.2	1.4	0.1	1.8	7*
	0.1	0.1	0.7	0.2	0.1	0.8	0.2	0.2	7*
	0.1	0.1	0.2	0.5	0.2	0.1	0.2	0.1	7*
	0.1	0.4	0.1	0.1	0.1	0.3	0.4	0.2	0.1
	0.1	0.1	0.1	0.1	0.2	2.6	0.1	0.5	0.4
	0.1	0.1	0.1	0.1	0.4	0.1	0.4	0.1	7*
	0.1	0	0.2	0.4	0.2	0.2	0.5	4.1	0.1
	0.1	0.1	0.1	1.2	0.1	0.2	0.9	0.3	7*
Ave (mg/Kg)	0.1	0.3	0.87	0.29	0.18	0.65	0.46	0.91	3.93
SD	1.46 E-17	0.57	0.20	0.35	0.09	0.80	0.33	1.24	0.17
Households with $FB_{(1+2)}$ >5***mg/ Kg	0	0	1	0	0	0	0	0	6
	a**	a	ab	ab	b	b	b	b	b

*Values are much greater than other households in same district

** Districts with similar letters beneath them have similar $FB_{(1+2)}$ concentrations. Kasungu had the lowest and Chikhwawa the greatest $FB_{(1+2)}$ contamination of maize ($p \leq 0.05$).

*** codex proposed maximum allowed limit

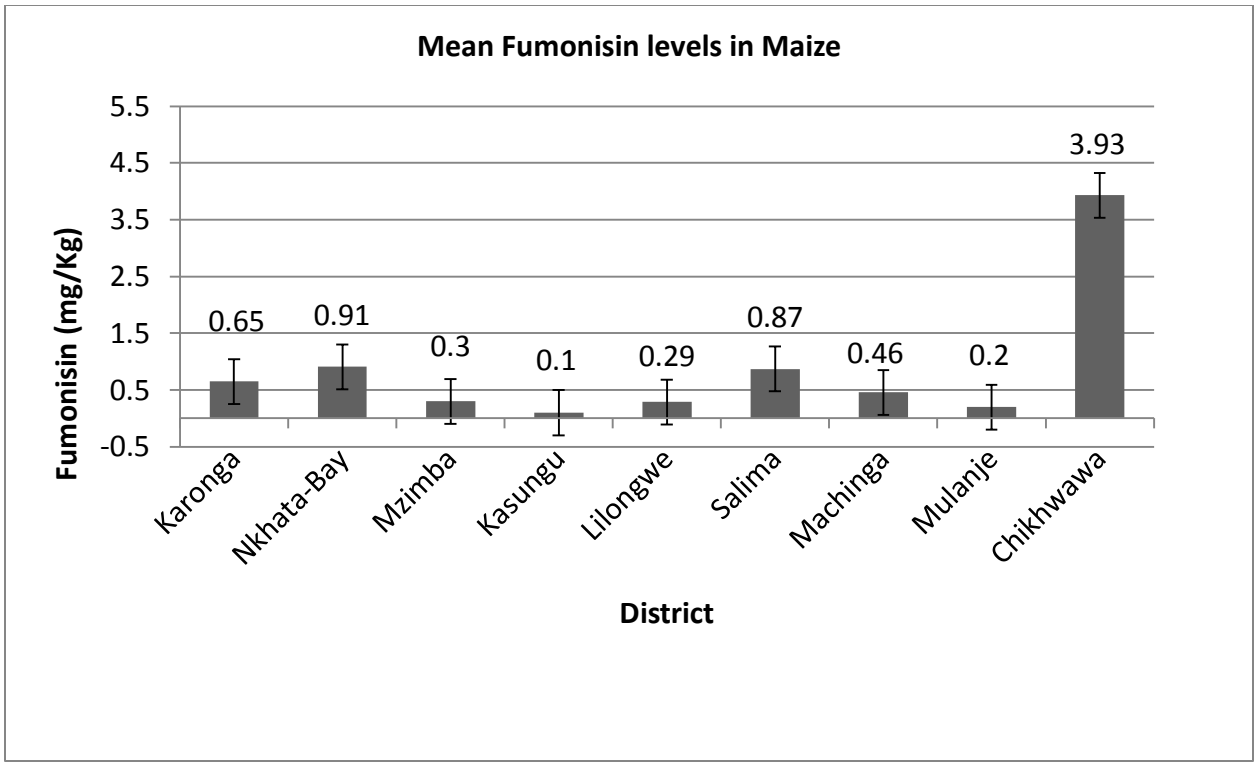

Figure 10. Average fumonisin concentrations in maize in nine districts representing a cross section of Malawi

The maximum fumonisin ($B_1 + B_2$) concentration in rice was 7 mg/kg, and the mean fumonisin concentration was 0.4 mg/kg ± 0.7 (Table 10.). As in maize, the maximum concentration of fumonisin in rice was observed in the Chikhwawa district with two households having contamination levels greater than 5 mg/kg, the Codex proposed maximum allowed limit, (Table 11). In spite of this there was no significant difference ($p \leq 0.05$) in fumonisin contamination, (Table 13) between the districts.

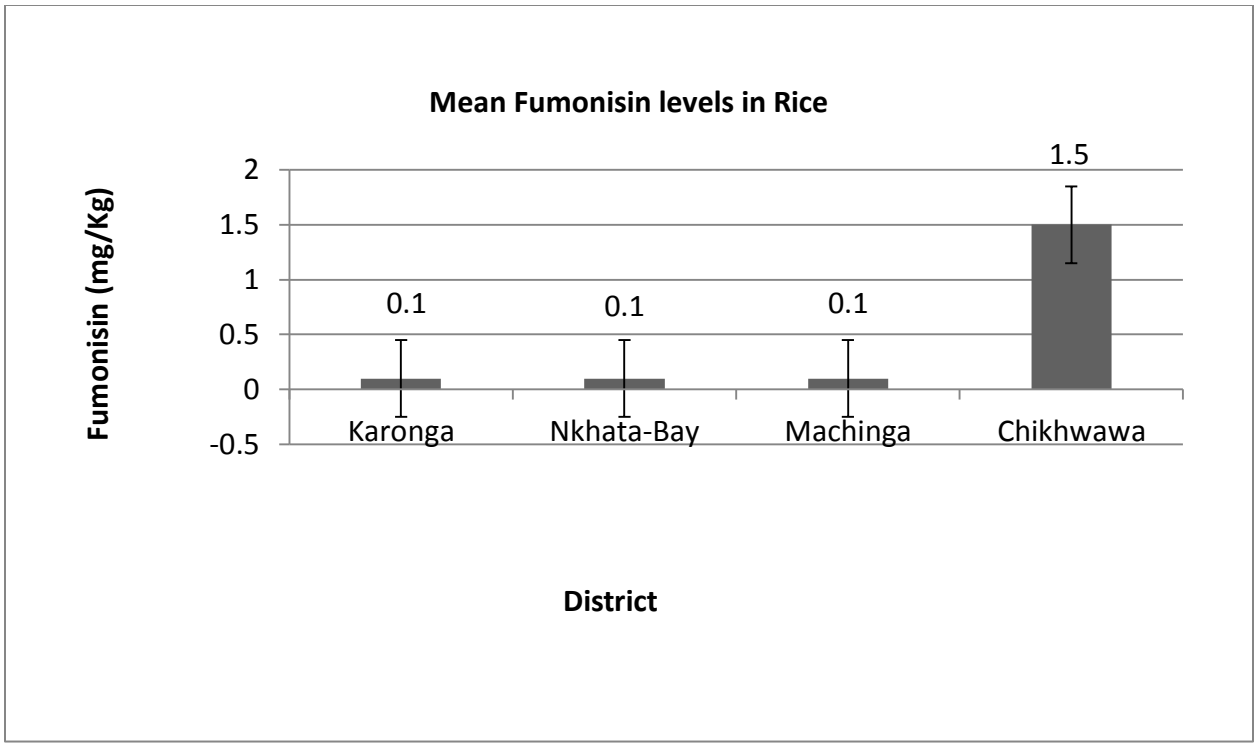

Figure 11. Average fumonisin concentrations in rice in four districts

Forty-one out of forty-three rice samples collected in all four districts (95%) had non-detectable levels of fumonisin ($B_1 + B_2$).

Table 13. Statistical comparison of fumonisin contamination in rice in the Districts sampled

	District			
	NKHATA-BAY	MACHINGA	KARONGA	CHIKHWAWA
	0.1	0.1	0.1	7*
	0.1	0.1	0.2	0.1
	0.1	0.1	0.1	0.1
	0.1	0.1	0.1	0.1
	0.1	0.1	0.1	0.1
	0.1	0.1	0.1	0.1
	0.1	0.1	0.1	7*
	0.1	0.1	0.1	0.1
	0.1	0.1	0.1	0.1
	0.1	0.1	0.1	0.1
ave	0.1	0.1	0.11	1.48
SD (mg/Kg)	1.46E-17	1.39E-17	0.032	2.91
Households with $FB_{(1+2)}$>5***mg/Kg	0	0	0	2
	a**	a	a	a

*Values are much greater than other households in same district leading to large standard deviations and skewed means.

** Districts with similar letters beneath them have similar $FB_{(1+2)}$ concentrations.

The maximum fumonisin ($B_1 + B_2$) concentration in groundnuts was 2.6 mg /kg, and the mean fumonisin concentration was 0.3 mg/kg ± 0.3 (Table 10.). The maximum contamination in groundnut samples was observed in the Chikhwawa district and the lowest in Kasungu. There was a significant difference ($p \leq 0.05$) in contamination between districts, (Table 14).

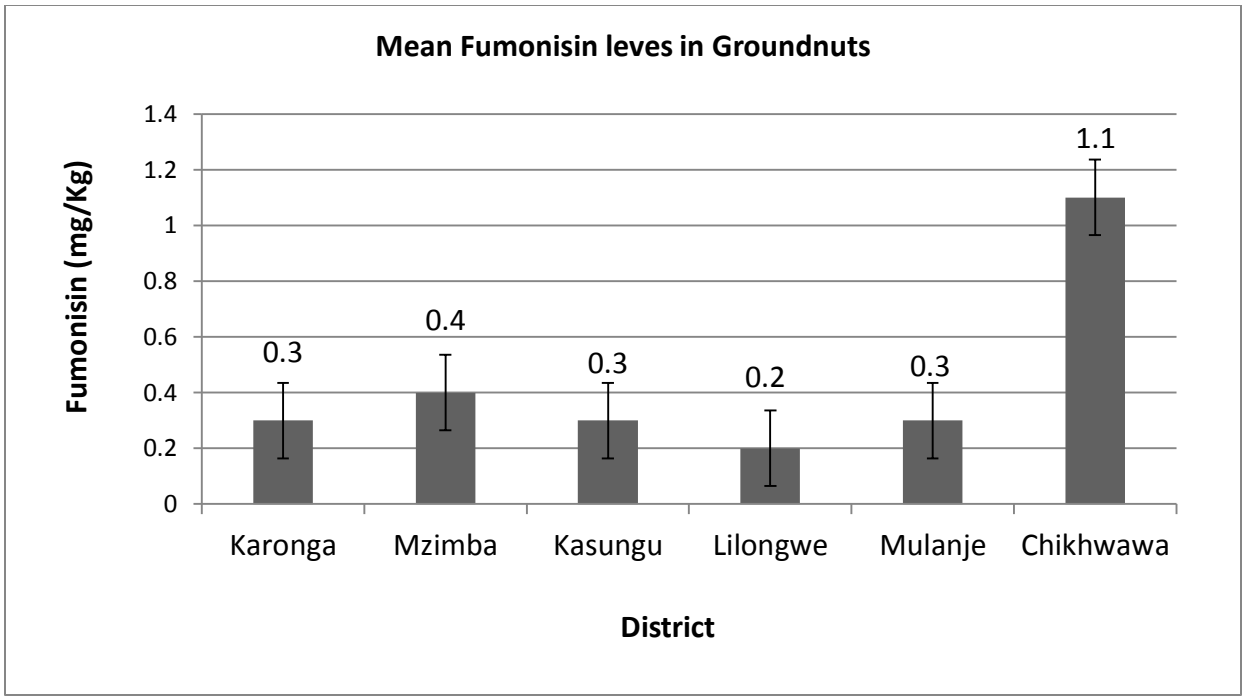

Figure 12. Average fumonisin concentrations in groundnuts in six districts

Table 14. Statistical comparison of fumonisin contamination in ground nuts in the Districts sampled

	District					
	KASUNGU	LILONGWE	MULANJE	MZIMBA	KARONGA	CHIKHWAWA
	0.1	0.2	0.1	0.2	0.2	2.3
	0.1	0.2	0.2	0.2	0.3	1.5
	0.1	0.1	0.7	0.2	0.4	0.8
	0.1	0.1	0.3	0.3	0.3	2.1
	0.1	1.1	1.1	0.4	0.3	1.2
	0.1	0.1	0.2	0.3	0.3	1.1
	0.1	0.1	0.1	0.3	0.3	0.1
	0.1	0.1	0.1	0.3	0.3	0.2
	0.1	0.2	0.1	0.3	2.6	1.3
	0.1	0.1	0.3	0.3	0.2	0.4
ave	0.1	0.23	0.32	0.28	0.52	1.1
SD (mg/Kg)	1.46E-17	0.31	0.33	0.063	0.73	0.75
Households with $FB_{(1+2)}$>5***mg/ Kg	0	0	0	0	0	0
	a**	ab	b	bc	c	dc

*Values are much greater than other households in same district

** Districts with similar letters beneath them have similar FB $_{(1+2)}$ concentrations.

Currently, fumonisins are not being regulated in Malawi and as such, levels of maximum contamination have not yet been set. Meanwhile, Codex Alimentarius has yet to pass a standard for fumonisins in food, although there is a proposed maximum limit of 5 mg/Kg. Only about 10% of maize, 0% of ground nuts and 5% of rice samples exceeded the maximum tolerable limit for the European Union and United States (Table 11). These results compare very well with

those reported in other studies by Madbouly, et al., (2012) in Egypt and Kimanya, et al., (2008) in Tanzania. Similar results were also reported by Fandohan, et al., (2005) in Benin with total fumonisins ranging from 0.6-2.4 mg/Kg in Zimbabwe and the Transkei region of South Africa, (Gamanya, et al., 2001 and Shephard, et al., 2007). In two of the samples from the Chikhwawa district, both aflatoxins and fumonisins were present in high concentrations in one of each maize and rice samples. The levels were 105.6 µg/Kg and 7 mg/Kg in rice for aflatoxins and fumonisins respectively and 140 µg/Kg and 7 mg/Kg in maize for aflatoxins and fumonisins respectively. This is in agreement with results from Tanzania where aflatoxins and fumonisins were found to coexist (Kimanya, et al., 2008).

Malawi is a maize-deficient country. The staple food shortage in the country was estimated at 700,000 tons in 2002 (Akinifesi, et al., 2006). The major causes of food shortage in Malawi include sporadic rainfall and frequent droughts, high fertilizer costs and lack of farm input loan facilities. Food safety in Malawi is subject to issues of food security, especially where food shortages are caused by natural phenomena such as drought. Many subsistence farmers in Malawi and in Africa in general (Shephard, 2003) are reliant on the consumption of home-grown crops, irrespective of the quality considerations normally applied in the developed world. Even with adequate crops, poor traditional storage facilities often lead to deterioration of these crops. Given these harsh realities, it is not surprising that mycotoxin contamination, (aflatoxins in particular) of staple foods in this study was detected in most of the samples collected. Although only recognized during the previous century, human and animal mycotoxicoses resulting from fungal contamination have presumably existed for centuries in Africa (Shephard, 2003), none of which have been reported in Malawi either due to lack of proper documentation or due to ignorance at the local level, or as noted from the results most of the contamination is at low

levels which may lead to chronic effects after a long term of exposure. Symptoms due to low, chronic intakes are difficult to associate with mycotoxin consumption.

Matumba et al., (2009) reported that most of the traditional methods of processing maize reduce aflatoxins by an average of 40% with the best process reducing aflatoxin levels by 80%. Although most of the maize was contaminated, 82% of the aflatoxin contamination was less than 3 μg/Kg. With a 40% reduction in contamination by processing, acute mycotoxicoses from aflatoxin and fumonisin is unlikely. Apart from outbreaks of acute aflatoxicosis, aflatoxin exposure is thought to substantially contribute to the disease burden of African communities due to chronic consumption of low levels of aflatoxins.

Studies on the correlation between the incidence of primary hepatocellular carcinoma and human exposure to aflatoxins in a number of African countries (Kenya, Mozambique, and Swaziland) helped demonstrate the role of aflatoxin as a human carcinogen (William et al., 2004). The relationship between aflatoxins and the childhood disease of kwashiorkor is not clear. Although kwashiorkor is widely thought to be a form of protein energy malnutrition, some characteristic features of the disease are known to be among the pathological effects caused by aflatoxins in animals. The prevalence and level of human exposure to mycotoxins in Malawi is not well documented nor assessed. However, the prevalence and level of human exposure to aflatoxins on a global scale has been reviewed and the resulting conclusion was that approximately 4.5 billion persons living in developing countries, including Malawi, are chronically exposed to largely uncontrolled amounts of the toxin (Williams, et al., 2004). In locations where aflatoxin exposure has been studied, chronic low level intakes result in poor nutrition and immunity status (Williams, et al., 2004). The aflatoxin exposure and the toxic effects of aflatoxins on immunity

and nutrition combine to negatively affect health factors, including HIV and AIDS which is currently a major issue in Malawi.

This is the first time fumonisins have been studied in Malawi, and as seen in Table 10 and in Figures 10-12, levels greater than 6 mg/Kg were detected in both maize and rice. These are the same staple crops with high aflatoxin contamination as well. With these findings it means people in the affected areas are at risk of the negative effects of mycotoxin ingestion. The fact that fumonisins have been detected it is a clear indication that other Fusarium mycotoxins are likely present (Sangare-Tigori, et al., 2006) putting people at even higher risk.

3.7 CONCLUSION

This study confirms that aflatoxins and fumonisins are widespread contaminants of maize and rice and that fumonisins are not common contaminants of groundnuts as compared to aflatoxins in food intended for human consumption in Malawi. It shows that populations in the rural areas of Malawi are at a high risk of exposure to unacceptably high levels of aflatoxins and fumonisins especially in the Chikhwawa and Machinga districts in the Southern part of the country where relatively high levels of both aflatoxins and fumonisins were observed in maize and rice. The findings of this study should trigger further research that will generate data on the aflatoxin and fumonisin exposure among Malawians especially children.

3.8 WAY FORWARD

There is need for further analysis using fully quantitative methods like high pressure liquid chromatography (HPLC) to fully quantify these mycotoxins and also specifically determine how much of each specific aflatoxin (AFB_1, AFB_2, AFG_1 AFG_2) and fumonisin (FB_1, FB_2) are present. This would be particularly interesting especially in Chikhwawa and Machinga which had more households with aflatoxin concentrations above the Malawi regulatory limit and where high contamination levels were observed for both aflatoxins and fumonisins in both rice and maize.

Based on our study, there clearly is a need to assess the extent of exposure in the rural population using biomarkers. People in rural areas should also be made aware of the existence of these contaminants in their food crops and the risks associated with them.

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