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Levels of Aflatoxins in Selected Spices Marketed in Dar es Salaam and Zanzibar, Tanzania

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Abstract

Aflatoxins are produced by fungi species known as *Aspergillus flavus* and *Aspergillus parasiticus* which invade crops like maize, oilseeds, and spices. This study investigated the levels of aflatoxins in four selected types of spices marketed in Dar es Salaam and Zanzibar in Tanzania to determine the incidence of human exposure to aflatoxin through the consumption of spices as food additives. A total of 72 samples of selected spices (25 cinnamon, 16 ginger, 20 cloves, and 11 mixed spices) were collected from farms (Zanzibar), markets and stores in Dar es Salaam and Zanzibar. Aflatoxins B_1, B_2, G_1, G_2, and total aflatoxins were determined using high-performance liquid chromatography with fluorescence detection. The findings revealed that 24 (33%) samples out of 72 were contaminated by aflatoxin B_1 but did not exceed the maximum tolerable level of 5 ngg^{-1}; whereas, total aflatoxins contaminated 53 (73.6%) samples out of 72, with 2 samples exceeding the maximum permissible limits of 10 ngg^{-1}. Although the detected levels were within acceptable limits, the risk of aflatoxicosis due to the consumption of spiced foods may be high; hence, necessitating regulatory bodies in the country to constantly monitor for aflatoxins in the marketed spices.

Keywords: Aflatoxins; Food safety; Spices contamination; Risk assessment; Liquid chromatography

Introduction

Aflatoxin contamination in commodities is currently a worldwide concern and is a serious problem that threatens human and animal health (Visconti et al. 2001). They are commonly found in groundnuts, dairy products, spices, grains, tea, peanuts, cottonseeds, sunflower, milk, rice, maize, beans, palm, copra and cocoa as well as in animal and fish feeds (Waliyar et al. 2015). Aflatoxins are secondary metabolites which are components of mycotoxins produced by species of the genus *Aspergillus* known as *Aspergillus flavus*, *Aspergillus parasticus* and *Aspergillus nomius* (Kamkar et al. 2011, Suriya et al. 2012, EFSA 2013). The growth of aflatoxins-producing fungi and aflatoxin contamination are a consequence of the interaction of different factors including fungal strain, humidity, temperature, substrate composition, damage and aeration (Al Hammadi 2015). Aflatoxins are known to cause different diseases, including cancer, fatty liver, immunosuppression, pulmonary and cerebral edema, heart and kidney diseases, convulsions and abdominal pain (Kyalo et al. 2023, Murokore et al. 2023). On its hand, AFB_1 is known to induce cancer in the liver, kidney, breast, small intestine, ovary, colorectal, and other organs of the body. Moreover, AFB_1 affects the biological functions of the brain, lungs, kidneys and blood coagulation (Murokore et al. 2023). Different studies on the contamination of aflatoxins in different food stuffs have been reported in Tanzania, and there is very little

information on spices contamination. For instance, occurrences of aflatoxins have been reported in maize, maize products, sunflower, cooking oil, and milk in different locations of Tanzania mainland (Kimanya et al. 2014, Magembe et al. 2016, Kimanya et al. 2016, Mtega et al. 2020).

The production of spices, like any other food crop, is affected by aflatoxin contaminations at various production stages. Spices are aromatic or pungent fragrance vegetable substances used in small amounts to alter or mask the flavour of the food. They are the most precious and interesting commodities which are used as flavouring, colouring and seasoning agents in food. They are mostly used in food preparation and processing throughout the world. Different types of spices are known, including clove, turmeric, garlic, black pepper, white pepper, chilli, cayenne, coriander, curry powder, paprika, cumin, ginger, cinnamon, nutmeg, and fenugreek. Traditionally, spices are valued for their distinctive flavours, colour and aroma. Spices are also known for their nutritional value. For instance, coriander, fenugreek, turmeric, and pepper are good sources of calcium, phosphorous, potassium, and sodium. Furthermore, spices are used as medicine, for example, clove is used for the treatment of toothache and also relieves upper respiratory infections (Leela 2008). Moreover, clove bud oil is used as an antibacterial, antifungal and antioxidant in the body (Leela 2008). Different regions of Tanzania produce different types of spices. For instance, Zanzibar produces ginger, cinnamon, clove, turmeric, garlic, black pepper, white pepper, chilli, cayenne, coriander, curry powder and paprika; Arusha produces nutmeg, ginger, cinnamon, clove, turmeric, garlic, black pepper, white pepper, chili, cayenne, coriander and paprika. However, not all spices marketed in different markets in Tanzania are produced within the country; some are imported from countries like India, South Africa, Kenya and Nigeria. Spices are among the food additives which are vulnerable to aflatoxin contamination. Although spices are used in small amounts they are recognized as significant sources of aflatoxins contaminations (Hashem and Alamri 2010). Tanzania is one of the East African countries which produce different types of spices such as cloves, cinnamon, black pepper, cardamom, ginger and cumin. However, to the best of our knowledge, there has been no study conducted on the levels of aflatoxins in these spices. This paper therefore, reports on the levels of aflatoxins in four selected types of spices: cinnamon, ginger, cloves, and mixed spices which are produced and/or marketed in Dar es Salaam and Zanzibar, Tanzania. The reported results are expected to trigger an alarm for closer monitoring of spices preparation and storage for minimized occurrence of aflatoxins in spices marketed in the country.

Materials and Methods

Sample collection

A total of 72 samples of spices comprising 25 cinnamon, 16 ginger, 20 cloves and 11 mixed spices were collected in duplicate from Dar es Salaam markets and stores (i.e., Kariakoo, Tandale and Tandika markets; Table 1), and four sampling sites in Zanzibar (Mwanakwerekwe market/stores, KZ spices farm, and ZT corporation (Table 2). Each sample was labelled for easy identification, packed in polyethylene bags to avoid moisture and refrigerated at a temperature of 4 ^{o}C, before being transported to the Tanzania Bureau of Standard (TBS) laboratory for analysis.

Table 1: Detail of Samples Collected from Markets and Stores in Dar es Salaam

	Number and types of collected spice samples				
Sampling sites (markets and store)	Cinnamon	Ginger	Clove	Mixed spice	Total
KR	3	2	3	4	12
KRs	2	-	2	-	4
TL	4	5	3	2	14
TK	4	3	2	2	11
TOTAL	13	10	10	8	41

Where: KR = Kariakoo market, KRs = Kariakoo stores, TL = Tandale market, TK = Tandika market.

Table 2: Detail of samples collected from markets, farm and store in Zanzibar

	Number and types of collected spices samples				
Sampling sites (Market, store and Farm)	Cinnamon	Ginger	Clove	Mixed spice	Total
MKm	5	3	3	3	15
KZf	3	3	2	-	15
KZs	4	-	3	-	7
ZTC	-	-	2	-	2
TOTAL	12	6	10	3	31

MKm = Mwanakwerekwe market, KZf = KZ spices farm, KZs = KZ Store and ZTC = ZT Corporation.

Chemicals

Chemicals and reagents used in different procedures of sample preparation were: acetonitrile (HPLC grade) and methanol (HPLC grade) from Carlo Erba Reagent, France. Trifluoroacetic acid (TFA) and phosphate buffer (pH 6-8) (prepared by dissolving PBS tablet (from Sigma-Aldrich) in 1 L of distilled water), glacial acetic acid, sodium hypochlorites 4%, and HPLC grade water (from AccuStandard, Japan). Sodium hydroxide (from Scharlau Chemie SA, Spain-EU) and aflatoxins standards (AFB_1, AFB_2, AFG_1 and AFG_2) were purchased from Sigma-Aldrich. The stock solution of aflatoxins was prepared in acetonitrile and methanol. The Immunoaffinity columns used for aflatoxins clean-up were AflaTest columns purchased from Sigma-Aldrich.

Extraction of samples

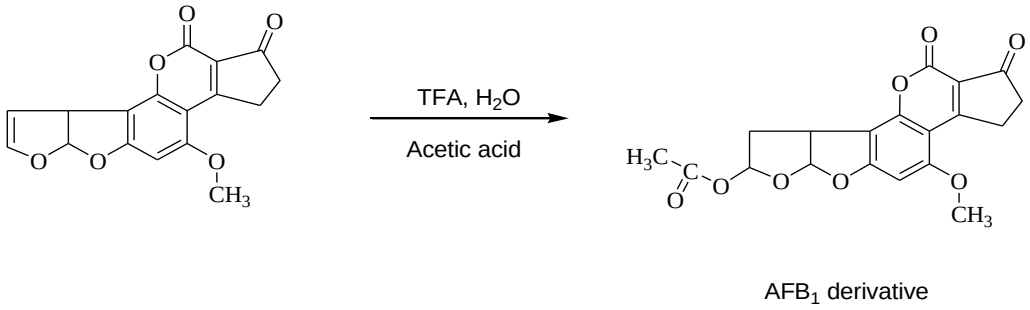
Cinnamon, clove, and ginger samples were ground to obtain a homogenous mixture and then subdivided to obtain representative sub-samples for analysis. Each 25 g of sub-sample from each sample was transferred into a blender jar and mixed with 100 mL of methanol: water (60:40 v/v). The blender jar was then covered and shaken vigorously at 150 revolutions per minute for 3 min by using a mechanical shaker. The extract was then filtered into a 250 mL Erlenmeyer flask using filter paper (Whatman No. 1) (Hussain and Sohail 2012).

Isolation and clean-up of aflatoxins

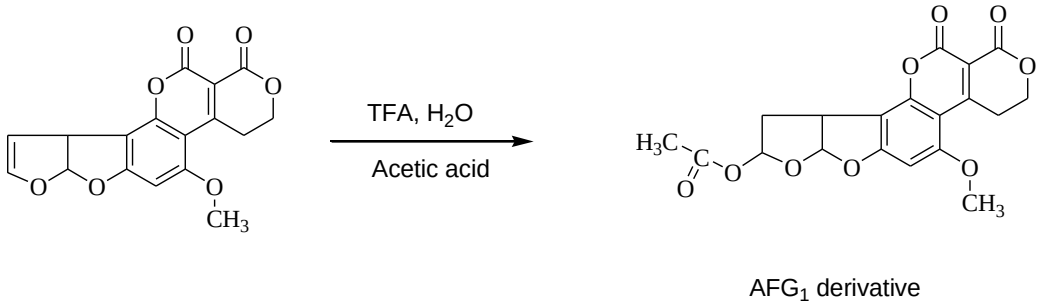
A portion of 4 mL of the extracts was taken and transferred into a beaker of 20 mL followed by the addition of 8 mL of phosphate buffer solution to dilute the sample at a pH of 6-8, adjusted using 0.2 M sodium hydroxide. The diluted extract was loaded on solid phase extraction (SPE) Immunoaffinity Columns, allowing the solution to pass through, then rinsed twice with 10 mL of HPLC grade water. The adsorbed aflatoxins were eluted with 1 mL of HPLC-grade acetonitrile, and the eluent was collected in vials. Finally, pressure was slightly applied to the column to remove any remaining liquid.

Derivatization, detection and quantification

To improve the detection of aflatoxins, pre-column derivatization was performed for the detection of aflatoxin B_1 and G_1. Derivatization was achieved by mixing 400 µL of the sample eluent with 600 µL of the derivatization agent (70:20:10 v/v) (water: TFA: acetic acid). The mixtures were well mixed by shaking using a vortex, reacted at 65ºC for 15 min and then cooled to room temperature before analysis in the HPLC (Fazekas et al. 2005). The derivatization reactions for aflatoxins B_1 and G_1 are shown in Schemes 1 and 2, respectively. The concentrations obtained for the derivatives of AFB_1 and AFG_1 in high-performance liquid chromatography with fluorescence detection (HPLC–FLD) were then recalculated to get the equivalent concentrations for the corresponding un-derivatized aflatoxins (AFB_1 and AFG_1) for proper comparisons. The conversion factor (CF) of aflatoxin and its derivative was calculated as the molecular weight of aflatoxin to that of the derivative using equation 1 (EPA, 1996).



Scheme 1: Derivatisation of AFB_1 using trifluoroacetic acid (TFA), water and acetic acid (Kok, 1994)



Scheme 2: Derivatisation of AFG_1 using trifluoroacetic acid (TFA), water and acetic acid (Kok, 1994)

$$CF = \frac{\text{Molecular weight of aflatoxin}}{\text{Molecular weight of aflatoxin derivative}} \quad (1)$$

HPLC conditions

After all stages of extraction, dilution, cleaning and pre-column derivatization, the extracts were analyzed using HPLC with Fluorescence Detector (FLD) (Model Agilent Chem station technology, series 1200). The mobile phase contained (50:40:10 v/v) water-methanol-acetonitrile ratio. The separations of aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2) were performed on the C_{18} column at a temperature of 40 ºC at a flow rate of 0.8 mL/min. The injection volume was 20 µL for both standard solution and sample extracts. Detection was achieved at an excitation wavelength of 365 nm and an emission wavelength of 450 nm. Separated peaks were identified and quantified based on standard aflatoxin mixture.

Determination of Percentage Recovery of the Method

The accuracy of the procedure was determined by calculating the percentage recovery of aflatoxins spiked blank samples. The mixed spice samples, which were observed to have not been contaminated with aflatoxins were used as blank samples that were spiked with a working solution of standard aflatoxins; AFB_1, AFB_2, AFG_1 and AFG_2 at the concentration of 5 $ngmL^{-1}$, then analysed in the same way as the samples. The recoveries of all aflatoxins were then calculated using Equation 2.

$$\%\text{Recovery} = \left(\frac{r - b}{s}\right) \times 100\%. \qquad (2)$$

where r = the recovered amount, b = blank concentration and s = the spiked amount

Data analysis

The obtained data were subjected to statistical analysis using MaxStat Lite. Thus, single factor analysis of variance (ANOVA) was applied to compare between mean aflatoxin concentrations and were considered not significantly different at *p*-values greater than 0.05.

Results and Discussion

HPLC method development and calibration

The qualitative and quantitative determination of aflatoxins was achieved by running the mixture of aflatoxins standard to establish the separation ability of the method. The aflatoxin standards were also used to obtain calibration curves to check the linearity and sensitivity of the detector.

Determination of percentage recovery of the method

The recoveries of all aflatoxins were determined to establish the accuracy of the method used and results are summarized in Table 3.

Table 3: Percentage Recoveries of Aflatoxins from Spices

	Spiked concentration (ngg^{-1})	Detected concentration (ngg^{-1})	% Recovery
AFG_1	5.000	4.285	85.7
AFB_1	5.000	5.657	113.14
AFG_2	5.000	4.454	89.08
AFB_2	5.000	5.023	100.46

The percentage recoveries for the identified aflatoxins were found to be in the range of 85.7 to 113.14% and thus, the recoveries were within the acceptable range of 70% to 120% (Shah et al. 2000).

Limit of detection (LOD) and limit of quantitation (LOQ) of the method

The limit of detection (LOD) and limit of Quantitation (LOQ) of the method for AFB_1, AFB_2, AFG_1 and AFG_2 were determined as per the methods reported by Armbruster et al. 2008 and the obtained values for various classes of aflatoxins are presented in Table 4.

Table 4: The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Each Analyzed Aflatoxin

	LOD ($ngmL^{-1}$)	LOQ ($ngmL^{-1}$)
AFG_2	0.1114	0.147
AFG_1	0.1044	0.110
AFB_2	0.1073	0.1123
AFB_1	0.1075	0.124

Quantified levels of aflatoxins

The concentrations of the analyzed samples were calculated using Equation 3. However, for the derivatized aflatoxins (i.e., AFG_1 and AFB_1), the concentration obtained in equation 3 was multiplied by the conversion factor "CF" obtained using Equation 1.

$$X,\ ng/g = \frac{A\ (ng/g) \times 1\ mL \times 100\ mL \times F}{4\ mL \times W\ (g)} \quad (3)$$

Where X = concentration of the analyte in sample, A = concentration of analyte in standard, F = dilution factor of the test sample *i.e.* 2.5, 100 mL = Volume of solvent used for extraction and W = weight of the sample taken, 4 mL = Volume of extract and 1 mL = Volume of acetonitrile for eluted aflatoxin.

Generally, it was observed that out of 72 analyzed samples; 53 samples were contaminated with total aflatoxins (TAF) whereas 24 samples were contaminated with aflatoxin B_1. Table 5 summarises the contamination levels for each type of spices by different kinds of aflatoxins.

Table 5: Detected aflatoxins in analysed spices

Spice	Number of Samples	Aflatoxin	Number of Contaminated Samples	Samples Exceeding EU Set Limits	Levels detected (ngg^{-1})
Cinnamon	25	AFB_1	7 (28%)	0	0.214 – 1.684
		TAF	18 (72%)	0	0.426 - 3.013
Clove	20	AFB_1	8 (40%)	0	0.289 – 2.564
		TAF	15 (75%)	1 (5%)	0.265 - 12.708
Ginger	16	AFB_1	5 (31.2%)	0	0.48 – 2.203
		TAF	12 (75%)	0	0.379 – 3.836
Mixed spice	11	AFB_1	3 (27.3%)	0	0.239 – 0.933
		TAF	8 (72.7%)	1 (9%)	0.239 – 19.109

The observed levels of aflatoxin B_1 in all spices from all sites did not exceed the limit of 5 ngg^{-1} set by the European Union/South Africa (Williams et al. 2004, Ataş et al. 2012) whereas the levels of total aflatoxins in analyzed spices did not exceed the limit of 10 ngg^{-1} set by the European Union/South Africa (Williams et al. 2004, Ataş et al. 2012) except for one sample of clove and one sample of mixed spice (Table 5). The mean concentration of TAF in mixed spice was observed to be higher, i.e. 4.0375 ngg^{-1}, followed by clove with a mean concentration of 2.134 ngg^{-1}, ginger with a mean concentration of 1.526 ngg^{-1} and finally cinnamon with a mean concentration of 1.145 ngg^{-1} for samples from Dar es Salaam. For samples collected from Zanzibar, ginger had the highest mean concentration, i.e., 1.985 ngg^{-1}, followed by cinnamon with a mean concentration of 1.636 ngg^{-1}, clove with a mean concentration of 1.036 ngg^{-1} and finally mixed spice with mean concentration of 0.7375 ngg^{-1}. It was, therefore, observed that the mean concentrations of total aflatoxins (TAF) contamination of each group vary with location.

It was specifically observed that the levels of total aflatoxins (TAF) in cinnamon varied with sites, of which the highest contamination was observed in samples collected from KZ farm with a mean concentration of 3.013 ± 2.613 ngg^{-1} and Tandika market with a mean concentration of 2.036 ± 0.808 ngg^{-1}. Followed by samples collected from the KZ store with a mean concentration of 1.254 ± 1.014 ngg^{-1}, Tandale market 0.948 ± 0.866 ngg^{-1}, Kariakoo stores with a mean concentration of 0.811 ± 0.576 ngg^{-1}, Mwanakwerekwe market 0.462 ± 0.398 ngg^{-1} and Kariakoo market 0.426 ± 0.022 ngg^{-1}. The higher levels of aflatoxins in samples collected from Tandika market, for example, could be attributed to the poor storage conditions of spices where the packaged spices were found kept on the floor, which might have contributed to the formation of moisture content in the spice packages. On the other hand, the low levels of aflatoxins detected in samples collected from Kariakoo market

might have resulted from good storage conditions and packaging of the spices. Furthermore, KZ farm showed higher levels, which might be attributed to different drying and handling conditions of the spices as some were found well dried while others were not.

For the individual types of aflatoxins, the levels detected ranged from 0.327 to 1.499 ngg^{-1} for AFB_1, from 0.659 to 5.382 ngg^{-1} for AFB_2, from 0.269 to 2.673 ngg^{-1} for AFG_1 and from 0.262 to 0.810 ngg^{-1} for AFG_2. The data showed that the mean concentration of AFB_2 was higher, followed by AFG_1, AFB_1 and AFG_2. The average concentrations of aflatoxins indicated in Table 6 were unevenly distributed revealing the variations of aflatoxin levels based on different factors such as storage, packaging and drying.

Table 6: Cinnamon mean concentration for AFB_1, AFG_1, AFG_2, AFB_2 and TAF in Dar es Salaam and Zanzibar sampling sites.

		AFG_1 (ngg^{-1})	AFB_1 (ngg^{-1})	AFG_2 (ngg^{-1})	AFB_2 (ngg^{-1})	TAF (ngg^{-1})
SITE	N	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
KR	3	ND	0.583 ± 0	0.539 ± 0.116	ND	0.426 ± 0.022
TL	3	ND	0.948 ± 0	ND	ND	0.948 ± 0.066
TK	2	2.673 ± 0	1.499 ± 1.009	ND	1.941 ± 0	2.036 ± 0.808
KRs	2	ND	ND	0.810 ± 0.576	ND	0.811 ± 0.576
MKm	3	0.269 ± 0	0.327 ± 0.152	0.262 ± 0.229	0.993 ± 0	0.462 ± 0.108
KZf	2	ND	0.645 ± 0	ND	5.382 ± 0	3.013± 1.613
KZs	3	1.849 ± 1.055	ND	ND	0.659 ± 0	1.254 ± 1.014

ND = Not detected, KR = Kariakoo market, TK = Tandika market, TL = Tandale market, KRs = Kariakoo store, MKm = Mwanakwerekwe market, KZf = KZ spices farm and KZs = KZ Store

The variations of aflatoxin levels in ginger revealed that AFB_1 had a higher mean concentration, followed by AFG_1, AFG_2, and AFB_2. It was further noted that the KZ farm exhibited different patterns of variation of AFB_1 and TAF, of which the levels of the two were higher than in any other samples (Figure 1). This might be attributed to the fact that ginger samples collected from the KZ farm, for example, were possibly contaminated with aflatoxin during transportation from the field as it was reported to take a long time, which might have caused accumulation of moisture content in the packaging bags (Hashem and Alamri. 2010). The Tandale market showed high contamination levels with AFG_1, which could be due to the poor storage conditions of the shops as most of the vendors keep the spices on the floor. This might contribute to the formation of moisture content leading to the growth of aflatoxins-producing fungi.

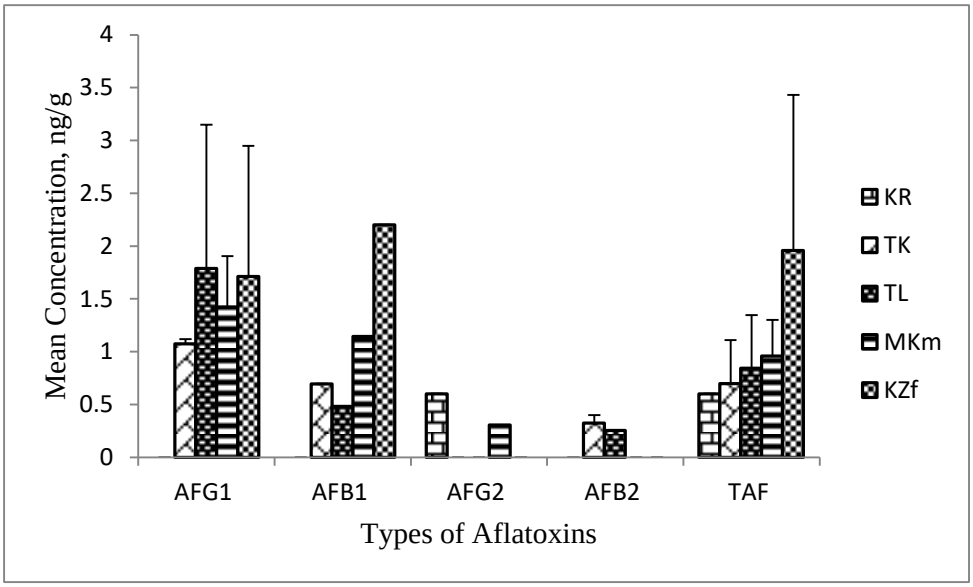


Figure 1: Ginger mean contamination for AFB_1, AFG_1, AFG_2, AFB_2 and TAF in Zanzibar and Dar es Salaam sampling sites

The variations of aflatoxin levels in cloves showed that the higher levels of total aflatoxins (TAF) were in clove samples collected from Kariakoo market, which registered the mean concentration of 5.671 ± 0.234 ngg^{-1}. Followed by samples from KZ stores 0.983 ± 0.442 ngg^{-1}, Kariakoo store 0.752 ± 0.543 ngg^{-1}, KZ farm 0.652 ± 0.213 ngg^{-1}, Tandale markets 0.597 ± 0.683 ngg^{-1}, Mwanakwerekwe market 0.574 ± 0.253 ngg^{-1} and Tandika market 0.265 ± 0 ngg^{-1}. For the individual aflatoxins, AFB_1 concentrations of the contaminated clove samples were within the acceptable limit of 10 ngg^{-1} for TAF and 5 $ngg^{-1}$for AFB_1 set by the European Commission and adopted by the Tanzania Bureau of Standards (TBS) (European Commission, 2006). The highest detected level was in the Kariakoo market, with a mean concentration of 1.199 ± 1.002 ngg^{-1}, followed by the Mwanakwerekwe market 0.816 ± 0.018 ngg^{-1}, KZ farm with a mean concentration of 0.803 ± 0 ngg^{-1}, Tandale market 0.737 ± 0 ngg^{-1}. In contrast, the AFB_1 in samples from Tandika and Kariakoo stores had concentrations below the detection limit. Other types of aflatoxins were detected, of which AFG_2 had the highest level, followed by AFG_1 and AFB_2, as shown in Table 7. It was further observed that among the sites on which clove samples were collected, only two sites were contaminated with AFG_2 (Kariakoo market and KZ store). In further analysis, it was observed that clove samples collected from Tandika market, Kariakoo stores and KZ store were not contaminated by AFB_1. On the other hand, clove samples from Tandale market, Kariakoo stores and KZ store were contaminated with AFG_1 whereas, AFB_2 was found in clove samples from Kariakoo market only. Table 7, shows that Tandika market was contaminated with AFB_2 only and had low levels compared to other sites. This might be explained by the fact that cloves in Tandika markets and shops were very few indicating a fast-moving commodity and hence short shelf time which contributes to less fungal accumulation. The observed variations might be due to the storage conditions and nature of the market surroundings that promote contamination due to human activities. On the other hand, clove samples from ZTC Zanzibar were found to contain aflatoxins below the limit of detection. This is attributed to the observed good storage condition of cloves as ZTC exports their spices to the international market, which requires good quality. However, one sample from the Kariakoo

market among the contaminated clove samples exceeded the European countries set maximum tolerable limit of 10 ngg^{-1} for total aflatoxins by having a mean concentration of 12.708 ngg^{-1}. This might be attributed to the storage conditions since the spices were kept on the floor which might have contributed to the formation of moisture content in the spice packages, which is one of the factors that influence the growth of aflatoxins producing fungi. Also, this might be associated with the long period of clove storage in the shops, which could lead to contamination. The levels of aflatoxins revealed by this study were lower compared with the levels reported from other countries; for instance, in the study conducted in Kenya by Mwangi et al. (2014) reported aflatoxins contamination of different spices in which clove showed moderate levels of TAF (ie., 7 ngg^{-1}) (Mwangi et al. 2014).

Table 7: Clove mean contamination for AFG_1, AFB_1, AFG_2, AFB_2 and TAF in Dar es Salaam and Zanzibar sampling sites

		AFG_1 (ngg^{-1})	AFB_1 (ngg^{-1})	AFG_2 (ngg^{-1})	AFB_2 (ngg^{-1})	TAF (ngg^{-1})
SITE	N	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
KR	3	ND	1.199 ± 1.002	10.144 ± 0	ND	5.671 ± 0.234
TK	1	ND	ND	ND	0.265 ± 0	0.265 ± 0
TL	3	0.379 ± 0	0.737 ± 0	ND	0.674 ± 0.203	0.597 ± 0.083
KRs	2	0.907 ± 0	ND	ND	0.598 ± 0.099	0.752 ± 0.143
MKm	2	ND	0.816 ± 0.018	ND	0.333 ± 0	0.574 ± 0.253
KZf	2	ND	0.803 ± 0	ND	0.501 ± 0	0.652 ± 0.213
KZs	2	0.983 ± 0	ND	0.804 ± 0	1.162 ± 0	0.983 ± 0.142

ND = Not detected, KR = Kariakoo market, TK = Tandika market, TL = Tandale market, KRs = Kariakoo store, MKm = Mwanakwerekwe market, KZf = KZ spices farm and KZs = KZ Store

The variations of aflatoxin levels in mixed spices showed that samples collected from Kariakoo market had higher mean concentration for the total aflatoxins of 7.464 ± 1.231 ngg^{-1} followed by Tandale market with 0.763 ± 0.240 ngg^{-1}, Mwanakwerekwe market with mean concentration of 0.738 ± 0.585 ngg^{-1} and Tandika market with mean concentration of 0.348 ± 0 ngg^{-1} as illustrated in Figure 2. However, the mean concentrations of total aflatoxins in samples of mixed spices from all sites were less than the limit set by European Countries for TAF, indicating that mixed spices from Zanzibar and Dar es Salaam were relatively less contaminated on average. However, one mixed spice sample from the Kariakoo market showed a higher contamination level of 19.109 ngg^{-1} of AFB_2 but had mean total aflatoxins of 7.464 ngg^{-1}, below the maximum tolerable level of 10 ngg^{-1}. The mixed spices in these shops were placed on the floor, which might have contributed to the accumulation of higher moisture content in the plastic packets and consequently favored growth of aflatoxins producing fungi, increasing the levels of aflatoxins.

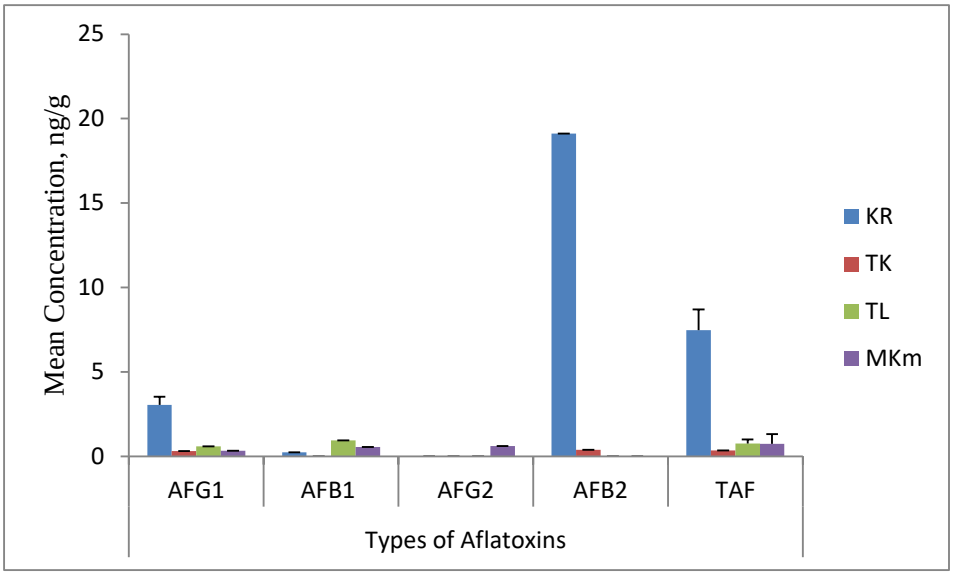


Figure 2: Mixed spices mean contamination of AFG_1, AFB_1, AFG_2, AFB_2 and TAF in Dar es Salaam and Zanzibar sampling sites.

Comparison of the levels of aflatoxin between spices from Dar es Salaam and Zanzibar

The study generally revealed that the mean concentration of TAF in mixed spices from Dar es Salaam had higher levels (2.974 ngg^{-1}) compared to samples collected from Zanzibar, which had a mean concentration of 0.738 ngg^{-1} (Figure 3). This can be explained by the fact that most of the mixed spices in Dar es Salaam markets were placed on the floor which might have contributed to the accumulation of higher moisture content in the plastic packets and consequently favoring the growth of aflatoxins producing fungi. Furthermore, the results revealed that the mean concentration of TAF in clove spices collected from Dar es Salaam was higher compared to the concentration of aflatoxins in clove samples collected from Zanzibar, i.e., 1.753 ngg^{-1} and 1.009 ngg^{-1}, respectively. This might be due to the prolonged shelf time of cloves in Dar es Salaam which might be partly caused by the relatively higher prices. However, in the case of Zanzibar, cloves are produced locally and hence less expensive, leading to short shelf time in shops compared to Dar es Salaam. On the contrary, most of the spices marketed in Dar es Salaam are imported from different countries such as India, thus, leading to contamination during storage and transportation as compared to Zanzibar, where most spices are bought and sold within the same neighborhoods. Contamination in cinnamon was observed to be a little higher in samples from Zanzibar compared to Dar es Salaam, where their mean concentration was 1.789 ngg^{-1} in Zanzibar and 1.101 ngg^{-1} in Dar es Salaam. Similarly, aflatoxins contamination in ginger from Zanzibar was higher with a mean concentration of 1.488 ngg^{-1} compared to ginger samples from Dar es Salaam, which had a mean concentration of 1.321 ngg^{-1}.

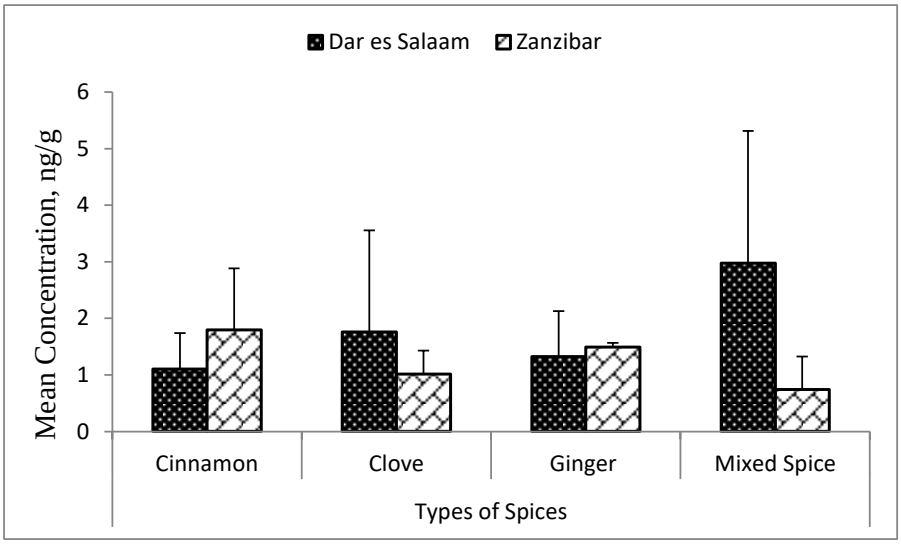


Figure 3: Mean Concentration for TAF in Dar es Salaam and Zanzibar Spices

Conclusion

The results showed that 53 (73.6%) samples were contaminated by total aflatoxins with only 2 samples (i.e., 1 clove and 1 mixed spice) whose levels exceeded the maximum permitted levels of 10 ngg^{-1} set by European Countries/South Africa. The mean aflatoxin levels of cinnamon and ginger in the Zanzibar farm were higher; 3.013 ngg^{-1} and 1.957 ngg^{-1}, respectively, while in Dar es Salaam and Zanzibar, markets had mean total aflatoxin concentrations of 1.000 ngg^{-1} for cinnamon and 0.776 ngg^{-1} for ginger, and clove in markets and store in Zanzibar and Dar es Salaam had shown to have higher mean levels; 1.322 ngg^{-1} while in KZ farmer in Zanzibar the mean level was 0.983 ngg^{-1}. It has to be noted that although aflatoxin levels in the analyzed spices were within acceptable limits, the risk of aflatoxicosis resulting from the continuous ingestion of these foods may be high. Therefore, these levels should trigger an alarm for closer monitoring of the preparation and storage of spices. The observed levels necessitate that the regulatory bodies in Tanzania constantly monitor for aflatoxins in spices marketed in different markets in the country. Furthermore, farmers and spices vendors should be trained on safe post-harvest drying and storage techniques to prevent the aflatoxin-producing fungi growth.

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