AFLATOXINS CONTAMINATION IN SPICES AND ASSOCIATED PREDISPOSING FACTORS IN MOROGORO REGION, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PUBLIC HEALTH AND FOOD SAFETY

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ABSTRACT

Aflatoxins contamination in spices can result to serious health impact to consumers. Little information is available about levels of aflatoxins in spices traded in Tanzania. This study investigated awareness on aflatoxins and handling, storage and packaging practices of spices in relation to aflatoxins contamination as well as levels in black pepper, cinnamon, cloves and turmeric in Morogoro, Tanzania. A total of 120 spices samples were collected from 52 spice dealers among spice traders and analyzed for aflatoxins B_1, B_2, G_1, G_2, total aflatoxins and moisture content. Aflatoxins were analyzed using HPLC with fluorescence detector. The association of aflatoxins contamination in spices with traders' demographic and sociological characteristics, awareness and handling practices were determined using a questionnaire. Results showed that 24.2% of the spice samples were contaminated with aflatoxins; whereby 11.7% was with aflatoxin B_1 ($AFB_{1)}$. Spices from Morogoro rural had high rate of aflatoxin contamination (33.3%) compared to those from Morogoro municipality (15.0%). Turmeric had the lowest contamination rate of AFB_1 (0.0%) and total aflatoxins (3.3%) while cloves had highest contamination with 20% and 50.0% for AFB_1 and total aflatoxins, respectively. Lowest aflatoxins contamination was 0.2 µg/kg in cloves while the highest was 164.9 µg/kg in black pepper. Percentage of spice samples exceeded European Union regulatory limit was nine point two percent for AFB_1 and 13.3% for total aflatoxins. Majority (96.2%) of spice traders store their spices and storage time varied from 0 to 100 weeks. Among spice dealers who were doing storage, 34.9% of their samples had aflatoxin contaminations. Half of the respondents who were doing sorting, 65.4% of them discarded the rejected spices. Aflatoxin contaminations for the samples from respondent who were not doing sorting was 61.5%. Occurrence of aflatoxins in spices was associated ($p>0.05$) with sorting, criteria for sorting, handling of rejected spices, storage practices, awareness on causes of spoilage and awareness on aflatoxins in food. It is concluded that spices in the study area were highly contaminated with aflatoxins some of them beyond the maximum limit set by EU. The study revealed limited awareness and knowledge on aflatoxins contamination in spices among spice traders. Awareness creation on aflatoxins contamination in spices and preventive strategies need to be considered to reduce aflatoxins contamination to safeguard health of spices consumers in Morogoro, Tanzania.

DECLARATION

I, Lilian Gabriel Peter 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.

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DEDICATION

I dedicate this dissertation to my husband, my lovely children Moreen and MicahKlodo and my young sister Vicky for their patience, strength, support and love throughout the study period.

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LIST OF ABBREVIATIONS AND SYMBOLS

AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
AFM_1	Aflatoxin M_1
AFM_2	Aflatoxin M_2
AFTs	Aflatoxins
AOAC	Association of Officiating Analytical Chemists
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GAP	Good Agricultural Practices
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis of Critical Control Points
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HPLC	High Performance Liquid Chromatography
ISO	International Organization for Standardization
ITC	International Trade Centre
LDCs	Least Developed Countries
LOD	Limit of Detection
LOQ	Limit of Quantification
MC	Moisture Content
NCDs	Non-communicable diseases
ORCI	Ocean Road Cancer Institute

SEM	Standard Error Mean
SPE	Solid Phase Extraction
TBS	Tanzania Bureau of Standards
TZS	Tanzania Standard
WHO	World Health Organization of the United Nations

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Mycotoxins are toxic secondary metabolites produced by species of filamentous fungi such as *Aspergillus*, *Penicillium* and *Fusarium* that can grow in a wide range of foods including cereals, nuts, spices, dried fruits, apples and coffee beans, often under warm and humid conditions (Naz *et al.*, 2016). Mycotoxin contaminations in foods are recognized as a serious problem in many parts of Africa due to their impacts on human and animal health, economy and trade (WHO, 2018). Aflatoxins are one of the highly toxic mycotoxins to human and livestock (WHO, 2018). Aflatoxins contamination in African foods and food commodities exhibits a serious threat to human and animal health over the past few decades (Chauhan, 2017). Increased risk of hepatocellular carcinoma in the presence of hepatitis B virus infection has been associated with aflatoxins contamination of food in most developing countries in Africa (Williams *et al.*, 2004). Chronic exposure due to low levels of aflatoxins contamination consumed regularly, may increase liver cancer risks and can suppress the immune system, particularly for the population with hepatitis B virus (HBV), while high levels can cause acute poisoning and even death to human (WHO, 2018).

Spices are group of plant substances valued for their high mineral content, with strong taste and aroma and being used in small amounts as flavoring agents in various foods, as antioxidants, colorants, and preservatives (El-Sayed and Youssef, 2019). Despite its importance, spices can be contaminated with mycotoxins due to their pre-harvest, post-harvest, and storage conditions (Potorti, 2020). Spices cultivated in tropic and sub-tropic areas can be exposed to contamination with toxigenic fungi and subsequently mycotoxins before and after harvest (Kabak and Dobson, 2016). This is due to high temperature, humidity, and rainfall which favor mould growth resulting into aflatoxins contamination (Hussain *et al.*, 2012). Aflatoxins contamination in spices has been reported in many countries including Hungary (Fazekas *et al*., 2007) and Kenya (Mwangi *et al*., 2014). In Tanzania, a study conducted on commonly used spices from local markets and Indian shops in Dar es Salaam revealed fungal contamination in red chill 18.37%, ginger (14.28%), curry powder (4.04%) and fenugreek (2.04%) (Temu, 2016).

Poor storage and handling practices along the value chain subject spices to fungi contamination (Nguegwouo *et al*., 2018). Studies have been conducted to determine the relationship between awareness, social demographic and economic characteristics on fungal contaminations in maize, groundnuts and animal feeds. The findings indicated that almost all (97%) of the respondents were not aware of the mould growth in stored maize and groundnuts. Also there was a significant association between awareness and socio-demographic and economic characteristics and mycotoxins contaminations (Magembe *et al*., 2016; Ngoma *et al*., 2017; Ayo *et al.*, 2018). Very little has been reported on the relationship between handling practices, awareness and the occurrence of aflatoxins in spices in Tanzania.

Aflatoxins contamination problem in Tanzania has been reported mostly on maize (Seetha *et al.*, 2017; Kamala *et al*., 2018) and groundnut (Kuhumba *et al*., 2018). Tanzania Initiative for Preventing Aflatoxin Contamination (TANIPAC) project has been established with the purpose of minimizing aflatoxins occurrence in the food system through an integrated approach in the maize and groundnuts value chains (AFDB, 2018). The overall aim of improving food safety and food security, which will ultimately improve the health and nutrition of the communities, improve agricultural productivity, and boost trade (AFDB, 2018).

1.2 Justification

There is increased reports of cancer cases in particular liver cancer with unknown cause some of them being associated with ingestion of aflatoxins contaminated foods (Kimanya *et al*., 2021). According to GloboCan (2020) there is increase on incidences for cancer cases in Tanzania in which new cancer cases for 2020 was 40,464 while the number of death also increases from 19,900 (WHO, 2014) to 26, 945 (GloboCan, 2020). Aflatoxin is the global food safety concern since they can contaminate food crops and pose a serious health threat to humans and livestock (WHO, 2018). There is limited information regarding aflatoxins contamination of spices traded in Tanzania. Thus data are needed for setting monitoring measures.

Although spices are valued for their distinctive flavours, colours and aroma and are among the most versatile and widely used ingredients in food preparation and processing throughout the world, they can be a source of microbial contamination such as moulds (Temu, 2016). Mycotoxins contamination has been reported in various food products in Tanzania including maize (Nyangi *et al*., 2016; Kamala *et al*., 2018; Sasamalo *et al.*, 2018), peanut-enriched complimentary flours (Kuhumba *et al*., 2018) while information on aflatoxin contamination in spices is limited. Temu (2016) reported fungal contaminants in common spices used in Tanzania, one of them being *A. flavus* which is capable of producing aflatoxins. Fundikira (2018) reported aflatoxins contamination of marketed spices in Dar es Salaam. This study aimed at assessing the storage and handling practices and levels of aflatoxins contamination in selected spices in Morogoro Tanzania.

The information from this study will establish the magnitude of the problem and facilitate establishment of appropriate strategies to prevention aflatoxins contamination in spices and exposure to consumers hence reducing the impacts associated with aflatoxins contamination in the country.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to carry out surveillance of aflatoxins contamination in commonly used spices and the predisposing factors in Morogoro region, Tanzania.

1.3.2 Specific objectives

The specific objectives of the study were to;

i. Determine presence of aflatoxins in cinnamon, black pepper, turmeric and cloves traded in Morogoro municipality and Morogoro rural districts.

ii. Assess awareness, handling, packaging and storage practices associated with aflatoxin contamination of spices in Morogoro municipality and Morogoro rural districts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Spice Production in Tanzania

Spices are one of the important commodities used all over the world as ingredients to flavour different types of prepared food items or beverages, condiment and as ingredients of medicines and cosmetics (Naz, 2016; Jiang, 2019; Thanushree *et al.*, 2019). Spices are consumed in small quantities, but used in a wide range of foods and food products representing a unique segment within the food sector (Székács *et al*., 2018). There are around 40 to 50 spices of global economic and culinary importance (FAO, 2005).

Between 1995 and 1999, Tanzania ranked the third among the Least Developed Countries (LDCs) by exporting 5 % of LDCs' total spice exports due to the fact that the climatic and soil conditions of Tanzania favors spice production (Maerere and Noort, 2014). Madagascar was the largest LDC exporter (72%) followed by Comoros (6%), but total LDC exports fulfilled only 5.5% of global import demand (ITC, 2014). Between 2006 and 2011, Tanzania's share in the global spice trade was only 0.36% (ITC, 2014). The major spices produced in Tanzania include clove, pepper, ginger, cardamom, cinnamon, chillies, coriander, lemon grass, nutmeg and vanilla (Maerere and Noort, 2014). The main spices producing regions include Tanga, Morogoro, Zanzibar, Pemba, Mbeya, Kilimanjaro, Kigoma, Ruvuma, Singida, Iringa and Coast region (Maerere and Noort, 2014). Both cultivation and trade in spices increased from the year 2014 to 2018 (FAOSTAT, 2020). The amount produced depended on the type of the spice and the season of the year (ITC, 2014). In 2014, cloves produced were 2800 Mt, peppers 3000 Mt and cinnamons 1500 Mt (ITC, 2014).

Spice have been used since ancient times as flavour and aroma enhancers, colorants, preservatives and traditional medicines (Singab and Eldahshan, 2015; Kabak and Dobson, 2016). Various spices can perform other functions as nutrient sources, antioxidants, antimicrobials, preservatives, insecticides and as medicinal plants for human use (Fasoyiro, 2015). Spices and herbs such as clove, rosemary, sage, oregano, and cinnamon are excellent sources of antioxidants given their high content of phenolic compounds (Embuscado, 2015). The active components in the spices are phthalides, polyacetylenes, phenolic acids, flavanoids, coumarines, triterpenoids, serols and monoterpenes which are powerful tools for promoting physical and emotional wellness (Sharma, 2015).

The nutritional composition of spices varies significantly (Ereifej *et al.,* 2015). The exact chemical composition of spices have been reported to be greatly influenced by many factors such as part of the plant used, its vegetative state, environmental conditions and harvesting technique (Cosentino *et al.,* 1999). Generally spices are high in ash and fibre, contain a good amount of minerals like potassium, manganese, sodium, iron, and magnesium (Moawad *et al.,* 2015). It is evident that frequent consumption of spicy foods was also linked to lower risk of death from cancer and ischemic heart and respiratory system diseases (Jiang, 2019). Despite of nutritional values, spices can be contaminated with mycotoxins due to their pre-harvest, post-harvest and storage conditions (Potorti, 2020). Factors that affect aflatoxins contamination include the climate of the region, the variety of the crop planted, soil type, minimum and maximum daily temperatures, pre and post-harvest handling and daily net evaporation (Bankole and Mabekoje, 2004; Algabr *et al.,* 2018)

2.2 Overview on Mycotoxins

Mycotoxins are secondary metabolites produced by different fungal species and are found in diverse agricultural crops worldwide and pose a severe threat to public health (Al-Jaal *et al*., 2019).. Fungal growth in spices may occur during harvesting, processing, storage and handling (Al-Jaal *et al*., 2019). Contamination of foods by fungi and subsequent synthesis of mycotoxins in situ is a global threat (WHO, 2018). Mycotoxins continue to pose a health concern via human exposure to contaminated food (Nleya *et al.,* 2018). Mycotoxins contamination is becoming a serious problem in Africa resulting to negative effects on human and animal health, economy and trade (Zain, 2011). The most frequently investigated mycotoxins in Tanzania are aflatoxins and fumonisins (Nyangi *et al.*, 2016; Kamala *et al.*, 2018; Sasamalo *et al.*, 2018).

Mycotoxins exposure from foodstuffs can trigger serious health hazards (e.g. cancers, deformity and mutation), which have been a global public health concern (WHO, 2018). In recent years, large-scale poisoning incidents and international trade disputes caused by fungal contamination are extremely common (Yang *et al*., 2020).

2.3 Aflatoxins Contaminations in Food

Aflatoxins are one of the mycotoxins regularly found in nuts (WHO, 2018), dried fruits (Wei, *et al.*, 2017), spices (Fundikira, 2018), oil seeds (Mohammed *et al.,* 2018), cereals (Kimanya, 2014; Sasamalo *et al*., 2018) milk and animal feed (Mohammed *et al*., 2016). Aflatoxins are highly toxic metabolites of several *Aspergillus* species widely distributed throughout the environment (Mutegi *et al.*, 2018). The aflatoxin producing moulds are widely spread in nature and have contaminated food supplies of human and animals (Kumar *et al.*, 2017). The toxin is produced by the fungus *Aspergillus flavus* (Spanjer, 2019). Its contamination can occur before harvest, during the time between harvesting and drying, and in storage (Spanjer, 2019). Aflatoxins exist in six forms, four occur in food; B_1, B_2, G_1 and G_2 while M_1 and M_2 are metabolites of B1 and B2; respectively (Nejad and Giri, 2015). The severity of toxicity associated with aflatoxins varies with the type, arranged from the highest to the lowest in the following order: $B_1 > G_1 > B_2 > G_2$ (Cort´es *et al.,* 2010).

In many lower-income countries, aflatoxins pose serious public health concern since the occurrence of these toxins can be considerably common and even extreme (Udomkun *et al.,* 2017). 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 (Mahato, 2019). The most prominent contamination has been faced in maize, groundnuts, pistachio nuts, chestnuts, cottonseed, wine-grapes, spices, and other grain crops (Iqbal *et al.*, 2012).

The contamination by aflatoxins have been reported in foods and feeds such as groundnuts, millet, sesame seeds, maize, wheat, rice, spices, cocoa and dried fruits due to fungal contamination occurred due to pre- and post-harvest conditions (Martinez-Miranda *et al.,* 2019; Mahato *et al.,* 2019). Aflatoxins and their metabolites in foods have been implicated in many human diseases including cancer (Williams *et al.*, 2004). Aflatoxins and fumonisins have been implicated to the increasing cases of esophagus cancer, ranked third cause of deaths caused by cancer in Tanzania and fifth in the world (GloboCan, 2018). Moreover, most reported cases of esophagus cancer were related to young age and people from rural areas (Mchembe *et al.*, 2013), this might be due to the fact that staple food in particular maize is the main dish in most of the rural areas and majority of people consume this staple foods such as porridge at early age.

Aflatoxin contamination in various crops also impacts trade and economic growth. Codex Alimentarious Commission established aflatoxin maximum level in food stuffs being 5 ppb for aflatoxins B1 and 10 ppb for total aflatoxins and commodities exceeding these aflatoxin thresholds levels cannot enter lucrative premium markets, particularly in developed countries where aflatoxins are strictly monitored and regulated (Wu, 2015). Wu (2006) estimated annual loss of $163 million on average to US maize growers from aflatoxin.

The best way to avoid contamination is prevention, for which control strategies are proposed. Poor adoption of scientific practices across the spice supply chain is the prime cause of mycotoxins contaminations (Thanushree *et al*, 2019). Strategies to control mycotoxins in Africa can be broadly divided into: stopping the infection process (host plant resistance, bio-control); control of environmental factors (temperature, rainfall, relative humidity, evapo-transpiration, soil type) including efforts to build predictive models; crop management strategies (Good Agricultural Practices (GAP), pre and post-harvest management); post-harvest strategies (harvesting, drying, storage, use of plant extracts and preservatives) and decontamination (sorting, processing). The most effective way to reduce aflatoxins contamination in susceptible crops is particularly by stopping or reducing the growth of the causative toxigenic fungi (Liu *et al.*, 2020). Fungal infection at post-harvest handling can be minimized by quick and hygiene drying process, sortation and separation of physical damage/injury with intact seeds/kernels and keep dried stored-spices in sanitized container or polyethylene bag (Nurtjahja, 2019).

Regulatory limit for aflatoxins specific for spices is not yet established in Tanzania, however, many of the spices dealers used European Union provide the limit of 5 μg/kg and 10 μg/kg for aflatoxin B_1 and total aflatoxins respectively (EC, 2020). Limits are available for other food products such as maize grain (TBS, 2017), milled maize products (TBS, 2018), ground nuts (TBS, 2018) and peanut butter (TBS, 2014).

2.4 Aflatoxin Contamination Status in Tanzania

In Tanzania, aflatoxin contamination has been reported in maize (Kimanya *et al.*, 2010; Kimanya *et al.*, 2014; Amri and Lenoi, 2016; Magoha *et al.*, 2016; Kamala, 2018); in cassava (Manjula *et al*., 2009); in spices (Fundikira, 2018); complementary flours (Kuhumba *et al*., 2018); oil seeds (Mohammed *et al*., 2018) and in animal feed (Kajuna *et al.*, 2013). Majority of the Tanzanian are exposed to mycotoxins at an early age (Kimanya *et al*., 2009). The recent deaths in 2016 in Tanzania were attributed to aflatoxins contaminations in maize (WHO, 2018; while deaths in 2019 were suspected being caused by aflatoxicosis (WHO, 2019).

Tropical countries have high temperature, humidity, and rainfall, conditions which are favorable to aflatoxins contamination (Hussain *et al*., 2012). Different studies conducted in Tanzania revealed aflatoxins contamination in different food in which Kamala *et al*. (2015) in his study reported that 95% of maize samples collected from Eastern zone were detected with aflatoxins. Furthermore, Abt Associates Inc. (2013) reported that the levels of aflatoxin B1 and the level of total aflatoxins exceeded the respective Tanzanian limits of 5 ppb and 10 ppb in 20% of the 20 groundnut samples from Manyara (Northern zone) and in 20% of the 40 samples from Mtwara (Southern zone). The investigation also found that the levels of aflatoxin B1 and total aflatoxins exceeded the respective regulatory limits in 18% of the 40 samples of groundnuts from Geita (Western zone). The results on the aflatoxin contamination levels in the 101 rice samples from the three main rice-producing districts of Tanzania (Kilosa, Morogoro, Mbarari, Mbeya and Misungwi, Shinyanga), were ranged from 0.01-3.83 ppb. Mohammed *et al*. (2018) conducted a study on aflatoxins in sunflower seeds and sunflower oil in which the study revealed aflatoxin contamination of sunflower seeds and unrefined sunflower oil samples from Singida, Tanzania in which about 80.9% (17/21) of sunflower oil and 15% of sunflower seed samples were contaminated with AFB_1.

Another study conducted by Mohammed *et al*. (2016) in Singida, Tanzania reported 65% of the sunflower-based seedcake feed were contaminated with AFB_1 and 83.8% of cow's milk samples were contaminated with AFM_1. Kuhumba et al. (2018) determined the level of aflatoxins in peanut-enriched flours from selected markets in Tanzania in which aflatoxins B_1, B_2, G_1, and G_2 were present in all samples from all regions and from all manufacturers in 71% of samples had total aflatoxins above the acceptable levels of 10 μg/kg.

Evaluation on commonly used spices from local markets and Indian shops in Dar es Salaam revealed fungal contamination of 18.37% in Red chill, (14.28%) in ginger and 2.04% curry powder and fenugreek (Temu, 2016). Fungal contamination revealed in this study might be due to poor handling, storage and packaging practices including insufficient drying of spices. However, the study didn't evaluate the levels of aflatoxins. Fundikira, (2018) conducted a study on four marketed spices in Dar es Salaam and her findings revealed aflatoxin contamination of cinnamon (43.3%), cloves (70%), ginger (56.7%) and cardamom (60%).

2.5 Aflatoxins Contamination in Spices

Despite of spices being widely used ingredients in food preparation and processing throughout the world, can suffer fungal contamination in which their additions to ready to-eat foods may result in the proliferation of fungi which can be pathogenic (Hashem and

Alamri, 2010). The rate of production and the levels of contamination heavily depend on several abiotic factors and the condition of the spice. On the whole, mycotoxin infected spices are not safe for human consumption (Thanushree *et al*., 2019).

Contamination of spices by fungus is one of the major problems that can affect the human health and also degrade the quality and taste of the spices (Potorti, 2020). Spices can be contaminated with mycotoxins during pre-harvest, postharvest, and storage (Potorti, 2020). Spices usually remain close to the ground for drying, a fact that predisposes to fungal contamination, as well as moisture transferred from the tropical environment can allow their multiplication and synthesis of mycotoxins (Garcia *et al*., 2018).

Spices may get fungi contamination due to poor storage and handling practices throughout the commodity value chain (Nguegwouo *et al*., 2018). Soil and air is the main inoculum source for causing contamination in crude spices while in field (Moorthy, 2016). Aflatoxins contamination of spices has been reported in many countries including Hungary (Fazekas *et al*., 2007) and Kenya (Mwangi *et al*., 2014) while information on aflatoxin contamination in spices is limited regardless of its widely usage in the country. Fundikira (2018) reported levels of aflatoxins in selected spices in Dar es Salaam, Tanzania.

2.6 Effects of Aflatoxins on Health

A. flavus and *A. parasiticus* are species of moulds, which are responsible for the production of aflatoxins and are crucial in the pathogenesis of human diseases (Kowalska *et al*, 2017). Aflatoxins are highly carcinogenic, hepatotoxic, mutagenic, teratogenic and are correlated with immunosuppression, reduced nutrients absorption and stunting of infants, and are fatal in high doses (Sarma *et al.*, 2017). IARC, 2002, termed aflatoxin as a known human carcinogen that has been shown to be a causative agent in the pathogenesis of hepatocellular carcinoma (HCC). Aflatoxins are associated with both acute toxicity and chronic toxicity in humans and animals (Spanjer, 2019). Aflatoxins are chemically and thermally stable during food processing, including cooking, boiling, baking, frying, roasting and pasteurization (Thanushree *et al.*, 2019). In many lower-income countries, aflatoxins pose serious public health issues since the occurrence of these toxins can be considerably common and even extreme. Acute high exposure leads to outbreaks of aflatoxicosis, presenting as nausea, vomiting, abdominal pain and fever leading to liver failure that is potentially fatal (Azziz-Baumgartner *et al.*, 2005).

Chronic exposure due to low levels of contamination consumed regularly, may increase liver cancer risks and can suppress the immune system, particularly for the population with hepatitis B virus (HBV), while very high levels of aflatoxin can cause acute poisoning and even death to human (WHO, 2018). In high exposures aflatoxins cause acute toxicity particularly to the liver with growth retardation, DNA fragmentation metabolic disorders, cytotoxicity, tissue necrosis, eventually leading to organ failure and death depending upon the dose and duration of exposure (Benkerroum, 2020).

WHO estimates that 38 million people die annually from non-communicable diseases (NCDs) including cancer with more than three-quarters of these deaths occurring in low-income and middle-income countries (WHO, 2018). Intensive exposures of Aflatoxin B_1 at a concentration in excess of 2 ppm are reported to cause non-specific liver problems and death within few days, whereas chronic effect of AFB_1 leads to immunosuppression and nutritional deficiency (Chauhan *et al*., 2016). Multiple aflatoxicosis outbreaks have been documented and the most severe recorded outbreak was in Kenya in 2004, during which 125 deaths and hundreds of cases of acute hepatic failure occurred (CDC, 2004; Azziz-Baumgartner *et al*., 2005).

Other recent deaths attributed to aflatoxins were reported in 2016 in Tanzania (Kamala *et al.,* 2018) and in June 2019, (Anonymous, 2019). According to Anonymous (2019), a suspected aflatoxicosis outbreak was reported in Tanzania June to August, 2019 in which sporadic cases, presenting with symptoms and signs on abdominal distention, jaundice, vomiting, swelling of lower limbs, with a few cases of fever and headache, from Dodoma and Manyara regions in Tanzania. A total of 53 cases and 8 deaths have been reported as from Chemba, Kondoa and Kiteto Districts, (Anonymous, 2019). According to WHO, adults are more tolerant to acute exposure than children (WHO,2018). The consumption of food containing aflatoxin concentrations of 1 mg/kg or higher has been suspected to cause aflatoxicosis. Based on past outbreaks it has been estimated that, when consumed over a period of 1–3 weeks, an AFB_1 dose of 20–120 µg/kg per day is acutely toxic and potentially lethal (WHO, 2018). Codex Alimentarious Commission established a limit of 5 ppb for aflatoxin B_1 and 10 ppb for total aflatoxins in food stuffs (CAC, 1995).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Morogoro region in the districts of Morogoro rural and Morogoro municipality in Tanzania from December, 2019 to January, 2020. The districts are administratively divided in 29 wards, with a population size of 286 248 (NBS, 2012). Morogoro municipal is divided into 19 administrative wards with estimated population of 315 866 (NBS, 2012). Morogoro region was selected because was one of the major spice producing regions in Tanzania. Morogoro rural was selected because it had a large production of spices among the seven districts in Morogoro (Maerere and Noort, 2014) while Morogoro urban was selected as it is a primary market for most of spices from Morogoro rural district.

3.2 Study Design and Population

The study involved a cross sectional descriptive study. The study population was people who traded spices in the districts of Morogoro rural and Morogoro municipality. Samples were collected from four wards of Mtamba, Tawa, Kinole and Mkuyuni in Morogoro rural and from the markets of Mawenzi, Manzese, Kilakala and Kikundi in Morogoro municipality. Traders who participated in the study were randomly selected using a list provided by the respective ward leaders, extension and trade officers. Selection criteria for the traders were;- being a spice trader, trading spice types of turmeric, clove, cinnamon, black pepper either single type or more than one type of these spices. Other criteria used were also availability of enough samples of the spices and the willingness of the traders to participate in the study. A total of 52 traders were randomly selected for spice sampling and structured questionnaire were administered to them (Appendix 1). Responses were on demographic characteristics, handling, storage and packaging practices of spices dealers and awareness on Aflatoxins. A total of 120 samples (30 each for turmeric, black pepper, clove and cinnamon) were collected for aflatoxins contamination determination after the interview of the spices traders.

3.3 Sample Collection

Four types of spices (clove, turmeric, cinnamon and black pepper) were collected from Morogoro rural and Morogoro municipality in December 2019 and January 2020. A total of 120 whole dried spices were collected in clean polythene bags from 52 randomly selected spice traders. The 52 randomly selected traders were interviewed using a semi-structured questionnaire (Appendix 1). Responses were on demographic characteristics, handling, and storage and packaging practices of spices dealers. Spice samples were labeled, transported to the Tanzania Bureau of Standards (TBS) food laboratory and stored at temperature of 4 ºC prior to moisture content and aflatoxins analysis.

3.4 Moisture Content

Moisture content of the spice samples was determined following AOAC (2005) procedures using official method No. 941.11. Ten grams of grounded spices was weighed in tarred moisture dish with a cover and evenly distributed. With cover removed, the dish and cover were placed in an air- dry oven (HERAEUS) maintained at 105 °C until constant weight and dried for 4 hours. Thereafter, the dishes were covered and placed in a desiccator for 30 min to reach ambient temperature. The moisture content (MC) was determined by the differences of weight before and after drying the sample in an oven as follows;

$$\%\ \text{Moisture content} = \frac{(M_1 - M_2) \times 100}{(M_1 - M)}$$

Where;

M_1 = mass in g of the dish with spices before drying

M_2 = mass in g of the dish and spices after drying

M= mass in g of the empty dish

3.5 Aflatoxins Analysis

3.5.1 Sample preparation

Cloves, cinnamon, turmeric and black pepper were ground separately using grinder to obtain a homogenous mixture and then sub-divided to obtain representative sub samples for analysis.

3.5.2 Extraction

Samples were extracted according to procedure described in TBS (2020). All individual samples were analyzed independently. Each ground spice sample was placed into Erlenmeyer flask and weighed using the calibrated analytical balance to 25 ± 0.1g. Using a measuring cylinder, 100 ml of methanol: water (70:30 v/v) as extraction solvent was added to the 250 ml Erlenmeyer flask containing the sample. The flask was covered with aluminium foil and placed on the gyratory shaker (Stuart® Orbital Shaker SSL1, Cole-Parmer LLC, USA) at 250rpm for 30 minutes, then using a filter paper Whatman No. 1, the extract was filtered into a 250 ml Erlenmeyer flask according to the procedure described in TBS (2020).

3.5.3 Dilution

The sample extract (4 ml) was transferred to 15 ml centrifuge tube, and added 8 ml of distilled water. The mixture was vortexed (Talboys® Hvy Dty Vortex, Troemner LLC, USA) at a speed of 2500 rounds for 1 min to get a homogeneous mixture.

3.5.4 Isolation and clean-up of aflatoxin

The diluted extract was loaded and allowed to pass through Solid Phase Extraction (SPE) immunoaffinity columns and the sample loaded columns were rinsed twice with 10 ml of HPLC grade water. The adsorbed aflatoxins were eluted with 1 ml of HPLC grade methanol and the eluents were collected in vials. Finally, pressure was slightly applied on top of the column to remove any remaining liquid. Thereafter, 0.3 ml of the elute was mixed with 0.6 ml of water and 0.1 ml of acetonitrile and the mixture was vortexed for 30 seconds at a speed of 2500 rpm (TBS, 2020).

3.5.5 Sample analysis for aflatoxins

After extraction, dilution, cleaning and elution and post-column derivatization, the extracts were analyzed using HPLC with fluorescence detector (FtLD) (Model Agilent Chem Station Technology, series 1200, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA). The mobile phase contained water: methanol: acetonitrile (60:30:10, v/v). The separation of aflatoxins (B_1, B_2, G_1 and G_2) was performed on the C18 column at 30 ^{o}C at a flow rate of 1.2 ml/min. The injection volume was 50 µL for both standard solution and sample extracts. After separation, AFG_1 and AFB_1 were derivatized for detection with fluorescence detector at an emission wavelength of 450 nm and an excitation wavelength of 365 nm (TBS, 2020).

3.6 Method validation

A mixture of aflatoxin standards solution (G_1, G_2, B_1 and B_2) (all from Fisher Chemical, Bishop Meadow Road, Loughborough, Leicestershire) was prepared and analysed at the concentration indicated in (Table 1) to establish a four point calibration curve (Fig.1 - 4). The conditioning of the HPLC system was the same. The calibration curve was constructed to check the linearity and quantification of aflatoxin. To validate the extraction method and chromatographic performance, all four spice sample were spiked with three concentrations (1, 3 and 5ppb) of standard solutions of aflatoxins B_1 B_2, G_1, and G_2 in triplicate and mean concentrations and percentage recovery were recorded. The limit of detection (LOD) and limit of quantitation (LOQ) of the HPLC method for $AFB_{1,}$ AFB_2, AFG_1 and AFG_2 were determined using equations in Table 2 together with the LOD and LOQ values obtained for the four types of aflatoxins;

Table 1: Calibration table for AFB_1, AFB_2, AFG_1 and AFG_2

No	RT[1]	Signal	Compound	Level	Amount	Area	Rsp.Factor[2]
				1	1	123.69	8.08E-03
1	5.047	FLD1 A	AFLA G_2	2	5	624.11	8.01E-03
				3	10	1337.1	7.48E-03
				4	15	1928.5	7.78E-03
				1	1	49.599	2.02E-02
2	6.01	FLD1 A	AFLA G_1	2	5	229.18	2.18E-02
				3	10	498.49	2.01E-02
				4	15	712.59	2.11E-02
				1	1	129.57	7.72E-03
3	7.02	FLD1 A	AFLA B_2	2	5	616.17	8.11E-03
				3	10	1351.5	7.40E-03
				4	15	1880.4	7.98E-03
				1	1	69.771	1.43E-02
4	8.597	FLD1 A	AFLA B1	2	5	299.24	1.67E-02
				3	10	595.23	1.68E-02
				4	15	813.89	1.84E-02

[1] = Retention Time
[2] = Response Factor

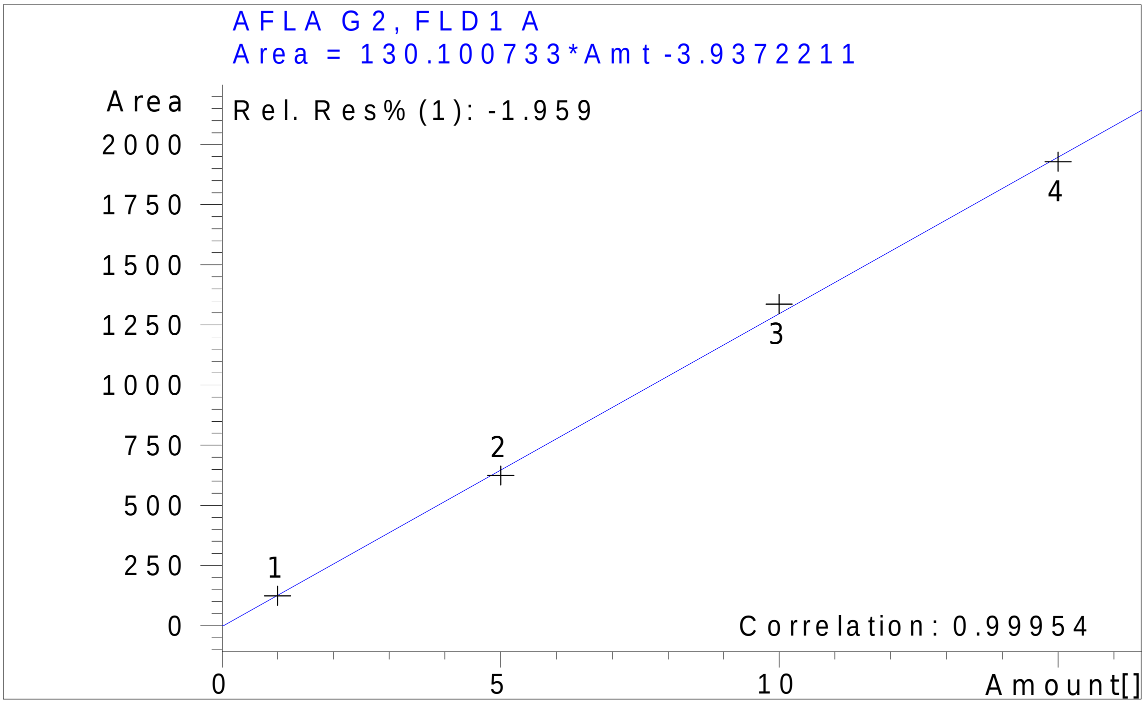

Figure 1: Calibration curve for AFLAG$_2$

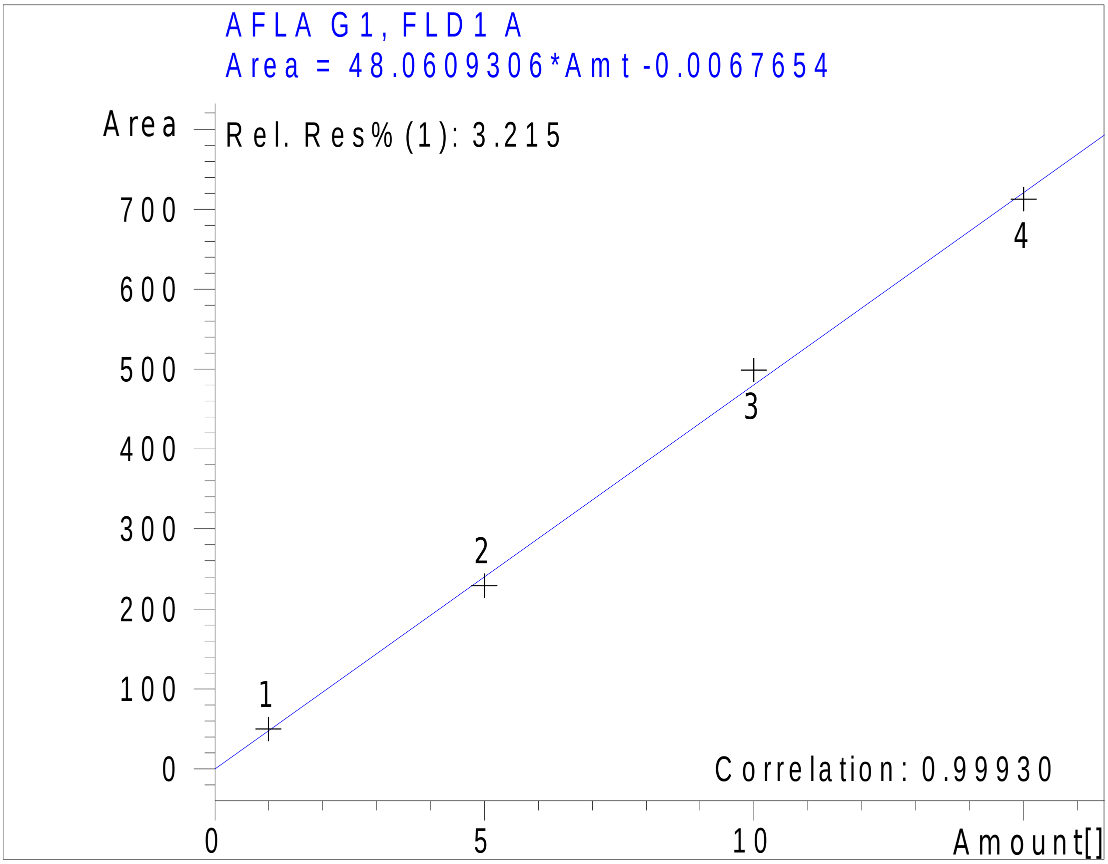

Figure 2: Calibration curve for AFLAG$_1$

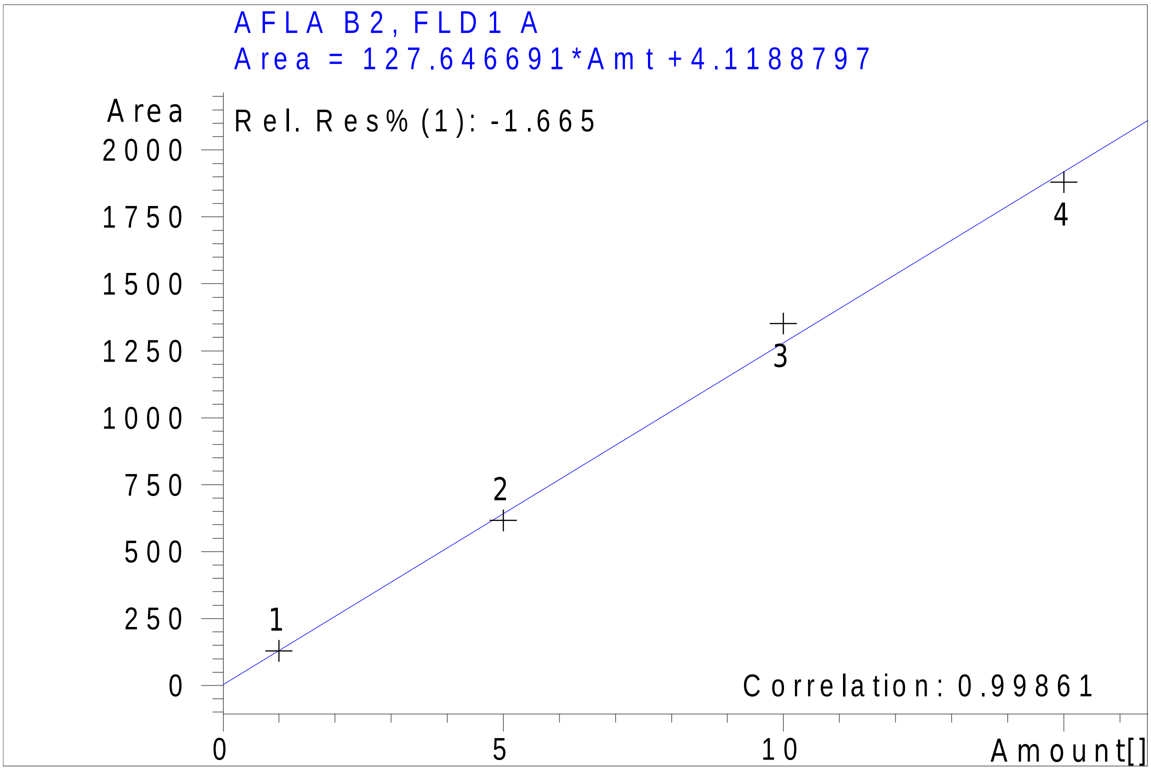

Figure 3: Calibration curve for AFLAB$_2$

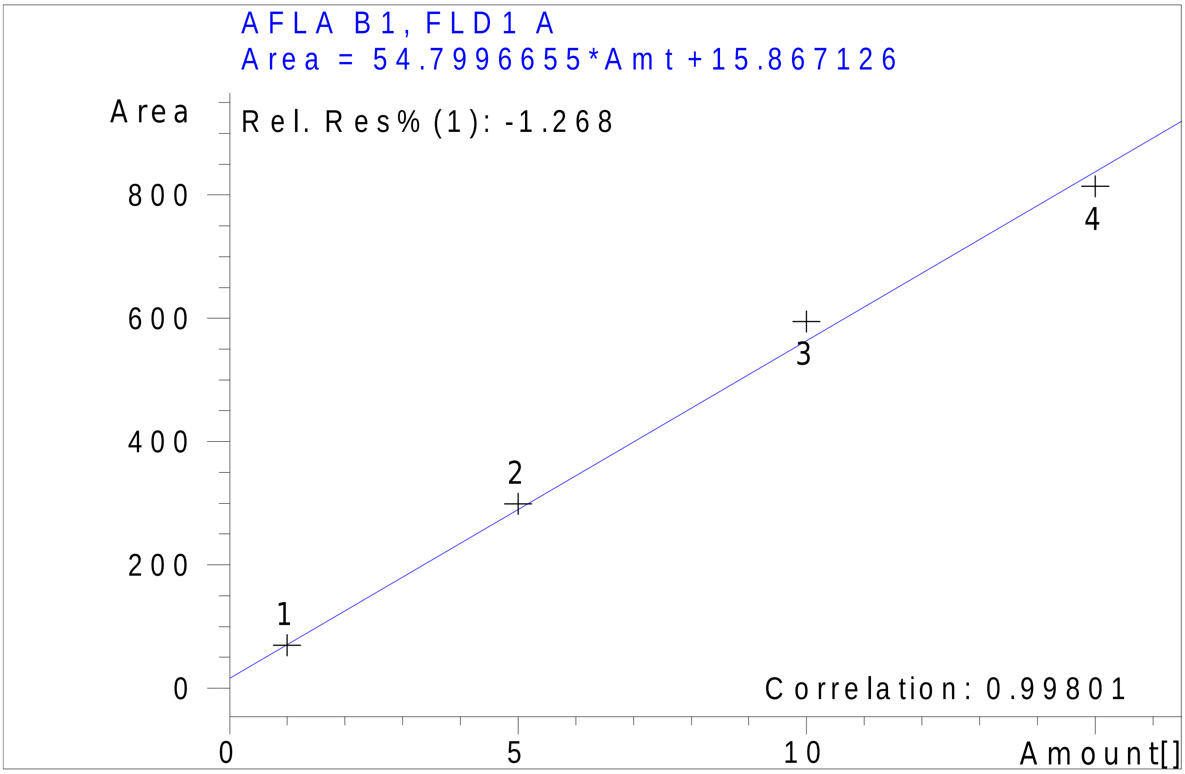

Figure 4: Calibration curve for AFLAB$_1$

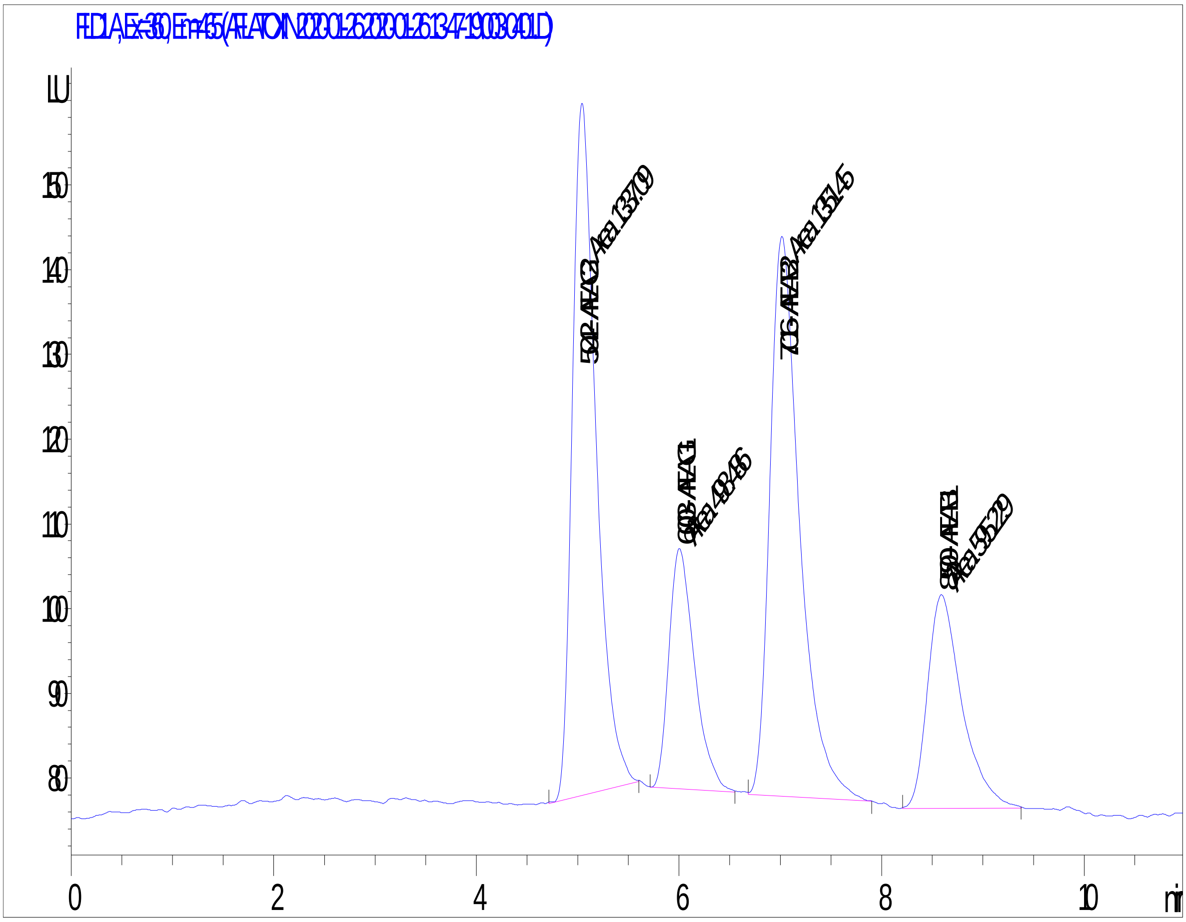

Figure 5: Standard chromatograph curve for 10 ppb

Table 2: The Limit of detection (LOD) and limit of quantitation (LOQ) for each analyzed aflatoxin

LoD	LoD = Mean + 3SD of the lowest concentration or blank	B_1: 0.16
		G_1: 0.13
		B_2: 0.13
		G_2: 0.13
LoQ	LoQ = Mean + 10 SD of the lowest concentration or blank	B_1: 0.29
		G_1: 0.21
		B_2: 0.18
		G_2: 0.16

Where SD = Standard deviation of the lowest concentration

3.7 Statistical Analysis

Data were cleaned, validated, organized, coded, and subjected to statistical analysis using IBM SPSS Statistics Version 20. One way Analysis of variance (ANOVA) was used to test significance of difference on the aflatoxins contamination levels in amongst the types of traded spices and study districts. The differences between means were detected using Duncan's multiple range tests in mean of AFG_2, AFG_1, AFB_2, AFB_1 and total aflatoxins. Also t test, Chi Square, multiple linear regression, correlation was performed to asses association between aflatoxins contamination and moisture content. Descriptive statistics such as frequency were used to present the awareness, handling, storage and packaging practices among spice traders. Chi square test performed to examine the statistical significance in the relationship existed between aflatoxins contamination in spices and awareness, handling, storage and packaging practices of the spice traders.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic Characteristics of the Spice Traders

Demographic characteristics of spice traders were as presented in Table 3. Majority (88.5%) of the respondents were males.

Table 3: Demographic characteristics of interviewed spice traders

Variable n= (52)	**N (%)**
Gender of respondents	
Male	46 (88.5)
Female	06 (11.5)
Age in years	
18-24	05 (9.6)
25-40	33 (63.5)
41-60	13 (25)
>60	01 (1.9)
Education level	
Completed primary education	24 (46.2)
Completed ordinary level secondary school	20 (38.5)
Completed high/advanced secondary school	03 (5.8)
Post-secondary/ tertiary education	01 (1.9)
Illiterate (No formal education)	04 (7.7)
Overall education level	
Attended school	48 (92)
Not attended school	04 (8)
Experience in spice business (years)	
0-10	39 (75)
11-20	10 (19.2)
21-30	2 (3.8)

4.2 Spice Dealers Social Demographic Practices and its Association with Aflatoxins Contamination

Results revealed that there was no significant association between aflatoxins contamination and social demographic characteristics of the respondents. Association existed (p-value 0.026) between districts surveyed and aflatoxins contamination (Table 4).

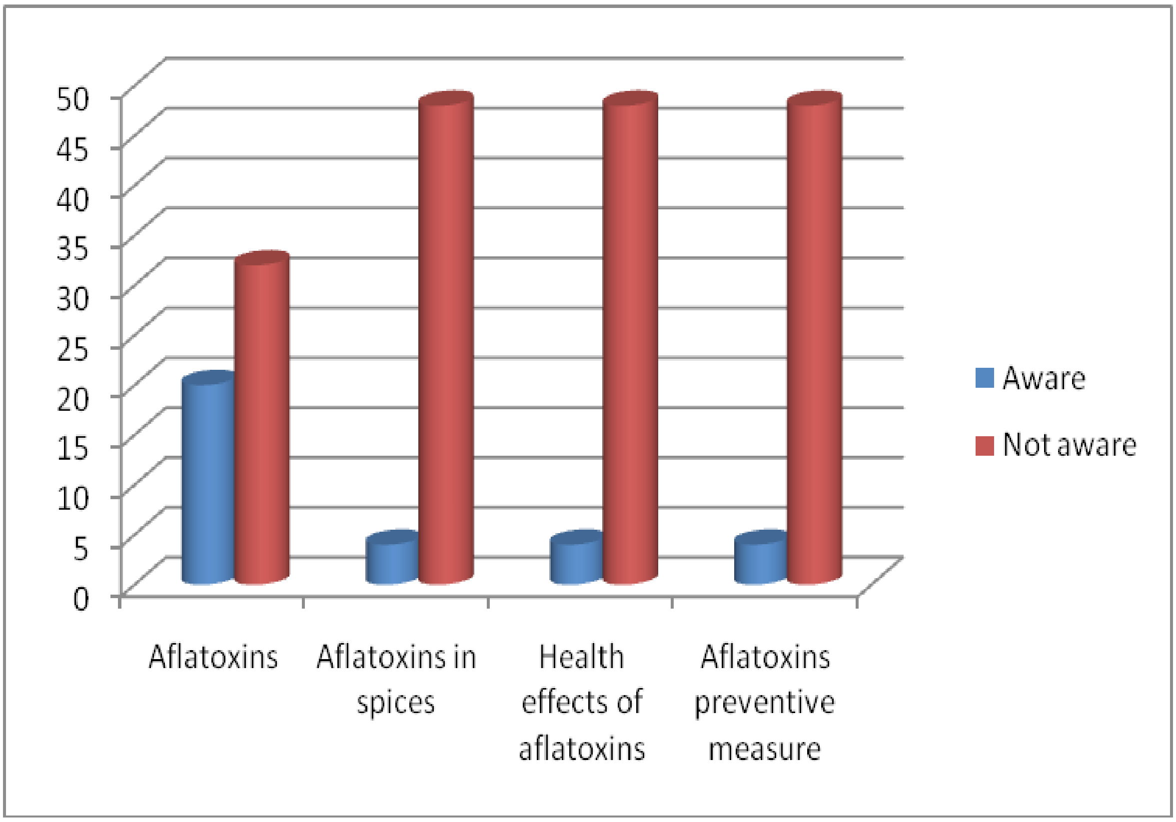

Figure 6: Aflatoxins awareness status of the 52 respondents

Table 4: Association of occurrence of aflatoxins in spices with the demographic characteristics of spice dealers in Morogoro rural and Morogoro municipality districts

Variable	Description	N=52	n (%)	Chi-square	p-value
District	Morogoro Rural	25	13 (52)	5.050	0.026
	Morogoro municipal	27	6 (22.2)		
Age	18-24	5	0(0)		
	25-40	33	12 (36.4)	7.065	0.07
	41-60	13	6 (46.15)		
	>60	1	1(100)		
Sex	Male	46	16 (34.8)	0.513	0.474
	Female	06	03 (50)		
Marital	Single	13	4 (30.7)	0.253	0.615
	Married	39	15 (38.5)		
Formal education	Formal education	48	19 (36.5)	4.849	0.05
	No formal education	04	0 (0)		
Education	Illiterate	04	0 (0)		
	Completed primary school	24	13 (54.2)		
	Completed secondary school	20	05 (25%)		
	Completed high/advance school	03	01 (33.3)	8.854	0.065
	Completed post-secondary/tertiary school education	01	0 (0)		
Experience	≤10 years	39	13 (33.3)	4.760	0.190
	11-20	10	05 (50)		
	21-30	02	0 (0)		
	31-40	01	01(100)		
Quantity of spices per week (Kg)	1-500	43	15 (34.9)	5.382	0.250
	501-1000	04	03 (75)		
	1001-1500	02	0 (0)		
	1501-2000	02	1 (50)		
	>2000	01	0 (0)		
	No	51	19 (37.3)		

4.3 Spice Dealer's Practices and their Association with Aflatoxins Contamination

Occurrence of aflatoxins in spices was associated with sorting and storage practices. Half (26/52) of the respondent were sorting and 34.6% of them (9/26), performed sorting based on maturity, presence of foreign materials and appearance. Among those who were doing sorting, the majority (17/26, 65.4%) discarded the rejected spices after sorting. Majority (61.5%) of those who were not doing sorting of their spice samples had aflatoxins contamination. Only 11.5% of those doing sorting their spice samples had aflatoxins contamination. Association's analysis revealed significant association (p- value 0.000) between sorting and aflatoxins contamination, criteria for sorting and aflatoxins contamination (p-value 0.002) and handling of rejected spices and aflatoxins contamination (p-value 0.041). The majority (96.2%) of the spice dealers was doing storage and the storage time for the 43 respondents (82.7%) varied between 0 to 100 weeks. About one third (34.9%) of spice dealers doing storage their samples had aflatoxins contamination and there was significant association between storage and aflatoxins contamination (Table 5).

4.4 Spice Dealers Knowledge and its Association with Aflatoxins Contamination

The majority (65.%) of the spice dealers were aware on the presence of toxins in food while 38.5% were aware on occurrence of aflatoxins in food and among them only 8% were aware on aflatoxins in spices, its health effects and preventive measures (Table 5 and Fig.6). The occurrence of aflatoxins in spices in the study area was significantly associated with lack of awareness on aflatoxins (p-value 0.002) and low knowledge on causes of food spoilage (p-value 0.016).

Table 5: Association of occurrence of aflatoxins in spice with the awareness, handling, storage and packaging practices in Morogoro rural and Morogoro municipality districts

Variable	Description	N=52	n (%)	Chi-square	pvalue
Sorting of spices	Yes	26	3 (11.5)	13.746	0.000
	No	26	16 (61.5)		
Criteria for sorting	No sorting	26	16 (61.5)	16.496	0.002
	Appearance	8	0 (0)		
	Maturity, foreign matters and appearance	09	02 (22.2)		
	Maturity and foreign matters	01	0 (0)		
	foreign matters and appearance	07	01 (14.3)		
What you do with rejects	No rejects	26	16 (61.5)	15.897	0.001
	Milling	06	0 (0)		
	Discarding	17	2 (11.8)		
	Milling and discarding	03	01 (33.3)		
Storage of spices	Yes	50	17 (34)	4.168	0.041
	No	02	02 (100)		
Storage time (weeks)	0-100	43	15 (34.9)	0.288	0.592
	>100	09	04 (44.4)		
Cleaning of storage facility	Yes	42	14 (33.3)	0.941	0.332
	No	10	05 (50)		
Packaging of spices	Yes	41	14 (34.1)	0.469	0.494
	No	11	05 (45.5)		
Awareness on presence of toxins in food	Aware	19	09 (47.4)	1.514	0.218
	Not aware	33	10 (30.3)		
Awareness on aflatoxins	Aware	20	02 (10)	9.871	0.002
	Not aware	32	17 (53.1)		
Awareness on aflatoxins in spices	Aware	04	02 (50)	0.327	0.567
	Not aware	48	17 (35.4)		
Awareness on health effects of aflatoxins	Yes	04	02 (50)	0.332	0.567
	No	48	17 (35.4)		
Awareness on preventive measure on aflatoxins	Yes	04	01 (25)	0.262	0.608
	No	48	18 (37.5)		
Knowledge on spoilage causes	Aware	42	12 (28.6)	5.799	0.016
	Not aware	10	07 (70)		
Obtained any training on food safety	Yes	01	0 (0)	0.576	0.448
	No	51	19 (37.3)		

N= total number of interviewed spice dealers

n= number of contaminated samples

4.5 Recovery of Aflatoxins in Spiked Sample

Results showed that all spiked spices have recovery of more than 85% (Table 6).

Table 6: Recovery of aflatoxins in spiked spice samples

Spice type		5 μ/Kg				3 μ/Kg				1 μ/Kg			
		G_2	G_1	B_2	B_1	G_2	G_1	B_2	B_1	G_2	G_1	B_2	B_1
Cinnamon	USP	0	0	0	0	0	0	0	0	0	0	0	0
	SP	4.87	5.75	4.41	5.01	2.94	3.00	2.91	2.66	0.98	1.06	0.94	0.965
	% Rec	97.44	114.94	88.16	100.11	97.87	100.02	97.018	88.60074	97.74	106.14	93.62	95.66
Turmeric	USP	0	0	0	0	0	0	0	0	0	0	0	0
	SP	5.56	4.80	5.77	4.78	2.87	3.26	2.93	2.71	0.87	1.11	1.05	0.86
	% Rec	111.24	95.96	115.38	95.65	95.83	108.81	97.56	90.41	87.23	111.22	105.04	86.11
Clove	USP	0	0	0	0	0	0	0	0	0	0	0	0
	SP	5.08	4.66	4.64	4.96	2.90	2.86	2.96	2.97	1.05	0.96	0.98	1.02
	% Rec	101.56	93.19	92.76	99.21	96.61	95.18	98.63	99.16	105.31	97.56	98.33	102.11
Black pepper	USP	0	0	0	0	0	0	0	0	0	0	0	0
	SP	4.38	5.24	4.69	4.74	2.94	3.00	2.91	2.66	0.98	0.91	0.97	0.88
	% Rec	87.61	104.87	93.87	94.85	97.87	100.02	97.02	88.60	97.89	91.2	96.64	87.73

USP= Un spiked spice sample Concentration (μ/Kg)

SP= Spiked spice sample Concentration (μ/Kg)

4.6 Moisture Content in Spices

A total of 120 samples were analyzed for moisture content and 35 (29.2%) exceeded regulatory limit for moisture contents. Turmeric had fewer samples (n=3, 10%) that had moisture content above the regulatory limit (12%) established by Tanzania Bureau of Standards and has the lowest moisture content of 7.37% (Fig.6) compared to other types of spices. Cloves had more samples (n=21, 70%) with moisture content exceeding regulatory limit of 12% and had the highest moisture content of 18.43% (Table 7 and Fig.7). Table 8 shows significant association between moisture content and the aflatoxins contamination in spices.

Table 7: Moisture content in the spices collected from Morogoro municipality and Morogoro rural districts

Type of spice	N	Mean (%)	Min. (%)	Max. (%)	Exceeded Tanzania regulatory limits 12% for T, B & CL; 14% for C
Turmeric (T)	30	10.8724	7.37	13.46	3 (10%)
Cinnamon (C)	30	11.9655	9.92	17.00	5 (16.7%)
Clove (CL)	30	13.9639	11.05	18.43	21 (70%)
Black pepper (B)	30	11.8612	10.23	14.93	6 (20%)
Total	120	12.1658	7.37	18.43	35 (29.17)

Table 8: Association of moisture content with the aflatoxin contamination

Variable	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	T	Sig.
Constant	-70.445	13.671		-5.153	0.000
Moisture	6.454	1.109	0.472	5.822	0.000

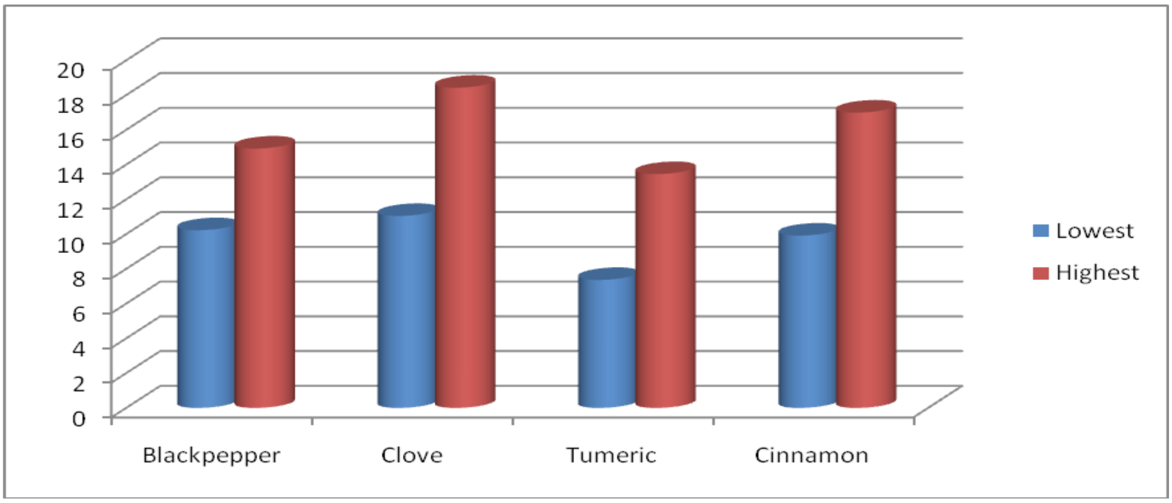

Figure 7: Highest and lowest moisture content percentage in the four spices

4.7 Aflatoxins Contamination in Spices

A total of 29 (24.2%) of the spice samples were contaminated with aflatoxins in which 11.7% were contaminated with aflatoxin B1. Turmeric had the lowest contamination by AFB_1 and total aflatoxins of 0.0% and 3.3%, respectively, while the highest contamination was observed in cloves being 20% and 50.0%, respectively. AFG_2, AFG_1 and AFB_1 were not detected in turmeric, while AFG_2 and AFG_1 were not detected in black pepper and cinnamon, respectively (Table 9).

Table 9: Percentage of aflatoxins contamination in turmeric, cloves, cinnamon and black pepper in Morogoro municipality and Morogoro rural districts

Spice	N	AFG_2		AFG_1		AFB_2		AFB_1		TOTAL AF	
		n	**%**	**N**	**%**	**n**	**%**	**n**	**%**	**N**	**%**
Turmeric	30	0	0.0	0	0.0	1	3.3	0	0.0	1	3.3
Black pepper	30	0	0.0	2	6.7	1	3.3	3	10	6	20
Cloves	30	1	3.3	9	30.0	3	10.0	6	20.0	15	50
Cinnamon	30	1	3.3	0	0.0	2	6.7	5	16.7	7	23.3
Total	120	2	1.7	11	9.2	7	5.8	14	11.7	29	24.2

N is the total number of samples analyzed for each spice

n is the total number of contaminated spice samples

Morogoro rural had the highest aflatoxins contamination (33.3%) compared to Morogoro Municipal (15.0%) while the difference in percentage contamination in total aflatoxins among the two districts at $p<0.05$ was significant (Table 10).

Table 10: Percentage of contaminated spice samples in Morogoro rural and Morogoro municipality districts

District	N	AFG2		AFG1		AFB2		AFB1		TOTAL	
		n	%	n	%	n	%	N	%	n	%
Morogoro Rural	60	1	1.7	5	8.3	6	10.0	9	15.0	20	33.3
Morogoro municipality	60	1	1.7	6	10.0	1	1.7	5	8.3	9	15.0
Chi square					0.100		3.793		1.294		5.502
Df					1		1		1		1
P					0.752		0.051		0.255		0.019

Table 11: Mean aflatoxin concentrations in turmeric, cloves, cinnamon and black pepper collected in Morogoro rural and Morogoro municipal districts (µg/kg)

Spice	AFG_2 (µg/kg)	AFG_1 (µg/kg)	AFB_2 (µg/kg)	AFB_1 (µg/kg)	Total (µg/kg)
Turmeric			0.8979+0.89793a		0.8979+0.89793a
Black pepper		4.089+3.935a	0.0067+0.0067a	5.897+5.4899.23a	9.99+6.63a
Cloves	0.039+0.039a	5.9247+3.504b	0.1105+0.1037a	8.2978+6.463a	14.3720+6.463a
Cinnamon	0.1283+0.1283a		3.73+3.66a	3.1722+1.405a	7.0308+3.8253a

Mean value of the aflatoxin (AFB_1, AFB_2, AFG_1, AFG_2) and total aflatoxins for turmeric, cloves, cinnamon and black pepper ranged from 0.0067± 0.0067a to 14.3720± 6.463a.

For total aflatoxins, cloves have high number of Aflatoxin contaminated sample (23.3%) which exceeded EU regulatory limit of 10 µg/kg while turmeric has low percent (3.3%). For AFB_1, cinnamon had high percent (16.7%) of contamination levels exceeding EU regulatory limit of 5 µg/kg while turmeric had no contamination. For contaminated samples, the lowest contamination was 0.201 µg/kg in cloves while the highest was 164.86 µg/kg in black pepper (Table 12).

Table 12: Total aflatoxins and AFB_1 contamination levels in spices that exceeded regulatory limits

Spice type	Type of aflatoxins	N	Positive samples		Exceed EU regulatory limits		Concentration range (µg/kg)
			N	%	n	%	
Turmeric	AFB_1	30	0	0.0	0	0.0	0
	Total		1	3.3	1	3.3	26.93
Cinnamon	AFB_1	30	5	16.7	5	16.7	10.33 - 26.94
	Total		7	23.3	6	20.0	3.85 -109.85
Cloves	AFB_1	30	6	20.0	4	13.3	0.58 – 147.49
	Total		15	50	7	23.3	0.201 – 147.49
Black pepper	AFB_1	30	3	10	2	6.7	3.38 – 164.86
	Total		6	20	2	6.7	118.11 -164.86

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study established high prevalence (24.2%) and high levels of aflatoxin contamination in spices traded in Morogoro municipality and Morogoro rural markets. The levels of aflatoxins contamination in evaluated spices ranged from 0.201 to 164.86 µg/kg (ppb) in which 11 sample (9.17%) exceeded the maximum limit (5 ppb) for AFB_1 while 16 samples (13.3%) exceeded maximum limit (10 ppb) for total aflatoxins set by European Commission. This implied that the spices consumers are at risk of aflatoxicosis if control measures are not considered. The observed prevalence was lower than what reported in Pakistan where the incidence was 61.5% aflatoxin contamination in spices out of which 53.66% samples exceeded the EU maximum limit in spices (Naz *et al.*, 2016). Also the results were lower when compared with the findings from the study conducted by Mwangi (2014) in Kenya in which 34 (73.9%) spice samples were contaminated with aflatoxins. The observed differences might be due to differences in climatic conditions between Morogoro, Tanzania; and Pakistan and Kenya as well as the handling practices.

However, the prevalence reported in this study (24.2%) were in line with the observation made by Garcia *et al.* (2018) that spices were highly susceptible to aflatoxins contamination since they had high *Aspergillus species* contamination in spices in Brazil. This indicates the global nature of the problem and might be due to the inadequate drying of spices as well as poor handling practices which favour the growth of fungus and aflatoxins production.

Turmeric had low percentage of aflatoxins contamination (3.3%) and is only contaminated with AFB_2. Probably turmeric has antimicrobial activity inhibiting fungal growth and mycotoxins production. Similarly, turmeric is reported to have a wide range of medicinal properties and the essential oils from turmeric exhibited significant inhibition of fungal growth as well as production of aflatoxins B_1 and G_1 (Sindhu *et al.*, 2011).

Findings from this study reveal that cloves have high aflatoxin contamination in which 15 (50%) of cloves samples were contaminated with aflatoxins compared to the other three types of spices and among them 7 samples (23.3%) exceeded the maximum limit for aflatoxins levels set by European Commission. The highest aflatoxin concentration was 147.5 ppb being higher than the levels of aflatoxin in cloves reported in Serbia (31.5 ppb) (Škrinjar *et al*., 2012) and in Kenya (7 ppb) (Mwangi, 2014). The higher aflatoxin contamination observed in cloves samples may be due to poor post-harvest practices such as inadequate storage and drying (Nurtjahja, 2019).

Observed prevalence of aflatoxin contamination in black pepper in this study was lower than what reported by Jeswal and Kumar (2015) where by 78.5% of black pepper samples were found to be contaminated with aflatoxins with the maximum amount of aflatoxins of 320 ppb. Also, the observed prevalence in this study was lower than that reported by Ozbey and Kabak (2012) in Turkey, in which aflatoxins were detected in 30.4% of black pepper samples. But the levels of aflatoxin contamination in black pepper in this study are higher when compared with the findings of Farid and Nareen (2013) in Iraq in which the maximum concentration obtained in black pepper was 5 ppb. This implies that spices, including black pepper can be contaminated with aflatoxin at different levels based on pre and post-harvest practices (Nurtjahja, 2019).

Seven samples of cinnamon were contaminated with aflatoxins (23.3%) and maximum concentration was 109.85 ppb. These findings were higher than what reported in a study conducted in Poland (1.79 ppb) (Waskiewicz *et al*., 2013); on a study conducted in Iraq (20.8 ppb) (Farid and Nareen, 2013) and Italy (2.27 ppb) (Romagnoli *et al.*, 2007). Five samples (16.7%) of cinnamon exceeded EU regulatory limit with concentration level of AFB_1 ranging from 10.33 - 26.94 ppb. However this was at lower side compared with the findings of the study conducted on spices in Turkey in which all samples of cinnamon (100%) exceeded EU maximum limit with concentration range of 49.4- 53 ppb (Tosun and Arslan, 2013). Also the finding is in lower to that of Fundikira *et al*. (2020) in which her study in Tanzania revealed higher percentage of cinnamon samples contaminated with Aflatoxin (43.3%).

Although most of the spice samples (75.8%) were not contaminated by aflatoxins, spices are highly susceptible to aflatoxins as observed in this study where by maximum concentration was 164.86 ppb which is at higher level to the acceptable maximum limit (05 ppb for AFB_1, 10 ppb for total aflatoxins). This indicated that consumers may be at risk to aflatoxicosis although the risk is depends on both consumption patterns and contamination levels (Ali *et al.*, 2015). Therefore, 13.3% (16/120) and 9.2% (11/120) of the samples were not fit for human consumption because they contained AFB_1 and total aflatoxins above the maximum allowable limit.

A total of 35 (29.2%) samples exceeded regulatory limit for moisture contents established by Tanzania Bureau of Standards (TBS, 2012; TBS, 2015; TBS, 2016; TBS, 2018). Highest moisture content observed in cloves (18.43%) which is at the higher side compared with the study conducted on spices in India in which the maximum moisture content was 12.37% (Kannaiyan *et al.,* 2011). This observed high moisture content in spice samples might be due to inadequate drying and poor handling as observed during the visit some of the dealers were kept their spices over the floor. The present study revealed a significant association existed between moisture content and the aflatoxin contamination in spices which is similar to the study conducted in spices in Iran in which the impact of moisture on aflatoxin level was observed, *P*=0.046 (Khazaeli *et al.,* 2017).

The aflatoxin contamination in spices found to be significantly associated with practices such as sorting and storage of spices and awareness of the respondents, however, no association was demonstrated in respect to respondents' demographic characteristics and aflatoxins contaminations in spices. Results showed that 50% of respondents were doing sorting of spices and had significant relationship with the aflatoxins contamination in tested spices being significantly higher in unsorted spices. Half of the respondents were not sorting for various reasons including lack of sorting equipment since they had bulk consignments, high demand from the market in which they considered sorting as wasting of time while others had no particular reasons. Findings from this study were similar to observations made by Hell and Mutegi (2011) that aflatoxin reduction is often achieved through proper drying, cleaning, sorting and physical segregation Furthermore, physical procedures such as cleaning, sorting, and hand picking were safe way of decontamination and do not alter the products significantly (Peng *et al*., 2018). Hand sorting is still the primary method to remove AFB_1 contaminations in spices. Sorting out of physically damaged and infected grains (known from colorations, odd shapes and size) from the intact spices can result in 40-80% reduction in aflatoxins contamination (Yang, 2019).

Results from this study further revealed among those who were sorting, 17 respondents (65.4%) discarded the bad spices which resulted into lower aflatoxins contamination while other respondents milled the rejects to get powdered spices. Therefore, sorting is an important factor in reducing aflatoxins contamination and it help to reduce impacts of aflatoxins contamination to human health if bad sorted spices are being discarded and not re-entered into the food value chain.

The study showed low awareness of occurrence of mycotoxins and aflatoxins in foods and spices and their health effects and preventive measures. The observed low awareness by respondents from this study is similar to other study which reported that majority of the farmers and processors (>50%) had limited knowledge about aflatoxins; contamination predisposing factors; dangers to animals and humans and mitigation strategies (Nakavuma *et al*., 2020). Also the findings were similar to the observation that majority of people in Tanzania and in Kenya are not aware of mycotoxins contaminations in foods (Kiama *et al*., 2016; Shirima, 2016). These findings were similar to the study conducted in India which reported that 64% of respondents (farmers and middle income consumers) were not aware about mycotoxins in foods (Yeole and Deshmukh, 2013) and also similar to the study conducted by Fundikira *et al*. (2020) in Tanzania which reported significant majorities (96.7%) of the respondents were not aware of aflatoxins contamination of spices during storage and its effect on health.

Significant association existed between lack of awareness on aflatoxins by spice dealers and aflatoxins contamination in spices. More than half (53.1%) of the respondents who were not aware on existence of aflatoxins in foods, their spices samples were found to be more contaminated with aflatoxins. This study also showed significant association between knowledge on causes of spoilage in food and aflatoxin contamination in food. This might be due to the facts that understanding causes of spoilage in food can help in preventing occurrence of that spoilage in food. Strosnider *et al.* (2006) reported that raising awareness of aflatoxins and disseminating relevant information to individuals is an important part of any intervention strategy.

The majority of the respondents (96.15%) were doing storage and 34.9% of them their samples were found to be contaminated with aflatoxins. There was also a significant relationship between storage and aflatoxins contamination and this might be due to the fact that storage conditions influence aflatoxins contamination. Similar observation was reported by other scholars that aflatoxins contamination of foods increased with storage period (Kaaya and Kyamuhangire, 2006). This might be due to storage conditions creating favoring environmental for fungi growth as reported by Villers (2017) that in the tropics, conventional storage of peanut for 2 months in Mali caused aflatoxins levels to rise 200% while conventional storage of maize for 3 months in Uganda caused aflatoxins levels to rise 300%.

Despite of about half (44.2%) of the respondent completed primary school education, no association existed between the level of education and aflatoxins contamination. The observed limited knowledge and awareness may result into high prevalence of aflatoxins contamination in spices. Among the educated, those with higher education had samples with lower aflatoxin contamination although the difference was not significant. Various studies indicate that education and awareness are crucial factors in alleviating the problems of aflatoxins contamination in developing countries (Strosnider *et al.*, 2006). The results from the study conducted by Udomkun *et al.* (2018) in Democratic Republic of Congo showed that knowledge and awareness about aflatoxins mostly were influenced by the level of education. Therefore, despite of lack of association in this study, education is an important tool in eradicating aflatoxins contamination in spices. For this reason, awareness creation along the spice value chain is needed in order to prevent and reduce impacts of aflatoxicosis to spice consumers.

The incidence of aflatoxins in foods and feeds is relatively high in tropical and subtropical regions, where the warm and humid weather provides optimal condition for the growth of moulds (Chiewchan *et al.,* 2015). Finding from this study revealed a significant association between increased in moisture content and aflatoxins contamination in spices similar to the study done by Khazaeli *et al.* (2017) which reported substantial impact of moisture on aflatoxin level in herbs and spices from different regions of Iran. The results was also similar to this study where cloves have higher number of samples which exceeded allowable maximum level of moisture content (TBS, 2012) and have higher number of aflatoxins contaminated samples compared to the turmeric which had fewer number of samples exceeded allowable maximum level of moisture content (TBS, 2015). Presence of samples with higher moisture content might be due to poor handling in particular inadequate drying of the spices. The correlation observed in this study also in line with study conducted in spices in Italy (Pesavento *et al.,* 2016).

5.2 Conclusion

High levels of aflatoxins contamination have been detected in selected spices traded in Morogoro region, Tanzania. Cloves had high prevalence of aflatoxins contamination compared to other three evaluated spices. Some samples exceeded the maximum limit for aflatoxins levels in spices according to EU set standards.

The study showed limited knowledge and awareness by spices traders on aflatoxins in spices in the study area although there was no significant association with aflatoxins contamination. Significant associations were demonstrated between practices such as storage and sorting with aflatoxins contamination in spices.

5.3 Recommendations

Based on the findings of this study, the following actions are proposed;

i. There is a need for more research on aflatoxins status in other types of spices traded in the region. Studies should also be extended in other regions of the country.

ii. Due to the problem of aflatoxins contamination and its effect to the public, awareness on aflatoxins and its effect should be created to the spices traders and general public.

iii. Good Agriculture Practices should be observed by farmers and Good Hygienic and Good Storage Practices should be followed by all actors along the spices value chain to prevent aflatoxins contamination.

iv. The Government of Tanzania through Tanzania Bureau of Standards should establish regulatory levels for aflatoxins B1 and total aflatoxins and ensure its implementation so that spices traded in the region has aflatoxins level conforming to the established standards to safeguard the health of consumers.

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APPENDIX

Appendix 1: Questionnaire to Assess Awareness, Handling, Storage and Packaging Factors Associated with Aflatoxin Contamination of Spices

Questionnaire number:………………………
Date of interview:…………………………….
Location:………………………………………
District:………………………………………..
Name of interviewer:………………………….

A. RESPONDENT PARTICULARS

1. Age of respondent……………………………..
2. Gender of respondent (Tick) (1) Male (2) Female
3. What is your level of education? Tick one
   (1) Never attended school/no formal education
   (2) Did not complete primary school education
   (3) Completed primary school education
   (4) Did not complete secondary school education (ordinary)
   (5) Completed secondary school education (ordinary)
   (6) Did not complete high school education (advanced)
   (7) Completed high school education (advanced)
   (8) Post-secondary/tertiary education

4. What is your marital status? Tick one
   (1) Single (2) Married living together (3) Divorced (4) Widowed (5) Separated (6) Cohabiting

B. RESPONDENT PRACTICES

5. How long have you been selling spices (years)?.................

6. Where do you purchase/procure spices?...............................

7. How much spies you purchase in a week in terms of weight (Kg)?..........

8. When purchasing/procuring your spices which criteria do you use for selection?

Tick appropriate response

Criteria	**Response**
Visual inspection	
Mould infested	
Cleanliness	
Moistness	
Foreign matters	
Others (Specify)	

9. (a) Do you know the causes of spoilage?

(1) Yes (2) No

9. (b) If yes mention the causes you know…………………………………..

9. (c) Can you identify spoiled spices?

(1) Yes (2) No

9. (d) If yes mention how you identify spoiled spices………………………

10. (a) Do you do any sorting of these spices?

(1) Yes (2) No

10. (b) If yes, what aspects do you consider when sorting?…………………………

10. (c) What do you do with bad sorted spices (rejects)?……………………

11. (a) Do you store the sorted (good) spices?

(1) Yes (2) No

11. (b) If yes, please mention how you store spices

11. (c) For how long do you store your spices? ………………..

11. (d) Do you clean storage facilities before using them?

(1) Yes (2) No

11. (e) Explain how you clean your storage facility……………………………..

12. How do you pack your spices?……………………………………………

C. RESPONDENCE AWARENESS OF AFLATOXIN CONTAMINATION

13. Have you heard toxins that may present in food which can be caused by moulds?

    (1) Yes (2) No

14. Where do you get this information?

    (1) Hospital (2) Colleague (3) Mass media (4) Others (Specify)

15. Have you heard aflatoxin before?

    (1) Yes (2) No

16. Are you aware of aflatoxin that can contaminate spices?

    (1) Yes (2) No

17. (a) Are you aware of the health effects of aflatoxin to human being?

    (1) Yes (2) No

17. (b) If yes please mention

    (1) cancer
    (2) liver cirrhosis
    (3) Stunting of children
    (4) Vomiting
    (5) Others (specify)…………………………….

18. (a) Have you attended any training on food safety (food handling, storage and packaging)?

    (1) Yes (2) No

18. (b) If yes, specify which training you attended…………………………………...

Thank you for filling this questionnaire