AFLATOXIN CONTAMINATION OF MARKETED SPICES IN TANZANIA: A CASE STUDY OF DAR ES SALAAM

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ABSTRACT

Aflatoxin contamination of spices, namely ginger, cinnamon, cardamom and cloves marketed in three districts of Ilala, Temeke and Kinondoni in Dar es Salaam region, Tanzania was investigated in the year 2017/18. Aflatoxin B_1, B_2, G_1 and G_2 and total aflatoxins were determined in 120 spice samples using immuno-affinity high performance liquid chromatography and post column derivatization. Fifty eight percent of the spice samples were contaminated and the mean total aflatoxins level in ginger, cinnamon, cloves and cardamom was 2.67, 2.88, 2.79 and 2.26 (µg/kg), respectively. Aflatoxin B_1 level in ginger, cinnamon, cloves and cardamom was 0.65, 0.41, 0.40 and 1.09 (µg/kg), respectively. About 10% of the contaminated spices had total aflatoxins above the acceptable EU regulatory level of 10µg/kg and the highest level was 11.9µg/kg, whereas 20.4% of contaminated spices contained aflatoxin B_1 above the acceptable EU regulatory level of 5µg/kg, of which the highest level was 11.23µg/kg. Thirty respondents, from whom the spice samples were collected, were interviewed through a structured questionnaire to assess their awareness on aflatoxins, handling and storage practices of spices. The majority (96.7%) of the participants had neither heard of aflatoxins nor attended any training related to food handling and storage. None of the participants were aware of the ill-health effects of aflatoxins on humans and animals. The odds of respondents with age between 36 and 44 years (OR = 0.326, 95%CI = 0.113 - 0.940, p = 0.038) was significantly associated with aflatoxin contamination of spices collected compared to other age groups. The odds of spices that were purchased from farmers (OR = 0.178, 95% CI = 0.061 - 0.525, p = 0.002) was also significantly associated with aflatoxin contamination of spices compared to other sources. The odds of storing spices for length of more than 14 days (OR = 3.608, 95%CI = 1.099 - 11.845, p = 0.034) was significantly associated with aflatoxin contamination of spices as compared to storing for shorter periods of time. It was concluded that the prevalence of aflatoxin contamination of the spices was high and the level of awareness on aflatoxins was very low in the study population. Hence, there was need for raising awareness and sensitization of stakeholders involved in spices and spice value chain.

DECLARATION

I, Sambwe Fundikira 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.

______________________________ ____________________

Fundikira Sambwe Date

(MSc. Food Quality and Safety Assurance)

The above declaration is confirmed;

_________________________ ____________________

Prof. Jovin K. Mugula Date

(Supervisor)

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DEDICATION

This dissertation is lovingly dedicated to my husband, Mr. Mark Ntangeki Lwakatare for his support, encouragement and constant love and to my children Daniella, Kenzo and Gabriella for their love and patience during my absence.

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LIST OF ABBREVIATION AND ACRONYMS

AFB_1	Aflatoxin B_1
AFB_2	Aflatoxin B_2
AFG_1	Aflatoxin G_1
AFG_2	Aflatoxin G_2
CI	Confidence Interval
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization
FtLD	Fluorescence Detector
HBV	Hepatitis B Virus
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ITC	International Trade Centre
LDC_S	Least Developed Countries
LOD	Limit of Detection
LOQ	Limit of Quantification
µg/kg	Microgram per Kilogram (ppb)
MTL	Maximum Tolerable Limits
RPM	Revolution per Minutes
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TBS	Tanzania Bureau of Standards
USA	United States of America

USDA	United States Department of Agriculture
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Mycotoxins are biologically active secondary metabolites produced by a variety of moulds such as *Aspergillus*, *Penicillium*, and *Fusarium* that can grow in a wide variety of foods including cereals grains, nuts, oilseeds, fruits, dried fruits, vegetables, cocoa and coffee beans, wine, beer, as well as herbs and spices (Khan *et al*., 2014). Mycotoxins can also be found in animal-derived food such as meat, eggs, milk, and milk derivatives if animals consume contaminated feed (Bryden, 2012; Marin, 2013)

It has been reported that 5-10% of agricultural products in the world are contaminated by moulds to the extent that they cannot be consumed by animals and humans (Tosun *et al*., 2013). There are currently more than 400 mycotoxins worldwide, but groups of mycotoxins considered potentially dangerous or of economic importance are the aflatoxins, ochratoxins, citrinin, zearalenone and trichothecenes (Ismaiel and Papenbrock, 2015).

Aflatoxins are one of the highly toxic secondary metabolites derived from polyketides produced by mould species such as *Aspergillus flavus*, *A. parasiticus*, and *A. nomius* (Payne and Brown, 1998). The moulds produce four main types of aflatoxins: aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). Aflatoxin B_1 (AFB_1) is the most carcinogenic mycotoxin. The International Agency for Research on Cancer (IARC) classified aflatoxins B_1 (AFB_1) as Group 1 human carcinogens (IARC, 2002). They are found in various foods including cereals, oilseeds, spices and nuts (Weidenborner, 2001; Reddy, 2010; Iqbal *et al*., 2012).

1.2 Production of Spices in Tanzania

Production of the major spice crops in Tanzania is undertaken in different areas with favourable environmental conditions. Spice crops such as cardamom and ginger, which belong to the same family, require heavy rainfall of over 1500 mm per annum, a temperature range of 20 - 35^{o}C and an altitude of between 300 and 1000 m above sea level (ITC, 2001). Pepper, clove and nutmeg are favoured by high relative humidity conditions and cannot tolerate excessive heat or dryness. Cinnamon requires intense sunshine (ITC, 2001). The production data on spices varies from one spice to the other depending on the season. The amount of cloves (*Syzygium aromaticum* (L.) produced in the year 2015 was 3,500 Mt, and a total of 9,500 Mt of ginger (*Zingiber officinale*), cardamom *(Elettaria cardamomum)* and cinnamon (*Cinnamomum zeylanicum*) was produced in the year 2014 (ITC, 2001).

1.3 Nutrition Value of Spices

Spices have strong flavours which make them to be used in small quantities; and tend to add few calories to food, even though many spices, especially those made from seeds, contain high portions of fat, protein and carbohydrate by weight, while others can contribute portions of micronutrients to the diet (USDA, 2013). When used in larger quantity, spices can also contribute a substantial amount of minerals, including iron, magnesium and calcium to the diet (Authority Nutrition, 2016). Most spices have substantial antioxidant activity, owing primarily to phenolic compounds, especially flavonoids, which influence nutrition through many pathways, including affecting the absorption of other nutrients (Ninfali *et al*., 2007). These antioxidants also can act as natural preservatives, preventing or slowing the spoilage of food, leading to a higher nutritional content in stored food.

1.4 Socio–economic Role of Spices

Spices are important cash crops and play a major role in the country economy. They provide foreign earnings; improve income and livelihood of people through employment. A wide range of spice crops are cultivated in Tanzania due to the existence of favourable climate and soil conditions. The most important spice crops produced for the local and export markets are cloves, pepper, chillies, cinnamon, cardamom, ginger, coriander, vanilla, garlic, lemongrass and red onions (ITC, 2001).

Globally, annual imports of culinary herbs and spices are in excess of US$ 2.0 billion with an annual growth rate of 8.5%. However, import markets for spices are concentrated with European Union (EU) and United States of America (USA), purchasing more than half of the total world exports (Jaffee, 2004; ITC, 2001). Between 1995 and 1999, Tanzania ranked third among Least Developed Countries (LDCs) by exporting 5% of LDCs' total spice exports. Madagascar was the largest LDC exporter (72%) followed by Comoros (6%), but total LDC exports fulfilled only 5.5% of global import demand (ITC, 2001).

1.5 Ill-health Effects Caused by Aflatoxins

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 (Lutfullah and Hussain, 2012).

1.6 Aflatoxin Contamination in Spices

Fungal contamination of spices usually occurs when spices are not properly dried or when stored in a highly humid environment (Lamanaka *et al.*, 2007). Many studies have been undertaken with regards to aflatoxins contamination in spices globally.

A study conducted in Nyahururu town in Kenya reported that 34 out of 46 spices samples collected and analyzed by using ELISA kit were contaminated with aflatoxins. Over 50% of samples analyzed contained aflatoxin <10 μg/kg. The highest quantities of aflatoxins were found in cayenne, paprika and cumin (99.6, 99.0 and 98.0 μg/kg, respectively). similarly relatively high levels of aflatoxins (31.5 μg/kg) were also found in chilli (Mwangi *et al.,* 2014).

Temu (2016) conducted a study on fifty samples of sixteen commonly used spices in Tanzania that were collected from different markets in Dar es Salaam to determine fungal contaminants associated with these spices. The fungi isolates were identified as *Lichtheimiaramosa, L. corymbifera, Rhizomucorpusillus, R. tauricus, A. aculeatinus, A. parasiticus, A. flavus, A. tubingensis, A. fumigatus, A. niger* and *A. nomiu*s. Red chilli had high level of fungal contamination (18.37%) followed by ginger (14.28%) while curry powder were less contaminated (2.08%).

There is inadequate information on aflatoxin contamination in commonly consumed spices in Tanzania, as well as factors associated with contamination. Most of the documented studies were on risks of fungal and mycotoxin contamination in staple crops and their control and mitigation measures.

1.7 Problem Justification

Aflatoxin contamination of agricultural produce is a growing concern globally, particularly in the food safety perspectives. In the past few decades incidences of aflatoxicosis have been reported within sub-Saharan Africa including the 2004 event in Kenya and 2016 aflatoxin outbreaks in Chemba and Kiteto districts of Tanzania (Lewis *et al*., 2005; WHO, 2017). Chronic exposure due to low levels of contamination consumed regularly, increases 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 humans (Makun *et al*., 2011).

Spices are also increasingly popular on the Tanzania market. Their uses are not limited to food but extend to industrial and medicinal uses. However, these commodities are susceptible to aflatoxin contamination due to the environmental conditions they are exposed to throughout the commodity value chain. A study carried out in India reported that 47% of the medicinal herb and spice samples collected from various markets had aflatoxin levels above acceptable regulatory limits (10 µg/kg for total aflatoxin) set by WHO (Aiko and Mehta 2013). Aflatoxin contamination of spices has also been reported in many countries including Hungary (Fazekas *et al.,* 2005), Morocco (Zinedine *et al*., 2006) and Kenya (Mwangi *et al*., 2014).

In Tanzania, mycotoxin contamination has been reported in various products including maize (Sasamalo et al., 2018; Nyangi *et al.*, 2016; Kamala *et al.*, 2015; Kimanya *et al*., 2010; Shirima *et al.*, 2013), cassava (Manjula, 2009); market-cured fish (Mugula and Lyimo, 1992); fermented porridge (Nyamete *et al*., 2016) and sunflower seeds and seed cake (Mmongoyo *et al*., 2017). However, there is limited information on aflatoxin contamination in spices which are also widely consumed with major staple foods, snacks and beverages in the country. Thus, understanding aflatoxin contamination of spices will help to educate farmers, traders, consumers and public on the problems of aflatoxin and how to control along the product value chain. Therefore, the aim of the study was to establish the safety of spices marketed in Tanzania, specifically in the most populous and commercial in capital city of Dar es Salaam.

1.8 Objectives

1.8.1 Overall objective

The main objective of this study was to carry out surveillance on aflatoxin contamination and safety of selected marketed spices in Tanzania

1.8.2 Specific objectives

The specific objectives of this study were to:

i. Determine the levels of aflatoxin contamination in cloves, cinnamon, cardamom and ginger collected from various markets in Dar es Salaam.
ii. Assess the awareness, handling, storage and packaging factors associated with aflatoxin contamination of marketed spices in Dar es Salaam.

The findings of this research work were reported in two manuscripts presented as chapter two and three.

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CHAPTER TWO

2.0 AFLATOXIN CONTAMINATION OF CLOVES, CINNAMON, CARDAMOM, AND GINGER MARKETED IN DAR ES SALAAM, TANZANIA

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Abstract

The aim of this study was to determine the levels of aflatoxins in spices, particularly cinnamon, ginger, cardamom and cloves marketed in Dar es salaam, Tanzania. The samples (n=120) were collected in Dar es Salaam and analyzed for aflatoxin B_1, B_2, G_1 and G_2 and total aflatoxins using immuno-affinity high performance liquid chromatography and post column derivatisation. Fifty eight percent of the spice samples were contaminated and the mean of total aflatoxin level in ginger, cinnamon, cloves and cardamom was 2.67, 2.88, 2.79 and 2.26 (μg/kg), respectively. Mean levels for aflatoxin B_1 in ginger, cinnamon, cloves and cardamom was 0.65, 0.41, 0.40 and 1.09 (μg/kg), respectively. About 10% of the contaminated spices had total aflatoxins above the EU acceptable levels (10 μg/kg), and the highest level was 11.9 μg/kg. Moreover 20.4% of the samples analysed had aflatoxin B_1 above the EU acceptable levels of 5μg/kg and the highest level was 11.23μg/kg. The study indicated that spices analyzed (ginger, cinnamon, cloves and cardamom) could pose a food safety hazard to consumers. Thus there is a need for creation of awareness and control of aflatoxins in spices value chains in Tanzania.

+**Keywords**: aflatoxins, spices, cinnamon, ginger, cardamom, cloves, food safety

2.1 Introduction

According to FAO (2005), spices can be defined as "vegetable products used for flavouring, seasoning and imparting aroma in foods". The European Spice Association (2014) defined culinary herbs and spices as "edible plant parts that are traditionally added to foodstuffs for either their natural flavouring and/or visual properties". Spices are also consumed for their associated health benefits including antioxidant, anti-allergen and antimicrobial effects (Toma and Abdulla, 2013).

Spices are the most versatile plant substances widely used all over the world (Hashem and Alamri, 2010). They are also known for their nutritional value, e.g., coriander, fenugreek, turmeric and pepper are good sources of calcium, phosphorous, potassium and sodium (Authority Nutrition, 2016). Moreover, spices are widely used as medicine, for example, clove is used for the treatment of toothache and also relieves upper respiratory infections (Parthasarathy *et al.*, 2008). Also ginger is traditionally used for various human ailments, to facilitate digestion and to treat stomach upset, diarrhea and nausea (Parthasarathy *et al.*, 2008; Stipanuk, 2000).

Furthermore, cinnamon is used as a flavoring agent in chewing gums, sweets, ice cream and beverages, also useful in the treatment of parched mouth, bronchitis, diarrhea, heart disease, antimicrobial, ant diabetic, antioxidant and urinary disease. The seeds, oils and extracts of cardamom are thought to have impressive medicinal properties and have been used in traditional medicine for centuries (Parthasarathy *et al.*, 2008).

Spices are usually produced in countries with high temperatures and high humidity (Martins *et al*. 2001) that favour growth of mycotoxigenic moulds. Mycotoxin contamination of spices has been reported in Nyahururu, Kenya and in Morocco (Mwangi *et al.*, 2014; Zinedine *et al.*, 2006). They could be contaminated with mycotoxigenic moulds during pre-and/or post-harvesting, drying, handling, packaging, storage and transportation (Banerjee and Sarkar, 2003, Tassaneeyakul *et al*., 2004). In addition, aflatoxins may be found in spices and herbs, which are very important ingredients used in different types of food and/or medicine all around the world (Hashem and Alamri 2010, Hammami *et al.*, 2014).

Mycotoxins are secondary fungal metabolites produced by various toxigenic genera such as *Aspergillus*, *Fusarium* and *Penicillium*. There are currently more than 400 types of mycotoxins worldwide, but groups of mycotoxins considered potentially dangerous are the aflatoxins, fumonisins, ochratoxins, citrinin, zearalenone and trichothecenes (Ismaiel and Papenbrock, 2015). It is estimated that approximately 25% of the world's agricultural commodities are contaminated to some extent with mycotoxins (Zain, 2011). Such studies revealing necessarily high occurrences and concentrations of mycotoxins suggest that mycotoxins are a constant concern Aflatoxin is the most common mycotoxin in spices, and its contamination in food has been reported by several studies including spice products (Jalili and Jinap, 2012).

Aflatoxins are highly toxic secondary metabolites produced by the *A.flavus*, *A.parasiticus* and *A.nomicus* with detrimental effects on human and animal health, including carcinogenicity, hepatogenicity, mutagenicity, immune suppression and neo plasticity (Varga *et al.*, 2011). There are four main types of aflatoxin; AFB_1, AFB_2, AFG_1 and AFG_2.

2.2 Materials and Methods

2.2.1 Sample collection

As the exact population of spice retailers was unknown, the sample size was estimated using the Kothari equation (Kothari and Garg, 2014): - $n = z^2P(1-P)/e^2$

Where, n= sample size, Z= standard variate at a given confidence level, for this study a 95% confidence level = 1.96 at and e = acceptable error (the precision/ estimation error) set at 8% (0.08) for this study. Thus, $1.96^2 \times 0.05 (1 – 0.05)/ 0.08^2 = 30$

Four types of spices (cinnamon, ginger, cloves and cardamom) were collected in Ilala, Temeke and Kinondoni municipalities in Dar es Salaam, Tanzania, between December 2017 and January 2018. A total of 120 dried and powdered samples were collected in clean polythene bags from 30 randomly selected retailers. In order to get the good representation of Dar e Salaam, higher number of samples was taken from Ilala districts due to the fact that most of the big markets/retailers as situated in Ilala. The samples were coded, transported to the Tanzania Bureau of Standards (TBS) food laboratory and stored at temperature of 4ºC prior to aflatoxin analysis.

Table 2.1: Number of samples collected from each municipality market

Sampling sites (markets)	Cinnamon	Ginger	Clove	Cardamom	Total
Kinondoni	8	8	8	8	32
Temeke	9	9	9	9	36
Ilala	13	13	13	13	52
Total	30	30	30	30	120

2.2.2 Aflatoxin analysis

2.2.2.1 Chemicals and standards

The chemicals and reagents used in different procedures of sample preparation were of chromatography grade. These included: acetonitrile (HPLC grade, methanol (HPLC grade), water (HPLC grade) (all from Fisher Chemical, Bishop Meadow Road, Loughborough, Leicestershire), aflatoxins standards (AFB_1, AFB_2, AFG_1 and AFG_2) and immunoaffinity columns (AflaTest from Romer Labs GmbH, Technopark 1, 3430 Tulin, Austria).

2.2.2.2 Extraction of samples

Cinnamon, clove, cardamom and ginger samples were ground separately to obtain a homogenous flour mixture and then sub-divided to obtain representative sub samples for analysis. Each ground spice sample was placed into Erlymeyer flask and weighed using the calibrated analytical balance to 25 $\pm$ 0.1g. Using a measuring cylinder, 100 ml of methanol: water (70:30 v/v) as extraction solvent was added to the 250 ml Erlymeyer 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/30 min, then using a filter paper Whatman No. 1, the extract was filtered into a 250 ml Erlymeyer flask according to the procedure described by Hussain *et al*. (2012).

2.2.2.3 Dilution stage

The sample extract (4 ml) was transferred to 15 ml centrifuge tube, followed by addition of 8 ml of distilled water. Then, the mixture was vortexed (Talboys® Hvy Dty Vortex, Troemner LLC, USA) for 1 minute to get a homogeneous mixture.

2.2.2.4 Isolation and clean-up of aflatoxins

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. 0.3 ml of the eluate was mixed with 0.6 ml of water and 0.1 ml of acetonitrile and the mixture was vortexed for 30 seconds (ISO 16050).

2.2.2.5 HPLC system

After extraction, dilution, cleaning and elution and post-column derivatization, the extracts were analyzed using HPLC with fluorescence detector (FtLD) (Model Agilent ChemStation 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 (AFB_1, AFB_2, AFG_1 and AFG_2) was performed on the C_{18} column at a temperature of $30^{O}C$ 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 to allow their detection with fluorescence detector at an emission wavelength of 465 nm and an excitation wavelength of 360 nm.

2.2.2.6 Quality assurance

A mixture of aflatoxin standards solution (B_1, B_2, G_1, and G_2) was prepared and analysed at the concentration ranges indicated in (Table 2.2) to establish a four point calibration curve (Figures 2.1 -2.4). The diluent was the same as the mobile phase (Water 6: Methanol 3: acetonitrile 1). The calibration curve was constructed to check the linearity

(R^2=0.9999) and was also used for quantification of aflatoxins. All aflatoxins were well separated from each other as indicated in the standard sample chromatograms Figure 2.5

Table 1.2: Standard concentration and peak areas for AFB_1, AFB_2, AFG_1 and AFG_2 used for drawing calibration curves

#	RT	Compound	Level	Amount	Area	Actual concentration
1	5.106	Aflatoxin G1	1	1	368.82	1.05553
			2	5	1635.8	5.00774
			3	10	3255.7	9.98526
			4	15	4871.3	15.00354
2	6.018	AflatoxinG2	1	1	172.99	1.07494
			2	5	790.24	4.99263
			3	10	1567.6	10.00130
			4	15	2351.4	14.99660
3	6.716	AflatoxinB2	1	1	384.94	1.06342
			2	5	1713.9	5.03320
			3	10	3381.8	10.01536
			4	15	5041.9	14.97446
4	8.094	Aflatoxin B1	1	1	183.24	1.04660
			2	5	837.51	5.02996
			3	10	1656.6	10.01670
			4	15	2471.1	14.97577

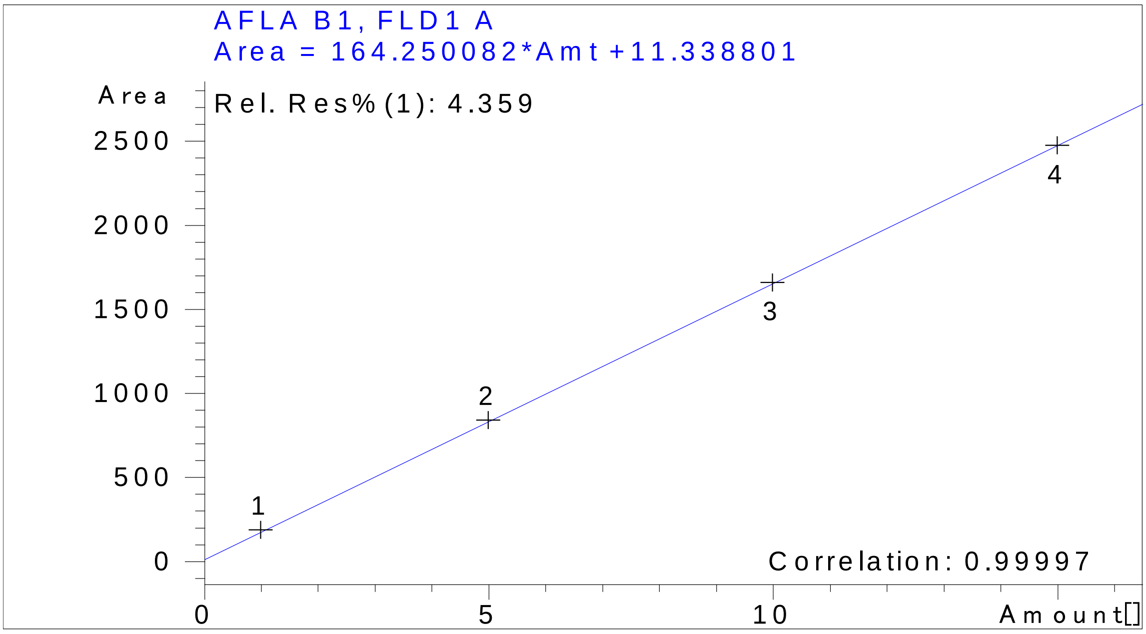

Figure 2.1: The Calibration curve for AFB_1 on spice

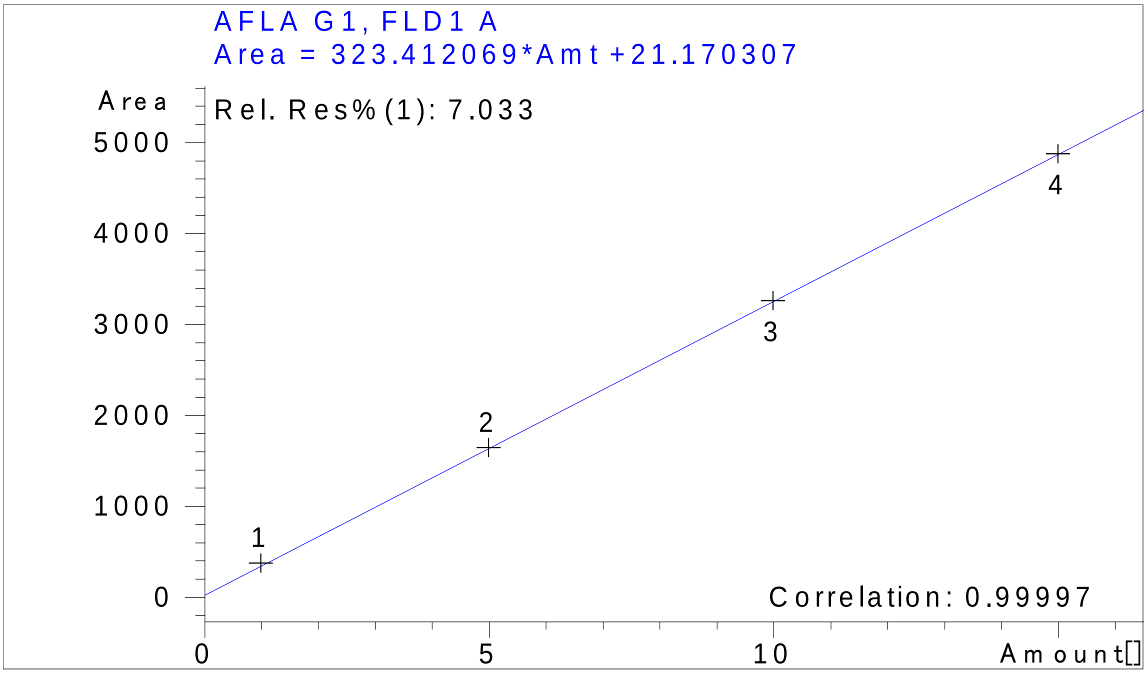

Figure 2 .2: The Calibration Curve for AFG_1 on Spice

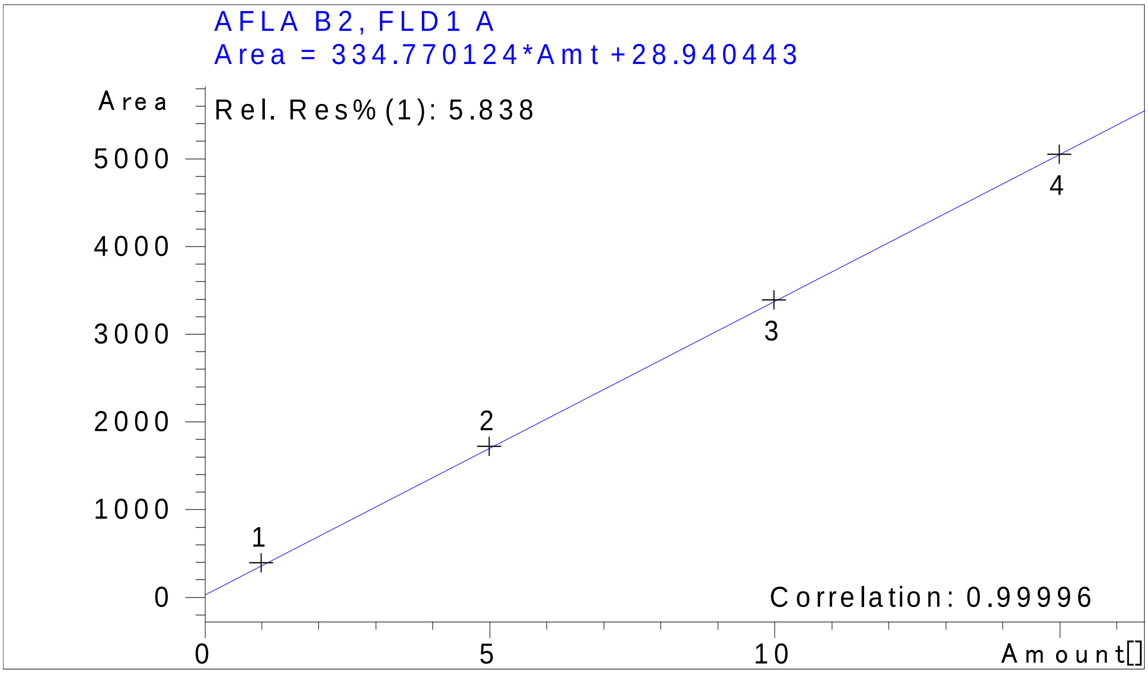

Figure 2.3: The Calibration Curve for AFB_2 on Spice

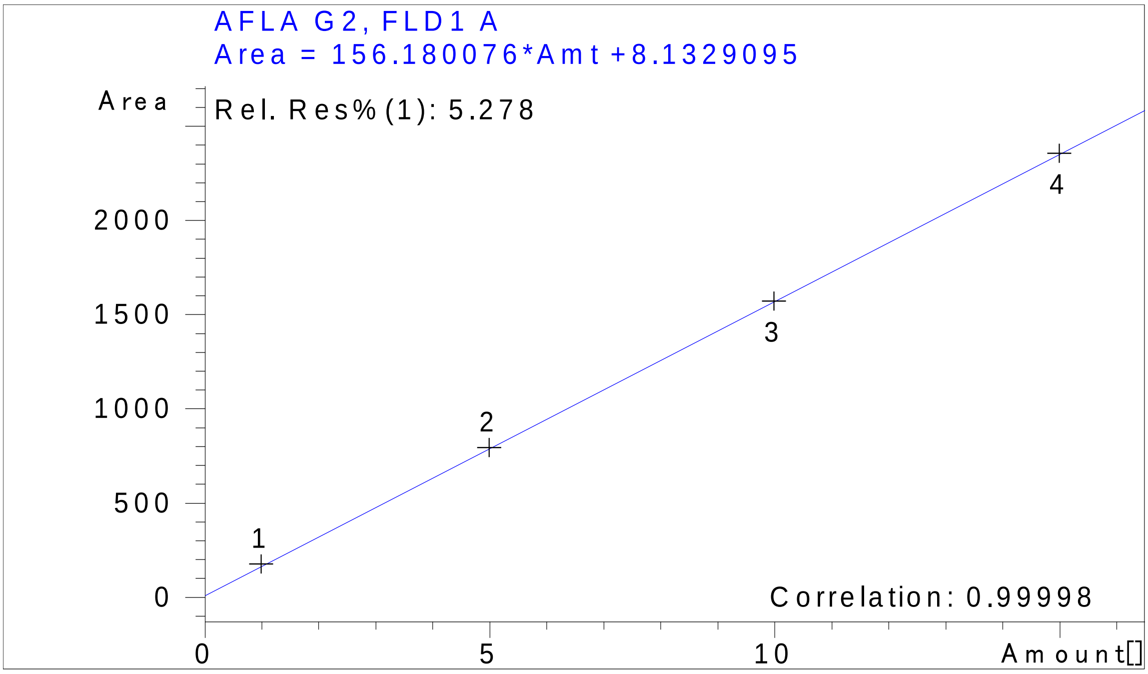

Figure 2.4: The Calibration Curve for AFG$_2$ on Spice

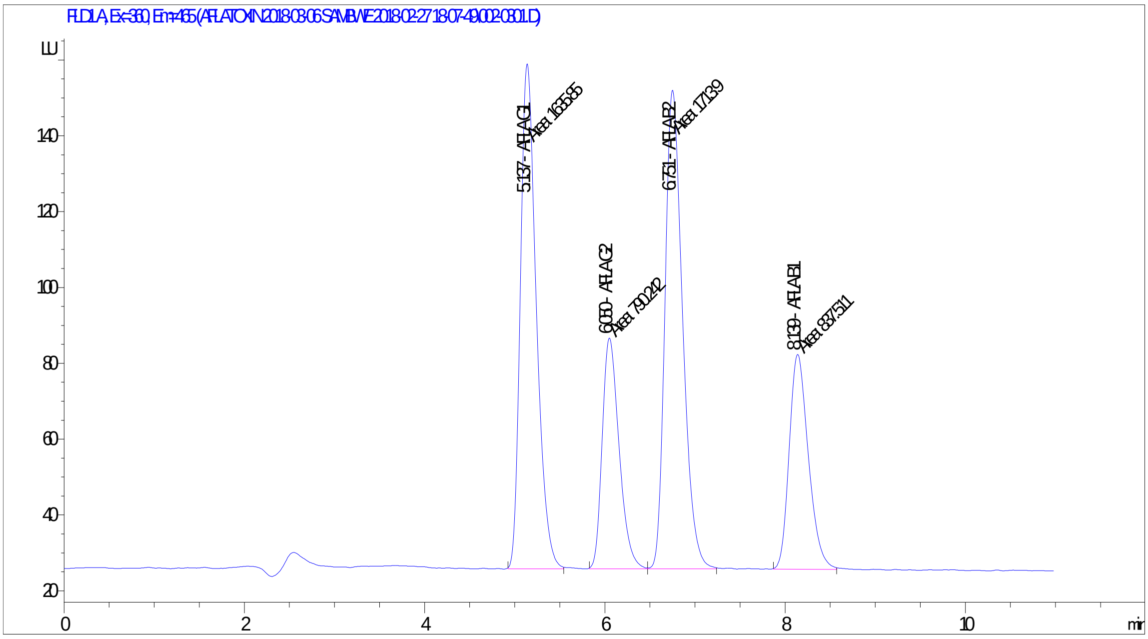

Figure 2.5: HPLC Chromatogram of aflatoxin standards (5 µg/kg)

2.2.2.7 Determination of the limit of detection and limit of quantitation of the HPLC method

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 2.1 and 2.2 respectively. The LOD and LOQ values obtained for the four types of aflatoxins are shown in Table 2.3.

LOD = Mean of the lowest concentration + 3SD………………………………..…… .. (2.1)

LOQ = Mean of the lowest concentration + 10 SD……………………………..………(2.2)

Where SD = Standard deviation of the lowest concentration (Armbruster *et al.* 2008).

Table 2.3: The Limit of detection (LOD) and limit of quantitation (LOQ) for each analyzed aflatoxin

	LOD (µg/kg)	LOQ (µg/kg)
AFG_2	0.13	0.16
AFG_1	0.13	0.21
AFB_2	0.13	0.18
AFB_1	0.16	0.29

2.3 Statistical Analysis

Data was analysed using Genstat Version 14.2, VSN International Ltd. One way Analysis of variance (ANOVA) was used to test significance difference on the aflatoxin levels amongst the types of marketed spices and study districts. The differences between means were detected using Duncan's multiple range tests in mean of AFB_1 AFB_2, AFG_1, AFG_2 and total aflatoxins.

2.4 Results

2.4.1 Recovery of Aflatoxins

The sensitivity of the method was determined by determining percent recovery of aflatoxins by calculating the percentage recovery of aflatoxins spiked blank spice samples.

The recoveries of all aflatoxins were greater than 70% (71.47 to 73.64 %) (Table 2.4) indicating the suitability and good performance of the approved aflatoxin extraction protocol and quantification (AOAC-RI050901) (Shah *et al.*, 2000).

Table 2.4: Recovery of aflatoxins from spiked spice samples

	Concentration of aflatoxins in blank sample (µg/kg)	Spiked concentration (µg/kg)	Detected concentration (µg/kg)	% Recovery
AFG_2	0.00	5.00	3.64	72.84
AFG_1	0.00	5.00	3.57	71.47
AFB_2	0.00	5.00	3.67	73.38
AFB_1	0.00	5.00	3.69	73.64

2.4.2 Aflatoxin contamination in spices

The mean values of aflatoxin (AFB_1, AFB_2, AFG_1 , AFG_2,) and total aflatoxins in cinnamon, cloves, ginger and cardamom ranged from 0.40±0.10 to 3.06±1.24 as shown in Table 2.5**.** The highest mean value for total aflatoxin was in cinnamon. However, there was no significant difference between the means at $p<0.05$.

Table 2.5: Mean aflatoxin concentrations (µg/kg) in cloves, cinnamon, cardamom and ginger collected in Dar es Salaam (Mean±SEM)

Spice	n	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins
Cinnamon	30	2.39±1.29[a]	1.83±0.75[a]	1.22±0.79[a]	0.41±0.25[a]	2.88±0.87[a]
Ginger	30	2.00±0.99[a]	0.49±0.13[a]	0.88±0.31[a]	0.65±0.39[a]	2.67±0.69[a]
Cloves	30	3.06±1.24[a]	0.65±0.18[a]	1.13±0.32[a]	0.40±0.10[a]	2.79±0.79[a]
Cardamom	30	1.53±0.42[a]	1.30±0.54[a]	1.54±0.92[a]	1.09±0.23[a]	2.26±0.60[a]

- Mean with different superscripts are significant different at $p<0.05$
- n is the total number of samples analysed for each spice

It was observed in Table 2.6 that, out of 120 spice samples collected from various markets in Dar es Salaam 69 samples (57.5%**)** were contaminated with aflatoxins, and out of which

49 samples (40.8%) were contaminated with AFB_1. The range of aflatoxin contamination was 0.13 - 11.22 µg/kg in cinnamon, 0.55 - 9.66 µg/kg in ginger, 0.16 - 11.23µg/kg in cloves and 0.23 - 11.90 µg/kg in cardamom. Cloves show high prevalence with AFB_1 and total aflatoxin with 63.3 and 70.0%, respectively. Cinnamon was the least susceptible to contamination with AFB_1 and total aflatoxin of 26.7 and 43.3%, respectively.

Table 2.6: Percentage of cloves, cinnamon, cardamom and ginger contaminated with aflatoxins in Dar es Salaam

Spice		Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total Aflatoxin
	N	n (%)	n (%)	n (%)	n (%)	n (%)
Cinnamon	30	8 (26.7)	6 (20.0)	5 (16.7)	3(10.0)	13(43.3)
Ginger	30	12 (40.0)	9 (30.0)	12(40.0)	7(23.3)	17(56.7)
Cardamom	30	10 (33.3)	5 (16.7)	12(40.0)	7(23.3)	18(60.0)
Cloves	30	19 (63.3)	7 (23.3)	4 (13.3)	3(10.0)	21(70.0)
Total	120	49(40.8)	27(22.5)	33(27.5)	20(16.7)	69(57.5)

- N is the total number of samples analyzed for each spice
- n is the total number of contaminated spices

From the samples that were found to be contaminated with aflatoxins, there were spices that exceeded the acceptable EU regulatory limits for aflatoxin B_1 (5 µg/kg) and total aflatoxin (10 µg/kg) with maximum concentrations of 11.23 µg/kg and 11.90 µg/kg respectively. In table 2.7, twenty percent of AFB_1 contaminated cardamom spices were found to exceed the EU regulatory limits for AFB_1 contamination and 11.1% of cardamom contaminated sample were found to exceed the EU regulatory limits for total aflatoxin while no sample of ginger exceeded maximum regulatory limit for total aflatoxin. Seventy one percent (71%) of all contaminated samples were contaminated by AFB_1 (0.15-11.23 µg/kg).

Table 2.7: Incidence of total aflatoxins and Aflatoxin B_1 (AFB_I)contamination in spices that exceeded EU regulatory limits

Spice	Aflatoxin tested	N	Positive samples n (%)	Exceed EU regulatory limits n (%)	Concentration range(μg/kg)
Cinnamon	AFB_1	30	8 (26.7)	1 (12.5)	0.39-11.22
	Total Aflatoxin	30	13 (43.3)	1 (7.7)	0.13-11.22
Cloves	AFB_1	30	19 (63.3)	1 (5.3)	0.15-11.23
	Total Aflatoxin	30	21 (70.0)	1 (4.8)	0.16-11.23
Ginger	AFB_1	30	12 (40.0)	2 (16.7)	0.18-9.66
	Total Aflatoxin	30	17 (56.7)	0 (0.0)	0.55-9.66
Cardamom	AFB_1	30	10 (33.3)	2 (20.0)	0.23-7.20
	Total Aflatoxin	30	18 (60.0)	2 (11.1)	0.23-11.90

- Positive samples are all analyzed samples with value > limit of detection (LOD)
- N is the total number of analyzed samples for each spice
- EU regulatory limits for AFB1 (5.0 μg/kg); for total aflatoxin (10.0 μg/kg)

In the three study districts of Kinondoni, Ilala and Temeke that the spices samples were collected for analysis, Ilala district was the one found to have the most contaminated samples (73.1%) while Kinondoni had the least contaminated samples (43.8%). Table 2.8 below shows number of contaminated spices across the three districts.

Table 2.8: Number of contaminated spice samples collected in Dar es Salaam districts

Districts	AFB_1		AFB_2		AFG_1		AFG_2		TOTAL AF	
	n	**%**	**N**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Kinondoni (N=32)	10	(31.3)	4	(12.5)	9	(28.1)	1	(3.1)	14	(43.8)
Ilala(N=52)	31	(59.6)	16	(30.8)	12	(23.1)	15	(28.8)	38	(73.1)
Temeke(N=36)	8	(22.2)	7	(19.4)	12	(33.3)	4	(11.1)	16	(44.4)

- n is the total number of contaminated samples
- N is the total number of samples analysed in each districts

The mean values for total aflatoxin contamination in the selected districts ranged from 1.47 ± 0.48 to 3.21 ± 0.52 μg/kg of which the highest aflatoxin mean value was in Ilala district. However there was no significant difference in aflatoxin contamination between the three locations that the spices where procured at $p<0.05$ (Table 2.9).

Table 2.9: Mean concentration of aflatoxins in spices in Dar es Salaam districts (mean +SEM)

District	AFB1 (µg/kg)	AFB2(µg/kg)	AFG1(µg/kg)	AFG2(µg/kg)	TOTAL(µg/kg)
Kinondoni	0.54±0.14[a]	1.02±0.79[a]	1.13±0.54[a]	0.91±0.00a	1.47±0.48[a]
Ilala	2.71±0.60[a]	1.11±0.35[a]	0.96±0.28[a]	0.58±0.19[a]	3.21±0.52[a]
Temeke	1.66±1.06[a]	0.83±0.19[a]	1.23±0.38[a]	0.57±0.32[a]	2.23±0.70[a]

Mean with different superscripts are significant different at $p<0.05$

2.5 Discussion

Spices have gained popularity due to their use in a wide range of meals to enhance flavor, color and aroma, especially in ready-to-eat meals and to create variety in the kitchen. However, spices may be contaminated with aflatoxins at every stage of development like pre-harvest, during drying, storage and transport (Elshafie *et al*., 2002).

This study was able to analyze and determine the level of aflatoxin in four different spices collected from various markets in Dar es Salaam. The results showed that 69 samples (57.5%) out of 120 spice samples collected were contaminated with total aflatoxins ranging 0.13-11.90 µg/kg of which 49 samples (40.8%) of the collected spices were contaminated with AFB_1 with the level ranging (0.15-11.23 µg/kg). AFB_1 have been detected more frequently compared to other types of aflatoxin (Table 2.6 and 2.8), this is similar with the study reported by Ozbey and Kabak, 2012. Prevalence of aflatoxin contamination obtained in this study was significantly high which indicates the risk of chronic exposure to the consumers.

The overall prevalence of aflatoxin contamination for this study (57.5%) was similar to the study reported by Haruna *et al.* (2016) on spice samples collected from Katsina central market, Nigeria whose prevalence was 57.1% of total aflatoxin contamination. This is contrary to studies reported by (Khazaeli *et al.* 2017) in Iran on commercial spices collected from various regions in Iran whose prevalence of aflatoxin contamination were 30.8% and 40.0% in which all of these samples were also contaminated with AFB1. However, the prevalence of this study was lower compared to the study conducted in Nyahururu retail market, Kenya which reported that 73.9% of the spice samples were contaminated with total aflatoxins (Mwangi *et al.*, 2014). This might have been due to good postharvest handling of the spices observed which resulted to low level of aflatoxin contamination. This was expressed by the ability of retailers to sort spoiled spices and further drying of the spices upon receipt. It have been reported that physical sorting is also another effective measure in the reduction of aflatoxins, as high as 40–80% (Bullerman and Bianchini, 2007).

Out of 30 cinnamon samples that were analysed for total aflatoxins, 13 (43.3 %) were contaminated by aflatoxins of varied concentrations ranging from 0.13 to 11.22 μg/kg with the mean concentration of 2.88 μg/kg. However, one sample (7.7%) of positive sample was observed with levels of total aflatoxins above the acceptable limit (10μg/kg) while for AFB_1, 1 sample (12.5%) exceeded the limit (5μg/kg) set by European countries/South Africa (Atas *et al.,* 2012; William *et al.*, 2014). In addition a study conducted by Mwangi *et al.* (2014) in Kenya reported high contamination levels of total aflatoxin in cinnamon with mean concentration of 15.1μg/kg. The results obtained in this study is slightly similar with findings reported by Al Juraifani, (2011) in Saudi Arabia which showed that 31 out of 50 cinnamon samples were found to be contaminated with AFB_1 but did not exceed maximum tolerable level of 5 μg/kg set by EU. Lower level of contamination of aflatoxin B1 in cinnamon was also reported by Ramagnoli *et al.* 2007 where by one cinnamon powder sample marketed in Italy contained AFB1 of 0.98μg/kg. This could be due to proper drying of spices after receipt, sorting of spices and the ability of the retailers to identify spoilage in spices which reduce the risk of fungi growth hence aflatoxin contamination.

There are several reports on the inhibitory effect of cloves on fungi (Bokhari, 2007) which is in contrast with observations made this study. About 21 (70 %) clove samples out of 30 collected samples from all sites were contaminated with total aflatoxins with mean concentration of 2.79µg/kg. This study revealed that one sample was observed with higher levels of total aflatoxins and AFB_1 of 11.23µg/kg and 11.23µg/kg respectively, above the acceptable limit (10µg/kg and 5µg/kg) set by European countries/South Africa. Higher levels above acceptable limits pose a risk to public health since consumption of the contaminated spices could lead to acute and/ chronic health conditions. (Atas *et al.,* 2012 and William *et al.,* 2014).

This is in contrast with the study done by Haruna (2016) which reported that clove from Tsohuwar Kasuwa market in Nigeria had higher contamination level > 20 µg/kg. Also a study done in Kenya, by Mwangi *et al.* (2014) reported higher aflatoxins contamination of different spices in which clove showed mean concentration of 7µg/kg for total aflatoxins. Agricultural practices and postharvest handling of the spices could also contribute to contamination by toxigenic fungi.

Among the four spices, cardamom had a prevalence of 60% where by 18 out of 30 samples were contaminated with aflatoxin. Also 33.3% of the contaminated samples were contaminated with AFB1. This might have been due to poor pre and post-harvest handing which resulted into aflatoxin contamination. This is in contrast with the study done by Elishafie *et al*. (2002) who did not detect any aflatoxins in cardamom.

The result also showed that 17 out of 30 (56.7%) of ginger samples were contaminated with total aflatoxins with mean concentration of 2.56 µg/kg, two samples (16.7%) were found to exceed maximum regulatory limit of 5 µg/kg for AFB1 and none exceeded for total aflatoxin. This poses a health risk to the public due to consumption of aflatoxin contaminated spices.

The levels of aflatoxins revealed by this study were lower compared with the levels reported from other countries; for instance in USA where botanicals used for medicinal and health-promoting purposes, including ginger were assessed for aflatoxins contamination and reported that ginger and ginger products were highly contaminated with aflatoxins at levels of 31 µg/kg (Trucksess and Scott, 2008). Also in Morocco aflatoxins were found in 10 out of 12 (83.3%) ginger samples analyzed and had aflatoxin level of 9.1 µg/kg (Zinedine *et al.* 2006) which is higher compared to this study.

However lower level of aflatoxin contamination in ginger have been reported in Korea by Cho *et al.* (2008) that only one of 7 ginger samples analyzed contained aflatoxins of 0.18 µg/kg. It is important to note that the variations reported in these previous studies, and those of the present study, are probably due to environmental factors and pre and post-harvest handling (Klich, 2007; Adzahan *et al*. 2009) which may affect fungal growth and thus mycotoxin contamination. Also Reddy *et al*. (2009) reported that hot and humid conditions are favorable for the growth of toxigenic fungi and ultimately aflatoxins production in agricultural products which is a typical condition in Dar es Salaam.

2.6 Conclusion

A surveillance of the four different types of spices (ginger, cinnamon, clove and cardamom) from three districts in Dar es Salaam was performed by determining the aflatoxin (AFB_1, AFB_2, AFG_1 and AFG_2) contents. The obtained results demonstrated that a significant number of samples were contaminated with aflatoxins.

It may be concluded that the contamination of aflatoxins in spices might have chronic ill-health effects in humans due to exposure to low dose of aflatoxins in spice sample which is normally consumed in small portions but on daily basis due to its increasingly use for culinary and therapeutic purposes. This study indicated the need for improvement in use of best practices along the value chain and need for regulatory authorities in Tanzania to continuously investigate and monitor aflatoxins contamination in marketed spice on regular basis to ensure that mycotoxins are not present at levels that may pose food safety risks to safeguard public health.

2.7 Recommendations

Aflatoxins continue to pose food safety risk to humans who are exposed to aflatoxins contaminated food, however, in this study it seems that the contamination of aflatoxins in analysed spices samples was low although it does not guarantee the safety of these products because spices are consumed in our daily lives, so regular monitoring is recommended.

Since the Tanzanian standards/ East African Standards for spice and spice products does not cover mycotoxin requirements eg.aflatoxins, this findings highlight the need for establishment of aflatoxins acceptable levels in spice standard for continuous monitoring in the market for assurance of public safety.

Further studies should be done to determine the levels of aflatoxins in spices other than those investigated in this study and contamination level of aflatoxins in composite spices in order to ensure the safety of all spices consumed in the country.

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CHAPTER THREE

3.0 AWARENESS, HANDLING AND STORAGE FACTORS ASSOCIATED WITH AFLATOXIN CONTAMINATION OF MARKETED SPICES IN TANZANIA: A CASE STUDY OF DAR ES SALAAM

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3.1 ABSTRACT

Mycotoxins particularly aflatoxins have been an economic and public health concern globally. In Tanzania, studies have been reported on the handling practices, levels of awareness and knowledge on common staple crops such as maize, groundnuts, complementary food and animal feeds. However, there have been limited studies that have focused on the levels of awareness and knowledge; and post-harvest practices with regards to market spices and their association with aflatoxin contamination. A structured questionnaire was used to interview 30 spice retailers to assess the awareness, handling, storage and packaging factors associated with aflatoxin contamination of marketed spices in Dar es Salaam. A significant majority (96.7%) of the participants had never heard of aflatoxins nor attended any training related to food handling and storage. None (0.0%) of the participants were aware of the health effects of aflatoxins in humans and animals. The odds of respondents with age between 36 and 44 years (OR = 0.326, 95%CI = 0.113 - 0.940, p = 0.038) was significantly associated with aflatoxin contamination of spices collected compared to other age groups. The odds of spices that were purchased from farmers (OR = 0.178, 95%CI = 0.061 - 0.525, p = 0.002) was also significantly associated with aflatoxin contamination of spices compared to other sources. The odds of storing spices for length of more than 14 days (OR = 3.608, 95%CI = 1.099 - 11.845, p = 0.034) was significantly associated with aflatoxin contamination of spices as compared to storing for lesser periods of time. It was concluded that, the level of awareness and knowledge about aflatoxins was very low in the study population. Hence, there is need for raising awareness to populations involved in spices and spice products.

Keywords: Aflatoxin, Awareness, Handling, Storage, Spices, food safety.

3.2 INTRODUCTION

Mycotoxins are biologically active secondary metabolites produced by a variety of moulds such as *Aspergillus*, *Penicillium*, and *Fusarium* that can grow in a wide variety of foods including cereals grains, nuts, oilseeds, fruits, dried fruits, vegetables, cocoa and coffee beans, wine, beer, as well as herbs and spices. Mycotoxins can also be found in animal-derived food if animals eat contaminated feed, namely meat, eggs, milk, and milk derivatives (Bryden, 2012; Marin, 2013). It has been reported that 5-10% of agricultural products in the world are contaminated by moulds to the extent that they cannot be consumed by animals and humans (Tosun *et al*., 2013).

There are currently more than 400 mycotoxins worldwide, however groups of mycotoxins considered potentially dangerous or of economic importance are the aflatoxins, ochratoxins, citrinin, zearalenone and trichothecenes (Ismaiel and Papenbrock, 2015).

Aflatoxins are one of the highly toxic secondary metabolites derived from polyketides produced by mould species such as *Aspergillus flavus, A. parasiticus*, and *A. nomius* (Filazi and Sireli, 2013; Omar, 2013). The moulds produce four main types of aflatoxin: aflatoxin (AFB_1, AFB_2, AFG_1, and AFG_2). Aflatoxin B_1 (AFB_1), the most carcinogenic mycotoxin, is typically produced in higher quantities. The International Agency for Research on Cancer (IARC) classified [a]naturally occurring mixes of aflatoxins as a Group 1 human carcinogens (IARC, 2002). They are found in various cereals, oilseeds, spices, and nuts (Weidenborner, 2001; Reddy, 2010; Iqbal *et al*., 2014).

The contamination of aflatoxins in foods and feeds is currently a significant problem in the world. Aflatoxins cause a number of adverse health effects in both animals and humans, resulting in economic loses and undesirable trade barrier for both raw materials and consumable products (Wu, 2006). Aflatoxins are of economic and health importance due to their ability to contaminate animal feeds, in particular cereals, nuts and oil seeds, and they are considered as one of the most dangerous contaminants of food and livestock feeds (Kaaya and Warren, 2005). 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 (Lutfullah and Hussain, 2012). The extent of food and feed contamination with aflatoxin vary with geographical location, agricultural and agronomic practice, and the susceptibility of food contamination with aflatoxins during pre-harvest, storage and processing period (Arapcheska *et al*., 2015).

Fungal contamination of crops including spices usually occurs when crops are not properly dried or when stored in a highly humid environment (Lamanaka *et al.*, 2007). Many studies have shown that factors that associate with aflatoxin contamination in developing countries include poor pre and post-harvest handling practices (Kamala *et al.* 2016) and other inefficient regulatory enforcement and educational programs (WHO, 2006).

Unnevehr *et al*. (2013) and Waliyar *et al*. (2008) reported that farmers and the general public in developing nations knew less about aflatoxins and the health effects associated with aflatoxin contamination. It is reported that over 5 billion people living in poor or developing countries globally are at risk of chronic exposure to aflatoxin through the consumption of contaminated foods (Wu *et al.*, 2011). The low levels of awareness and knowledge of aflatoxins among people living rural areas has contributed to this contamination and exposures because their own grown food are never subjected to any regulatory enforcement procedures (Cotty *et al*., 2007).

Aflatoxin contamination of agricultural produce is a growing concern globally, particularly in the food safety perspectives. In the past few decades incidences of aflatoxicosis have been reported within sub-Saharan Africa including the 2004 event in Kenya (Lewis *et al*. 2005) and 2016 aflatoxin outbreaks in Chemba and Kiteto districts of Tanzania (The Citizen, 2016; WHO, 2017). Chronic exposure due to low levels of contamination consumed regularly, increases 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 poisoning and even death to humans (Makun *et al*., 2012).

Spices are also increasingly popular in the Tanzania market. Their uses are not limited to food but extend to industrial and medicinal uses. However, these commodities are susceptible to aflatoxin contamination due to the environmental conditions they are exposed to throughout the value chain. Many studies have been reported on the factors associated with aflatoxin contamination including pre and post-harvest handling of crops, low levels of awareness and knowledge of consumers and producers/handlers of crops, educational and regulatory programs (WHO, 2006; Cotty *et al.*, 2007; Kamala *et al.*, 2016; Monyo *et al.*, 2010; Jolly *et al.*, 2006; Ayo *et al*., 2018).

In Tanzania, there have been studies reported on the awareness and knowledge of different populations with regards to aflatoxin contamination. These studies have shown an association between socio-demographic and/or economic characteristics of study populations and level of awareness of aflatoxins (Ayo *et al.*, 2018; Ngoma *et al.*, 2017; Magembe *et al.,* 2017). However, these studies are mainly concentrated on common staple crops such as maize, groundnut and animal feeds but limited studies have been reported on spices on the level of awareness and knowledge of spice handlers in Tanzania. Therefore, the aim of this study was to assess the awareness, handling and storage practices of retailers dealing with marketed spices and how these factors associate with aflatoxin contamination.

3.3 Materials and Methods

3.3.1 Study design

A cross sectional descriptive study was carried out between December 2017 and January 2018 in three districts of Dar es Salaam i.e. Ilala, Kinondoni and Temeke. This design was suitable because the study was aiming to capture and collect particular data regarding handling and storage practices and awareness of aflatoxin issues from the study population from whom the spice samples were to be collected.

3.3.2 Study population

The study population was people who were involved with the selling of spices in various markets from the three districts of Dar es Salaam. This group was selected because majority of consumers tend to purchase spices from them due to the accessibility and affordability. Additionally, these people were more likely to make informed decisions and respond to the study questions responsively.

3.3.3 Sample size estimation

Since the exact population of spice retailers was unknown, the sample size was estimated using the Kothari equation (Kothari and Garg, 2014): $n = z^2P\ (1\text{-}P)/e^2$

Where; n= sample size, Z= standard variate at a given confidence level, for this study a 95% confidence level = 1.96 at and e = acceptable error (the precision/ estimation error) set at 8% (0.08) for this study.

Thus the sample size of the study was

$n = 1.96^2 \text{ X } 0.05\ (1 – 0.05)/\ 0.08^2$

$n = 30$

3.3.4 Sampling techniques and procedures

Probability sampling techniques were employed. This technique was considered to be fit for the study because it gave equal chance to all the study respondents in the population to be selected and thus eliminating the possibility of bias in selection. It also provided a basis for the application of statistical theory of results. The study respondents were randomly selected in their respective markets through simple random sampling. Four samples of different spices (cinnamon, cloves, ginger and cardamom) were collected from each respondent after conducting the interview.

3.3.5 Data collection tools

The data of the study was collected using quantitative methods. An interview administered questionnaire (with closed and open ended questions) was used on the study respondents who consented on participation. This questionnaire was in English and translated in Kiswahili.

The questionnaire consisted of thirty questions that attempted to capture information on handling, storage practices of spices, knowledge and awareness of these respondents on aflatoxin and spoilage issues. The questionnaire was pretested on a random sample of 10 participants in Sinza Africa Sana Market to provide a clear indication on the response and relevance of the questions to the study objectives.

3.3.6 Statistical analysis

The data was analysed using Statistical Package for Social Sciences (IBM SPSS® Version 18, Chicago, IL, USA). The data was coded appropriately and fed into SPSS version 18 to determine the frequencies.

Also Logistic regression analysis was used to describe and determine the relationship or association between the independent variables and aflatoxin contamination. Data cleaning was primarily done to ensure there was no information missing. The analysis involved descriptive statistics to describe the sample population, socio demographic, awareness, handling and storage practices, in frequency tables. The chi square test was used for showing association between study independent variables such as socio demographic, awareness, handling and storage practices; and dependent variable (aflatoxin contamination) during statistical analysis.

3.4 Results

The demographic characteristics of the study respondents, awareness, storage and handling practices and factors associated with prevalence of aflatoxin contamination of the spices collected from the respondents were investigated.

3.4.1 Demographic characteristics of the respondents

This study targeted 30 respondents who retailed spice products in different markets within the three districts of Dar es Salaam, i.e., Ilala, Temeke and Kinondoni. All targeted respondents participated in the study and provided the samples used for mycotoxin analysis.

About 43% of the study respondents were from markets in Ilala district while about 27% were from Kinondoni district and 30% from Temeke (Table 3.1). The majority of respondents (83.3%) were males and (16.7%) were females. The respondents who never completed primary school education were 3.3%, while those with primary school education were 50.0% and, those with ordinary level secondary school education were 33.3%.

The average age of the respondents was 35 (34.55±9.59) and majority (40.0%) of the respondents were between 27 to 35 years old while only 13.3% of the respondents were over 45 years old. The average business experience of the respondents was 8.7 ± 7.5 years, of which 50.0% of the respondents reported to have less than 5 years of business experience in dealing with spices while 23.3% of respondents had more than 10 years of business experience in dealing with spices.

Table 3.1: Demographic characteristics of study respondents

Variable	Description	Frequency (N=30)	Percentage %
District	Kinondoni	8	26.7
	Ilala	13	43.3
	Temeke	9	30.0
Sex	Male	25	83.3
	Female	5	16.7
Age categories	18 – 26	6	20.0
	27 – 35	12	40.0
	36 – 44	8	26.7
	45 and above	4	13.3
Level of education	Did not complete primary education	1	3.3
	Completed primary education		
	Did not complete ordinary level	15	50.0
	Completed ordinary level	3	10.0
	Post-secondary/tertiary	10	33.3
		1	3.3
Marital status	Never married	10	33.3
	Married	20	66.7
Business years	Up to 5 years	15	50.0
	6 – 10 years	8	26.7
	More than 10 years	7	23.3

3.4.2 Handling and storage practices of spices

Table 3.2 indicates that 43.3% of the respondents purchased their spices from wholesale outlets which were almost similar to other respondents (40.0%) who purchased the spices from farmers. It was observed that 36.7% of the respondents further dried spices after receiving them, while 63.3% of the respondents did not further dry their spices after reception. About 76.7% of the respondents sorted spices before storage while 23.3% of respondents did not sort the spices.

It was observed that 46.7% of the respondents considered the physical quality (appearance, colour, cleanliness) of the spices during sorting while 30.0% of respondents considered insect infestation and mould aspects during sorting of spices. The mean storage duration of the spices was approximately 10 ± 2 days, of which 63.3% of respondents stored spices for less than 7 days, while 10.0% of respondents stored spices for more than 14 days. The mean quantity of spices purchased per week was 238.84 ± 300.07 kg, of which 50.0% of the respondents reported to purchase less than 200 kg of spices per week while 10.0% of respondents purchased more than 600 kg of spices per week.

About 63.3% of the respondents threw away the spices that appeared to be spoilt after sorting, while 3.3% of the respondents to either re-dried and sold or stored and milled the spoilt spices. About 96.7% of the respondents packaged for sale and 3.3% of the respondents did not package their spices prior to sale.

Table 3.2: Handling and storage practices of spices by respondents

Variable	Description	Frequency (N=30)	Percentage %
Where spices purchased	Farmers	12	40.0
	Outlets	13	43.3
	Others	5	16.7
Further Drying	Yes	11	36.7
	No	19	63.3
Quantity of spices purchased (Kg)	Less than 200	15	50.0
	200 – 400	2	6.6
	400 – 600	5	16.7
	More than 600	3	10.0
	Depend on demand	5	16.7
Storage length of spices (days)	Within 7 days	19	63.3
	7 - 14 days	8	26.7
	More > 14 days	3	10.0
Sorting of spices	Yes	23	76.7
	No	7	23.3
Criteria for sorting	Physical aspects	14	46.7
	Insect/Mouldy	9	30.0
	No sorting done	7	23.3
Packing of spices	Yes	29	96.7
	No	1	3.3
Bad sorted spices	Thrown away	19	63.3
	Recall to supplier	2	6.7
	Re-drying and sell	1	3.3
	Store and Mill	1	3.3
	None	7	23.4

3.4.3 Knowledge and awareness of spoilage and aflatoxin contamination

Table 3.3 shows that 83.3% of the study respondents knew the causes of spoilage in foods while 16.7% did not know the causes of spoilage in foods. The results show that 93.3% of the study respondents could identify spoiled spices where as 6.7% could not identify spoiled spices. Table (3.3) also shows that all the study respondents (100.0%) could identify well dried spices. However, the results shows that only 3.3% of the study respondents have ever heard of aflatoxins where as 96.7% of the respondents had not heard of the word aflatoxin. The results shows that all study respondents (100.0%) had not heard of toxins present in foods which can be caused by mould.

The results also show that only 3.3% of the respondents were aware that aflatoxins can contaminate crops during storage. In addition, about 3.3% of the respondents were aware of aflatoxin contamination in spices. There were no study respondents (0.0%) who were aware of the effects of aflatoxins in humans and in animals i.e. all of the study respondents (100.0%) were not aware of the effects of aflatoxins in humans and in animals. The table shows that 3.3% of the study respondents had attended training related to food handling and storage where as 96.7% of the respondents had not attended any training with regards to food handling and storage.

Table 3.3: Knowledge and Awareness of spoilage and aflatoxin contamination

Variable	Description	Frequency (N=30)	Percentage %
Know causes of spoilage	Yes	25	83.3
	No	5	16.7
Can identify spoiled spices	Yes	28	93.3
	No	2	6.7
Can identify well dried spices	Yes	30	100.0
	No	0	0.0
Heard of aflatoxins	Yes	1	3.3
	No	29	96.7
Heard of toxins present in food caused by mould	Yes	0	0.0
	No	30	100.0
Aware of aflatoxin contamination during storage	Yes	1	3.3
	No	29	96.7
Aware of aflatoxin contamination in spices	Yes	1	3.3
	No	29	96.7
Aware of the effects of aflatoxins in humans	Yes	0	0.0
	No	30	100.0
Aware of the effects of aflatoxins in animals	Yes	0	0.0
	No	30	100.0
Attended any training related to food handling and storage	Yes	1	3.3
	No	29	96.7

3.4.4 Prevalence of aflatoxin contamination in spices by characteristics of study respondents

The spices samples were collected from the study respondents of which these samples were analyzed for aflatoxin contamination in the laboratory. Four samples of different spices (cardamom, cloves, ginger and cinnamon) were collected from the respondents and analyzed for aflatoxin contamination. The laboratory analysis results showed that 69 samples (57.5%) of the 120 spices collected from the study respondents/spice traders were contaminated with aflatoxins.

Table 3.4 shows that the prevalence of aflatoxin contamination of these spices was higher among respondents who were from markets in Ilala district (73.1%) compared to those from Temeke district (47.2%) and Kinondoni district (43.8%). This observed difference was statistically significant (p = 0.010). The prevalence of aflatoxin contamination was higher among respondents who did not complete primary school education (100.0%) compared to those who completed primary education (68.3%), those who did not complete ordinary secondary school education (50.0%), those who completed ordinary level education (45.0%) and those who have post-secondary school/tertiary education (0.0%). This observed difference was statistically significant (p = 0.007). The prevalence of aflatoxin contamination was higher among respondents who did not attend any training related to food handling and storage (59.5%) compared to those who attended any training related to food handling and storage (0.0%). This observed difference was statistically significant (p = 0.018).

The prevalence of aflatoxin contamination was higher among those respondents who purchased their spices from farmers (64.6%) compared to those who purchase from outlets (59.6%) and from other sources (35.0%). However, this observed difference was not statistically significant (p = 0.073). The prevalence of aflatoxin contamination was higher among respondents who further dry their spices after purchase (65.9%) compared to those who do not further dry their spices after purchase (52.6%) However, this observed difference was not statistically significant (p = 0.156). The prevalence of aflatoxin contamination was higher among those respondents who do not sort their spices (71.4%) compared to those who sort their spices (53.3%). This observed difference was also not statistically significant (p = 0.089).

The prevalence of aflatoxin contamination was higher among respondents who reported to store their spices for more than 14 days (75.0%) compared to those who store their spices for 7 to 14 days (56.2%) and who store for less than 7 days (55.3%). This observed difference was not statistically significant ($p = 0.432$). The prevalence of aflatoxin contamination was higher among respondents who do not know the causes of spoilage in spices (70.0%) compared to those who know the causes of spoilage in spices (55.0%). This observed difference was however not statistically significant ($p = 0.215$).

Table 3.4: Prevalence of aflatoxin contamination by characteristics of respondents

Variables	Description	N=120	n (%)	Chi square	P-Value
District	Kinondoni	32	14 (43.8)	**9.195**	**0.010***
	Ilala	52	38 (73.1)		
	Temeke	36	17 (47.2)		
Age (yrs)	18 - 26	24	10 (41.7)	6.573	0.087
	27 - 35	48	29 (60.4)		
	36 - 44	32	23 (71.9)		
	45 and above	16	7 (43.8)		
Sex:	Female	20	11 (55.0)	0.061	0.804
	Male	100	58 (58.0)		
Education level	Did not complete primary education	4	4 (100.0)	**14.084**	**0.007***
	Completed primary education	60	41 (68.3)		
	Did not complete ordinary level	12	6 (50.0)		
	Completed ordinary level	40	18 (45.0)		
	Post-secondary/tertiary	4	0 (0.0)		
Business experience in spices	Up to 5 years	60	35 (58.3)	1.281	0.527
	6 - 10 years	32	16 (50.0)		
	More >10 years	28	18 (64.3)		
Where spices purchased	Farmers	48	31 (64.6)	5.224	0.073
	Outlets	52	31 (59.6)		
	Others	20	7 (35.0)		
Can identify spoiled spices	No	8	6 (75.0)	1.074	0.300
	Yes	112	63 (56.2)		
Know causes of spoilage in spices	No	20	14 (70.0)	1.535	0.215
	Yes	100	55 (55.0)		
Further drying of spices	No	76	40 (52.6)	2.010	0.156
	Yes	44	29 (65.9)		
Sorting of spices	No	28	20 (71.4) 49	2.899	0.089
	Yes	92	(53.3)		
Criteria for sorting spices	No sorting done	28	20 (71.4)	2.905	0.234
	Physical aspects	56	30 (53.6)		
	Insect and/or mould infestation	36	19 (52.8)		
Storage length of spices (days)	Within 7 days	76	42 (55.3)	1.680	0.432
	7 - 14 days	32	18 (56.2)		
	More > 14 days	12	9 (75.0)		
Attended any training on food handling & storage	No	116	69 (59.5)	**5.598**	**0.018***
	Yes	4	0 (0.0)		
Form of packing spices	Powder only	4	3 (75.0)	0.599	0.741
	Both/Sometimes powder or whole	112	64 (57.1)		
	No packing done	4	2 (50.0)		
Knowledge on aflatoxin issues	Less knowledgeable	96	54 (56.2)	0.614	0.738
	Somewhat knowledgeable	20	13 (65.0)		
	Highly Knowledgeable	4	2 (50.0)		
Type of spices	Cinnamon	30	13 (43.3)	4.467	0.215
	Ginger	30	17 (56.7)		
	Cloves	30	21 (70.0)		
	Cardamom	30	18 (60.0)		

N is the total number of samples collected and analyzed

* Statistically significant association between spice sample contamination and variable of the respondent

3.4.5 Logistic regression analysis

Logistic regression (multivariate) analysis was used to describe and determine the relationship or association between the independent variables and aflatoxin contamination of spices. Odds ratios determined associations between aflatoxin contamination and the respondents' characteristics (independent variables) at 95% confidence interval. P-values that were less than 0.05 ($P<0.05$) were considered statistically significant.

The odds of the spices collected from respondents being contaminated with aflatoxins varied significantly with *age*, where spices are purchased and storage length of spices (Table 3.5). The age of respondents (OR = 0.326, 95%CI = 0.113 - 0.940, p = 0.038) was weakly associated with aflatoxin contamination of spices collected. The source where the spices were purchased (OR = 0.178, 95%CI = 0.061 - 0.525, p = 0.002) was also weakly associated with aflatoxin contamination of spices. The storage length of spices (OR = 3.608, 95%CI = 1.099 - 11.845, p = 0.034) was strongly associated with aflatoxin contamination of spices.

Table 3.5: Multivariate analysis results for characteristics of respondents in association with aflatoxin contamination of spices

Variables	Factor	OR	95% Confidence Interval Lower limit	Upper limit	P-Value
Age group (years)	**36 - 44 yrs**	**0.326**	**0.113**	**0.940**	**0.038***
Sex		0.907	0.099	8.305	0.931
District		1.071	0.348	3.302	0.905
Education level		0.465	0.156	1.385	0.169
Marital status		1.052	0.117	9.484	0.964
Where spices purchased	**Farmers**	**0.178**	**0.061**	**0.525**	**0.002***
Can identify spoiled spices		0.789	0.005	125.815	0.927
Know causes of spoilage in spices		0.142	0.005	3.953	0.250
Further drying of spices		0.776	0.196	3.077	0.718
Sorting of spices		4.971	0.231	107.199	0.306
Criteria for sorting		0.374	0.076	1.831	0.225
How long sorted spices stored (days)	**More than 14 days**	**3.608**	**1.099**	**11.845**	**0.034***
Attended any training related to food handling and storage		0.000	0.000	0.000	0.999
Have you heard of the word aflatoxin before		1.231	0.048	31.766	0.900
Business years' experience		2.085	0.616	7.055	0.238
Packaging of spices		0.265	0.007	10.184	0.476
What form do you pack the spices		0.265	0.007	10.184	0.476
District		1.071	0.348	3.302	0.905

OR = Odds ratio, ***** = statistically significant at 95% confidence level ($p < 0.05$)

3.5 Discussion

The extent of handling and storage practices; knowledge and awareness of aflatoxin contamination and the association between these factors and aflatoxin contamination of the collected spices was investigated in this study.

3.5.1 Handling and storage practices

The responses to the questionnaires administered indicated that most of the respondents (63.3%) did not further dry their spices after receipt. Furthermore, the majority of the respondents (76.7%) sorted spices after receipt. About 47% of the participants (46.7%) acknowledged considering physical aspects during sorting such as inspecting the overall physical appearance, discoloration and cleanliness as compared to others (30.0%) who considered insect infestation and mouldiness of the spices. It has been reported that physical sorting is also another effective measure in the reduction of aflatoxins, as high as 40–80% (Bullerman and Bianchini, 2007). A large proportion of respondents (63.3%) stored spices for less than 7 days before selling them to consumers hence low chances of mould growth and aflatoxin contamination. An overwhelming majority (96.7%) of respondents packaged their spices for sale. It have been reported that long period of spices storage in the shops, increase the chances of aflatoxin contamination (Elshafie *et al*., 2002).

3.5.2 Awareness and knowledge on aflatoxin contamination

Significant majorities (96.7%) of the respondents had never heard of aflatoxins before and also were not aware of aflatoxin contamination of spices during storage. Thus, very few respondents (3.3%) had heard of aflatoxins. Spice retailers unawareness on aflatoxin contamination in this study could be a major contributing factor to aflatoxin contamination hence danger to public health. This is similar to observations reported by Ngoma *et al.* (2016) that among parents of young children in central Tanzania only 3.6% of the parents confirmed to have heard of aflatoxins; Similary Jolly *et al*. (2006) reported that only 7.7% of the respondents in four villages in Ejura Sekyedumase district of Ghana had heard of aflatoxins. In contrast, the results of this study were significantly lower than that reported by Ayo *et al*. (2018) that 28% of livestock farmers in Meru district in Tanzania had ever heard of aflatoxins. Kamala *et al*. (2016) reported that 20% of the respondents in Kilosa District of Tanzania had heard about aflatoxins. In addition, Marechera and Ndwiga (2014) reported 93% of the farmers in lower eastern Kenya had heard of aflatoxins in their respective areas. Lee *et al*. (2017) reported that 10.2% of the respondents from six provinces of Vietnam had heard of aflatoxins. Monyo *et al*. (2010) reported that 65% of groundnut farmers in Malawi were aware of aflatoxin. The disparity in awareness between this particular study and other reported studies may be attributed to several factors including the nature of the study population. For example in Kenya, where the aflatoxicosis events (Probst *et al.* 2007) which killed a number of people may have elevated the awareness of the public on aflatoxins. In Tanzania, 2016 aflatoxicosis outbreak in Dodoma region might have contributed to the increased awareness more specifically to maize and groundnuts value chain actors.

In this study, 16.7% of the respondents did not know the causes of spoilage of spices. This is higher than that reported by Marechera and Ndwiga (2014) in Kenya where 4.2% of farmers in lower eastern Kenya did not know the causes of aflatoxin contamination in food crops. None of the respondents (0.0%) in this study were aware of the effects of aflatoxins on quality of spices in contrast to what was reported by Ngoma *et al*. (2017) that only 1.4% of the respondents in central Tanzania were aware of the effects of aflatoxins.
Low / no awareness on the health effects of aflatoxins in humans may be an indication of poor public awareness. This is supported by the study done by Nyangaga, (2014) whereby few traders who had attended training related to handling and storage of food were more aware of aflatoxin and its health effects. Unawareness on the health effect of aflatoxin contamination in this study could pose health risk to public

About 94.9% of the respondents in the study reported by Jolly *et al.* (2006) did not know that aflatoxins caused ill- health effects. Furthermore, 93.3% of the respondents in this study were able to identify spoiled spices, which is similar to other studies done by Ayo *et al.* (2018) who reported that 93% of the livestock farmers in Meru district could identify mouldy feeds. Being able to identify spoiled spices may be contributed to the fact that these respondents were looking at physical aspect of the product in term of appearance but may not associate with safety aspect such as aflatoxin contamination in food. This could be supported by the fact that 56.2% (Table 3.4) of retailers who could identify spoiled spices had contaminated spices .This is an indication of low awareness on post-harvest management in reducing aflatoxin contamination. However, these results indicated higher percentage than other studies e.g. by Marechera and Ndwiga (2014) that reported that 81.7% of participants could identify fungal growth or symptoms associated with aflatoxin contamination. Jolly *et al*. (2006) reported that 89.4% of respondents in Ejura Sekyedumase district in Ghana were able to identify spoiled maize.

In this study, 3.3% of the study respondents had attended training on food handling and storage which was lower compared to study reported by Nyangaga, (2014) where 24% of human food and cattle feed traders in Nairobi markets in Kenya had attended organized workshops and seminars on training related to handling and storage of food and feed. Trainings on food handling and storage help to increase awareness on the best practices in the spice value chain hence might reduce the risks of aflatoxin contamination.

3.5.3 Association between respondents' characteristics/practices and aflatoxin contamination of spices

The results of this study show that the aflatoxin contamination of spices collected from respondents was significantly associated with age of the spice retailers. Traders who were between the ages of 36 and 44 years old were likely to have contaminated spices compared to younger peers and older peers respectively even though the odds of this were weak/low.

The study revealed that prevalence of aflatoxin contamination of the sampled spices from traders was higher in this particular age group compared to the other groups. This could be due to the fact that younger groups were more likely to be more learned or informed. Ayo *et al.* (2018) and Lee *et al*. (2017) reported that younger age groups were more aware of aflatoxins and had the ability and curiosity to learn. Hence, they were more likely to be aware of methods of preventing spoilage and aflatoxin contamination or seek further information. This is reflected in the study where, the 3.3% of respondents who had ever attended any training on food handling were between the ages of 18 and 26 years of the study participants.

This study revealed that the source of purchased their spices and storage duration of were significantly associated with aflatoxin contamination of spices. It was observed that spices sourced from farmers were likely (but at lower odds) to be contaminated with aflatoxins compared to those sourced from retail outlets and other sources such as own production. This might be due to poor post-harvest management by farmers where most of the farmers dry the spices on the ground which may cause an increase in water activity of the spices due to absorption of moisture from the soil and re-wetting by rain which may lead to growth of mould hence aflatoxin contamination (Kaaya and Kyamuhangire, 2006). Also most of the farmers do not do physical sorting which is an effective measure in reducing aflatoxin contamination (Bullerman and Bianchini, 2007).

It was also observed that storage duration of spices of more than 14 days was more likely (three times the odds) to be contaminated with aflatoxins compared to those stored for less than seven days and 14 days, respectively. These study findings were similar to studies reported by Adekoya *et al*. (2017) and Hell *et al*. (2003) indicating the relationship between storage and marketing practices and mycotoxin contamination. Sasamalo *et al*. (2018) observed the increase in aflatoxin levels with extended storage duration of stored maize in central Tanzania.

3.6 Conclusion

This study gave a preliminary insight into the handling and storage practices of traders dealing with spices within Dar es Salaam and level of awareness and knowledge of traders on aflatoxin issues. It was clear that the awareness of the spice traders was significantly low compared to other populations because a large majority of the study respondents neither had never heard of aflatoxins nor had ever attended any training on handling and storage of foods. The traders were not aware of the ill-health effects of caused by consumption of foods contaminated with aflatoxins on humans or animals. In addition, this study contributed information on aflatoxin awareness and safety hazards in spices in Tanzania.

Furthermore, this study determined the predictors/factors associated with aflatoxin contamination of spices sampled from the study respondents which were: respondent age; sources of where spices purchased and storage duration/length of spices. Considering the fact that the sampled spices were contaminated with aflatoxins and the majority of the respondents were not aware of presence of aflatoxins and lack of knowledge of their ill health consequences a few recommendations have been outlined to facilitate improvement in food safety of consumers and promotion of trade for improved livelihood.

3.7 Recommendations

Awareness on aflatoxin contamination should be extended to marketed spices and spice products because a lot of efforts have been geared towards other crops particularly maize, groundnuts and animal feed. Therefore, farmers and distributors of spices should be educated and sensitized on aflatoxin issues

Regulatory and educational programs should be further enhanced because producers and distributors of spices should be subjected to continuous surveillance and monitoring because these products are readily consumed by the general public through various dietary compositions. Sensitization of farmers and retailers/distributors could be achieved through regulatory authorities and government agencies.

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APPENDICES

Appendix 1: Questionnaire to assess awareness, handling, storage and packaging factors associated with aflatoxin contamination

My name is **Sambwe Fundikira.** I am a student pursuing MSc Food Quality and Safety Assurance studies at Sokoine University of Agriculture (SUA). In partial fulfilment of the degree requirements, I am carrying out a research on **Aflatoxin contamination of marketed spices in Tanzania: a case study of Dar es salaam**. This questionnaire is for selected spice retailers in Dar es Salaam. The aim is to obtain information on the **storage and handling practices and to assess awareness on marketed spices particularly cinnamon, cardamom, cloves and ginger**. This information will be useful in assessment of factors that affect aflatoxin contamination in spices. The information obtained is strictly confidential and will be used for academic purposes only, to facilitate the intended learning at the Sokoine University of Agriculture.

Questionnaire number…………………………………………………………………….

Date of interview…………………………………………………………………………

Location of market ……………………………………………………………………...

Name of District……………………………...

A: RESPONDENT PARTICULARS

1. Age of respondent (years) ………………………………………………………………

2. Sex of respondent ……………………………………………………………………….

3. What is your level of education?

    (1) Never attended school/no formal education

    (2) Did not complete primary school education

(3) Completed primary school education

(4) Did not complete secondary school education (ordinary)

(5) Completed secondary school education (ordinary)

(6) Did not complete high school education (advanced)

(7) Completed high school education (advanced)

(8) Post-secondary/tertiary education

4. What is your marital status?

(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 the spices?…………………………………………

7. How much spices do you purchase/procure in a week in terms of weight (kg)? ……..

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

Tick appropriate response for each spice mentioned

Criteria	**Type of spices**			
	Cinnamon	Ginger	Cloves	Cardamom
Visual inspection				
Mould infested				
Cleanliness				
Moistness				
Foreign matters				
Others (specify)				

9. Can you identify spoiled spices?

(1) Yes (2) No

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

(1) Yes (2) No

9(b) If Yes, please mention the causes you know of

…………………………………………………………

10. Do you know how to identify well dried spices?

(1) Yes (2) No

10(a) In what condition do you receive the cinnamon that you usually procure?

(1) Dry (2) Moist

10(b) In what condition do you receive the ginger that you usually procure?

(1) Dry (2) Moist

10(c) In what condition do you receive the cloves that you usually procure?

(1) Dry (2) Moist

10(d) In what condition do you receive the cardamom that you usually procure?

(1) Dry (2) Moist

11(a) Do you do any further drying of these spices after receiving them?

(1) Yes (2) No

11(b) If Yes, where do you normally dry the spices?………………………………………..

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

(1) Yes (2) No

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

12(c) What do you do with the bad sorted spices (rejects)?..

13(a) Do you store the sorted (good) spices?

(1) Yes (2) No

13(b) How do you store the sorted spices?...

13(c) Do you clean the storage structures before using them?

(1) Yes (2) No

13(d) How long do you store the sorted spices? ……………………………………….

14(a) Do you use any pesticides on spices during storage?

(1) Yes (2) No

14(b) If yes, please mention them…………… ……………………………………..

15(a) Do you do any packaging of spices?

(1) Yes (2) No

15(b) In what form do you pack the spices?

(a) Powdered (b) Whole

16 (c) Which type of material is used to pack spices?

....

C: RESPONDENT AWARENESS OF AFLATOXINS CONTAMINATION

Yes answer=1 and No answer=0

17. Have you attended any training related to food handling and storage?

(1) Yes (2) No

18. Have you ever heard of toxins that may present in crops that can be caused by moulds?

(1) Yes (2) No

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

(1) Yes (2) No

20. Where did you get this information?

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

(4) Others (Specify)…………………………………………………………………

21. Have you heard of the word mycotoxin (sumu kuvu) or aflatoxin before?

(1) Yes (0) No

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

(1) Yes (0) No

23. Are you aware of mycotoxins/aflatoxins (toxins) that can contaminate food?

(1) Yes (0) No

24. Are you aware of aflatoxins contamination in spices?

(1) Yes (0) No

25(a) Are you aware of the ill-health effects of aflatoxins in human beings?

(1) Yes (0) No

25(b) If yes, please mention them;

(1) Stunting of children

(2) Liver cirrhosis

(3) Cancer

(4) Vomiting

(5) Others specify……………………………………………………………………...

26(a) Are you aware of the effects of aflatoxins in animals?

(1) Yes (0) No

27(b) If yes, please mention them;

(1) Tumours

(2) Poor digestion

(3) Liver disease

(4) Stunting/loss of productivity

(5) Death

(6) Others specify…………………………………………………………………….

D: KNOWLEDGE ON AFLATOXINS CONTAMINATION

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

SN	Statement	Response	
28.1	Hot and humid climate in this area can promote growth of fungi	Correct (1)	Incorrect (0)
28.2	Improperly stored food can be contaminated by fungi	Correct (1)	Incorrect (0)
28.3	Aflatoxin is a type of fungi	Correct (1)	Incorrect (0)
28.4	Aflatoxins are cause caused by fungi	Correct (1)	Incorrect (0)
28.5	Poor storage conditions promote the presence of aflatoxins in foods.	Correct (1)	Incorrect (0)
28.6	Exposure to aflatoxin (mouldy food) can be harmful to health	Correct (1)	Incorrect (0)
28.7	Aflatoxins are only found in crops	Correct (1)	Incorrect (0)
28.8	Aflatoxins are only found in foods	Correct (1)	Incorrect (0)
28.9	Some liver diseases can be linked to intake of aflatoxins (mouldy foods)	Correct (1)	Incorrect (0)

28.10	Aflatoxins (mouldy food) can cause cancer	Correct (1)	Incorrect (0)
28.11	Use of pesticides can reduce fungi in storage	Correct (1)	Incorrect (0)
28.12	Sorting of spices reduces aflatoxins (mould) contamination	Correct (1)	Incorrect (0)
28.13	Cleaning of spices reduce aflatoxins (mould) contamination	Correct (1)	Incorrect (0)
28.14	Eating contaminated foods with aflatoxins (mouldy) can cause diseases	Correct (1)	Incorrect (0)
28.15	Eating contaminated foods with aflatoxins (mouldy) can cause death	Correct (1)	Incorrect (0)
28.16	Good agricultural practices will minimize aflatoxins in crops	Correct (1)	Incorrect (0)
28.17	Sorting of damaged spices is time consuming	Correct (1)	Incorrect (0)
28.18	Aflatoxins can be removed during cooking of foods	Correct (1)	Incorrect (0)
28.19	Sorting of spoiled spices is too costly?	Correct (1)	Incorrect (0)
28.20	Sorting of spices is hygienic?	Correct (1)	Incorrect (0)
28.21	Clean spices attract better prices?	Correct (1)	Incorrect (0)
28.22	Clean spices always sell faster	Correct (1)	Incorrect (0)