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Status of aflatoxins contamination in spices produced in Morogoro, Tanzania

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Abstract

Aflatoxins, the toxic secondary metabolites produced by fungi under *Aspergillus species* in foods, are capable of causing adverse health effects in humans and animals. Information on levels of aflatoxins in spices produced and traded in Tanzania is limited. This study investigated aflatoxins contamination levels in black pepper, cinnamon, cloves and turmeric spices produced and vended in Morogoro district, Tanzania. A total of 120 samples were collected and analyzed for aflatoxin B1, B2, G1, G2 and Total aflatoxins using High Performance Liquid Chromatography (HPLC) with fluorescence detector. Results showed that 24.2% (n=120) of the samples were contaminated with aflatoxins whereby 11.7% (n=120) had AFB1. Spices from Morogoro rural had significantly high (p=0.002) aflatoxins contamination (16.7%; n=120) compared to those from Morogoro Municipality (7.5%; n=120). In reference to each spice samples, turmeric had no AFBI contamination (0.0%) with lowest Total Aflatoxins (3.3%; n=30) while cloves had the highest contamination with 20% (n=30) and 50.0% (n= 30) for AFB1 and Total aflatoxins, respectively. The lowest detected contamination concentration was 0.201µg/kg in cloves while the highest was 164.86µg/kg in black pepper. The spices; Turmeric (0.0%; 3.3%); Cinnamon (16.7%); (20%); cloves (13.3%; 23.3%) and black pepper (6.7%; 6.7%) (n=30) exceeded Tanzania regulatory limit of 5 µg/kg and 10µg/kg for AFB1 and Total aflatoxins contamination levels, respectively. It is concluded that spices produced and marketed in Morogoro were contaminated with aflatoxins some of them beyond the maximum limit set by Tanzania Bureau of Standards risking the health of spices consumers. Awareness creation to spices traders on aflatoxins contamination and preventive measures need to be considered to safeguard health of spices consumers in Morogoro, Tanzania.

Key words: *black pepper; cinnamon; cloves; fungi; mycotoxins; turmeric*

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Introduction

Mycotoxins such as aflatoxins are toxic secondary metabolites produced by species of filamentous fungi such as *Aspergillus, Penicillium* and *Fusarium*. The fungi grow in a wide range of foods including cereals, nuts, spices, dried fruits, apples and coffee beans, often under warm and humid conditions (Naz *et al.,* 2016). Aflatoxins contamination in foods have been recognized as a serious problem in many parts of Africa due to their impacts on human and animal health, economy and trade (WHO, 2018). Aflatoxins are one of the highly toxic mycotoxins to human and livestock (Chauhan, 2017; WHO, 2018). Aflatoxins exist in six forms, four occur in food; B_1, B_2, G_1 and G_2 while M1 and M_2 are metabolites of B_1 and B_2; respectively (Chauhan, 2017). Of the aflatoxins, AFB_1, is the most frequent in occurrence and the most carcinogenic natural products formed in nature.

Increased risk of hepatocellular carcinoma in the presence of hepatitis B virus infection has been associated with aflatoxins contamination of food in most developing countries (Williams et al., 2004). Chronic exposure due to low levels of aflatoxins contamination consumed regularly increases the risks of liver cancer and suppresses the immune system, particularly for the population with hepatitis B virus (HBV). High levels can cause acute poisoning and even death to human and animals (WHO, 2018).

Spices are group of plant substances valued for their high mineral content, with strong taste and aroma. They are used in small amounts as flavoring agents in various foods, as antioxidants, colorants, and preservatives (El-Sayed and Youssef, 2019). In spite of being among the most versatile and widely used ingredients in food preparation and processing throughout the world, spices can be a source of microbial contamination such as mold producing mycotoxins in foods (Temu, 2016). Contamination with aflatoxins occurs during pre-harvest or post-harvest depending on the storage conditions (Potorti, 2020). Aflatoxins contamination has been reported in various food products in Tanzania including maize (Nyangi *et al.,* 2016; Kamala *et al.,* 2018; Sasamalo *et al.,* 2018), peanut-enriched complimentary flours (Kuhumba *et al.,* 2018). However, information on aflatoxins contamination in spices is limited.

Spices cultivated in tropic and sub-tropic areas can be exposed to contamination with toxigenic fungi and subsequently mycotoxins before and post-harvest (Kabak and Dobson, 2016). This is due to high temperature, humidity, and rainfall which favor mold growth resulting into aflatoxins production (Hussain *et al.,* 2012). Aflatoxins contamination in spices has been reported in many countries including Hungary (Fazekas *et al.,* 2007) and Kenya (Mwangi *et al.,* 2014). In Tanzania, fungal contamination was reported in commonly used spices obtained from local markets and Indian shops, one of them being *A. flavus* which is capable of producing aflatoxins (Temu, 2016). The study revealed fungal contamination in red chili (18.37%), ginger (14.28%), curry powder (4.04%) and fenugreek (2.04%) (Temu, 2016). Fundikira, (2018) conducted a study on four marketed spices in Dar es Salaam and her findings revealed aflatoxin contamination of cinnamon (43.3%), cloves (70%), ginger (56.7%) and cardamom (60%). Morogoro is one of the main producers for the spices in the country, raising the need to know the contamination levels, practices and traders' awareness about aflatoxins.

There is an initiative to minimize aflatoxins contamination in maize and groundnuts in Tanzania (AFDB, 2018). The efforts have not included spices probably due to limited information about aflatoxins contamination. This study assessed the contamination levels of aflatoxins in selected spices produced and marketed in Morogoro Municipality and Morogoro Rural, Tanzania. The results can contribute to setting guidelines to regulate safety of spices marketed in the country in respect to aflatoxins contamination to safeguard health of the spices consumers.

Materials and Methods

Study area

The study was conducted from December, 2019 to January, 2020 in Morogoro district in Morogoro region, Tanzania. The region is located in the Mid-Eastern part of Tanzania and it lies between 5°58′ and 10′ south of equator and between longitude 35°25′ and 38°30′ East Greenwich. Morogoro district is among the seven districts in the region others are Kilosa, Mvomero, Malinyi, Mlimba, Ulanga, Gairo and Ifakara town council. The district has two councils, which are Morogoro rural and Morogoro municipality. Morogoro district council had a population of 286 248 (NBS, 2012). Morogoro municipal is divided into 19 administrative wards with estimated population of 315 866 (NBS, 2012). Morogoro rural was selected because it had a large production of spices among the seven districts in Morogoro (Maerere and Noort, 2014) while Morogoro urban was selected as it is a primary market for most of spices from Morogoro rural areas which include cloves, black pepper, and cinnamon, turmeric, vanilla and cardamom.

Study design

The study adopted a cross sectional descriptive study design, where the data collected at a single point in time. A combination of probabilistic and non-probabilistic sampling methods used to select spice samples and spice traders. This involved multistage sampling to select region, district, wards/markets and then individual spice traders.

Study population

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

Sample collection

Four types of dried spices; clove (*Syzygium aromaticum*), turmeric (*Curcuma longa*), cinnamon (*Cinnamomum verum*) and black pepper (*Piper nigrum*) were collected into clean polythene bags. Spice samples were labeled and transported to the Tanzania Bureau of Standards (TBS) food laboratory located at TBS HQ offices in Dar es Salaam and stored at temperature of 4 °C prior to moisture content and aflatoxins analysis.

Aflatoxin Analysis

Sample preparation and Extraction

Cloves, cinnamon, turmeric and black pepper were ground separately using grinder to obtain a homogenous mixture and then sub-divided to obtain representative sub samples for analysis. Samples were extracted according to procedure described in ISO (2003). All individual samples were analyzed independently. Each ground spice sample was placed into Erlenmeyer flask and weighed using the calibrated analytical balance to 25 ± 0.1g. Using a measuring cylinder, 100 ml of methanol: water (70:30 v/v) as extraction solvent was added to the 250 ml Erlenmeyer flask containing the sample. The flask was covered with aluminium foil and placed on the gyratory shaker (Stuart® Orbital Shaker SSL1, Cole-Parmer LLC, USA) at 250rpm for 30 minutes, then using a filter paper Whatman No. 1, the extract was filtered into a 250 ml Erlenmeyer flask according to the procedure described in ISO (2003).

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.

Isolation and clean-up of aflatoxin

The diluted extract was loaded and allowed to pass through Solid Phase Extraction (SPE) immunoaffinity columns and the sample loaded columns were rinsed twice with 10 ml of HPLC grade water. The adsorbed aflatoxins were eluted with 1 ml of HPLC grade methanol and the eluents were collected in vials. Finally, pressure was slightly applied on top of the column to remove any remaining liquid. Thereafter, 0.3 ml of the elute was mixed with 0.6 ml of water and 0.1 ml of acetonitrile and the mixture was vortexed for 30 seconds at a speed of 2500 rpm ISO (2003).

Sample analysis for aflatoxin

After extraction, dilution, cleaning and elution and post-column derivatization, the extracts were analyzed using HPLC with fluorescence detector (FtLD) (Model Agilent Chem Station Technology, series 1200, 5301 Stevens Creek Blvd, Santa Clara, CA 95051, USA). The mobile phase contained water: methanol: acetonitrile (60:30:10, v/v). The separation of aflatoxins (B_1, B_2, G_1 and G_2) was performed on the C18 column at 30 ^oC at a flow rate of 1.2 ml/min. The injection volume was 50 µL for both standard solution and sample extracts. After separation, AFG_1 and AFB_1 were derivatized for detection with fluorescence detector at an emission wavelength of 450 nm and an excitation wavelength of 365 nm ISO (2003).

Method validation

A mixture of aflatoxin standards solution (G_1, G_2, B_1 and B_2) (all from Fisher Chemical, Bishop Meadow Road, Loughborough, Leicestershire) was prepared and analysed at the concentration indicated in (Table 1) to establish a four-point calibration curve (Fig.1 - 5). The conditioning of the HPLC system was the same. The calibration curve was constructed to check the linearity and quantification of aflatoxins. To validate the extraction method and chromatographic performance, all four-spice samples were spiked with three concentrations (1, 3 and 5ppb) of standard solutions of aflatoxins B_1 B_2, G_1, and G_2 in triplicate and mean concentrations and percentage recovery were recorded. The limit of detection (LOD) and limit of quantitation (LOQ) of the HPLC method for AFB_1, AFB_2, AFG_1 and AFG_2 were determined using equations in Table 2 together with the LOD and LOQ values obtained for the four types of aflatoxins.

Table 1

Calibration table for AFB_1, AFB_2, AFG_1 and AFG_2

No	RT[1]	Signal	Compound	Level	Amount	Area	Rsp.Factor[2]
1	5.047	FLD1 A	AFLA G_2	1	1	123.69	8.08E-03
				2	5	624.11	8.01E-03
				3	10	1337.1	7.48E-03
				4	15	1928.5	7.78E-03
2	6.01	FLD1 A	AFLA G_1	1	1	49.599	2.02E-02
				2	5	229.18	2.18E-02
				3	10	498.49	2.01E-02
				4	15	712.59	2.11E-02
3	7.02	FLD1 A	AFLA B_2	1	1	129.57	7.72E-03
				2	5	616.17	8.11E-03

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

				3	10	1351.5	7.40E-03
				4	15	1880.4	7.98E-03
				1	1	69.771	1.43E-02
4	8.597	FLD1 A	AFLA B_1	2	5	299.24	1.67E-02
				3	10	595.23	1.68E-02
				4	15	813.89	1.84E-02

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

Figure 1

Calibration curve for AFLAG$_2$

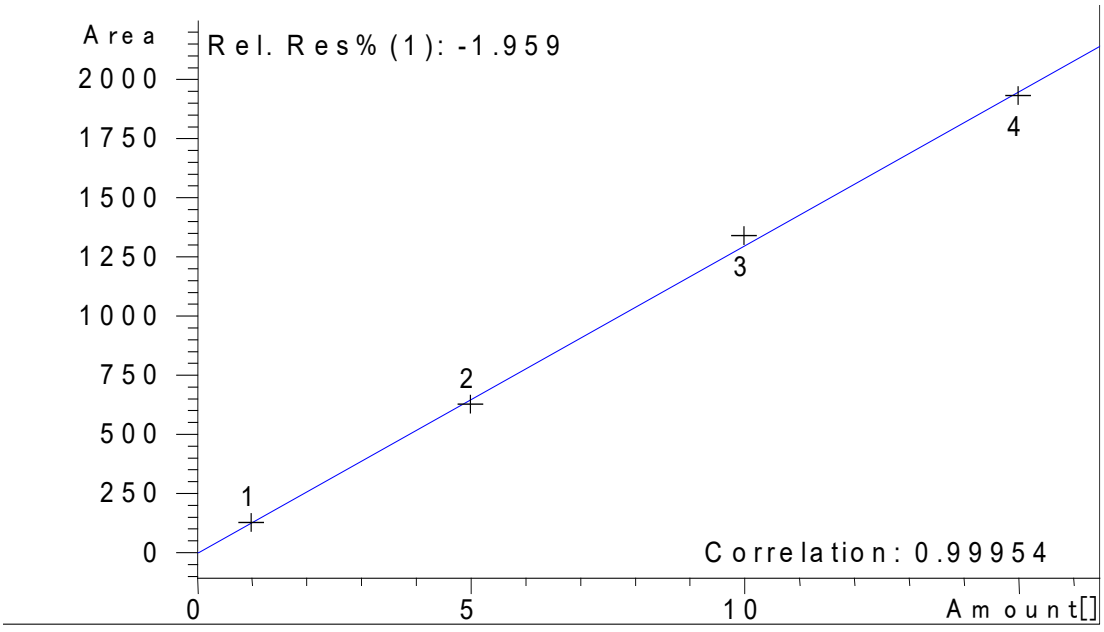

Figure 2

Calibration curve for $AFLAG_1$

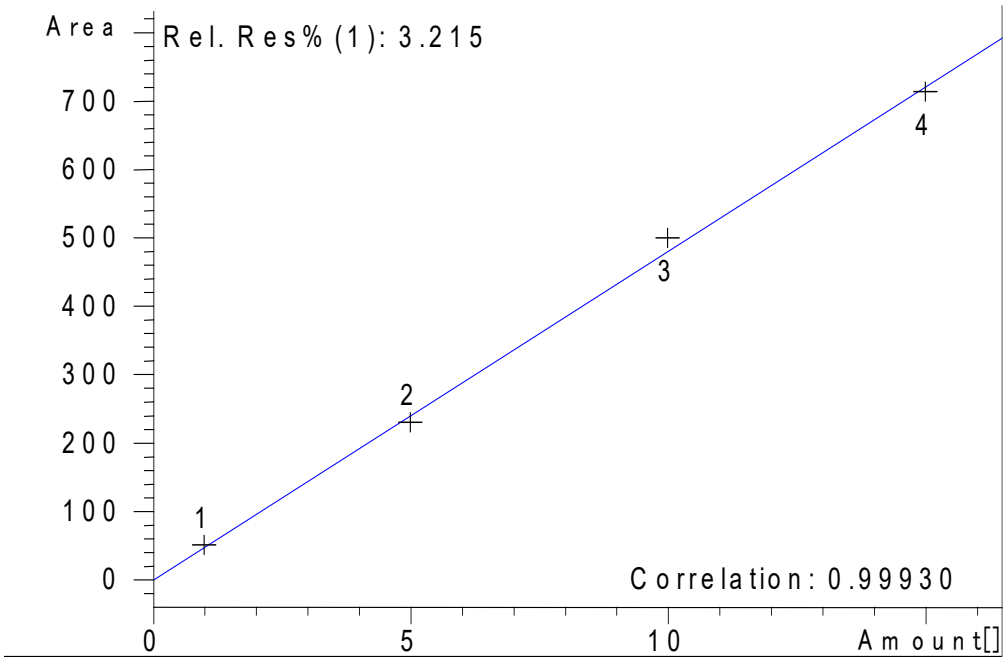

Figure 3

Calibration curve for $AFLAB_2$

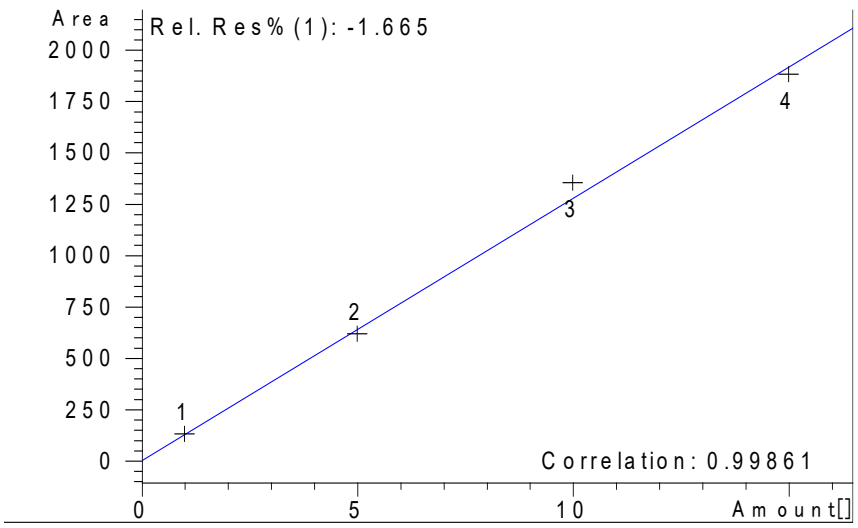

Figure 4

Calibration curve for AFLAB$_1$

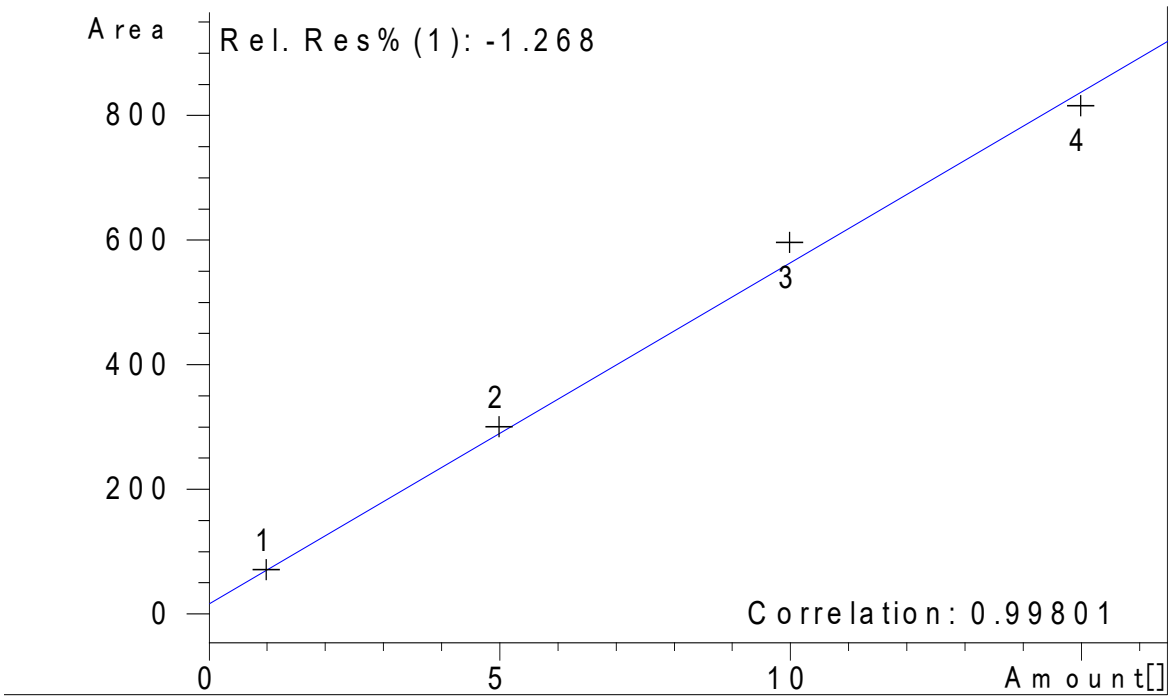

Table 2

The Limit of detection (LOD) and limit of quantitation (LOQ) for analyzed aflatoxins contaminations in analyzed spices

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

Where SD = Standard deviation of the lowest concentration

Results

Moisture Content in Spices

A total of 120 samples were analyzed for moisture content and 35 samples (29%) had moisture content exceeding the regulatory limit (Table 3). Turmeric had fewer samples (n=3, 10%) that had moisture content above the regulatory limit of 12% compared to other types of spices analyzed. Cloves have more samples (n=21, 17.5%) above the regulatory limit. A significant association (p = 0.00) between moisture content and the Aflatoxins contaminations in spices was demonstrated.

Table 3

Moisture content of the selected spices produced and marketed in Morogoro District, Tanzania

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

Aflatoxins contamination in spices

Twenty-nine (24.2%) of the 120 spice samples were contaminated with aflatoxins in which 14 (11.7%) were contaminated with aflatoxins B_1. Turmeric had the lowest contamination for AFB1 and Total aflatoxins of 0.0% and 3.3%, respectively, while the highest contamination was observed in cloves being 20% and 50.0%, respectively. FG_2, AFG_1 and AFB_1were not detected in Turmeric, while AFG_2 and AFG_1 were not detected in Black pepper and cinnamon, respectively, (Table 3).

Table 4

Number of Aflatoxins contaminated spices samples from Morogoro District, Tanzania

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

For Total aflatoxins, cloves had the highest number of contaminated samples (23.3%) which exceeded Tanzania regulatory limit of 10µg/kg while turmeric has low percent (3.3%). For AFB1, cinnamon had high percent (16.7%) of contamination levels exceeding Tanzania regulatory limit of 5µg/kg while turmeric had no contamination. For contaminated samples, the lowest contamination was 0.201µg/kg in cloves while the highest was 164.86 µg/kg in black pepper (Table 5).

Table 5

Number of Total aflatoxins and AFB_1 contaminated spices samples marketed in Morogoro District, Tanzania

Spice type	Type of aflatoxins	N	Positive samples n(%)	Exceed National regulatory limits n(%)	Mean ± standard deviation
Turmeric	AFB_1	30	0(0%)	0(0)	NA
	Total aflatoxins		1(3.3%)	1(3.3)	0.8979±0.89793a
Cinnamon	AFB_1	30	5(16.7%)	5(16.7)	3.1722±1.405a
	Total aflatoxins		7(23.3%)	6(20.0)	7.0308±3.8253a
Cloves	AFB_1	30	6(20.0%)	4(13.3)	8.2978±6.463a
	Total aflatoxins		15(50%)	7(23.3)	14.3720±6.463a
Black pepper	AFB_1	30	3(10%)	2	5.897±5.4899.23a
	Total aflatoxins		6(20%)	2	9.99±6.63a

Discussion

This study established high prevalence (24.2%) and high levels of aflatoxins contamination in spices traded in Morogoro municipality and Morogoro rural markets. The levels of aflatoxins contamination in evaluated spices ranged from 0.201 to 164.86 µg/kg (ppb) in which 11 samples (9.17%) exceeded the maximum limit (5µg/kg) for AFB_1 while 16 samples (13.3%) exceeding maximum limit (10µg/kg) for total aflatoxins set by TBS and European Commission (EC 2020/685, 2020). This implied that the consumers using these spices are at risk of aflatoxicosis if control measures are not considered. The observed prevalence was lower than that reported in Pakistan where the incidence was 61.5% aflatoxins contamination in spices out of which 53.66% samples exceeded the EU maximum limit in spices (Naz *et al.,* 2016). Also, the results were lower when compared with the findings from the study conducted by Mwangi (2014) in Kenya in which 34 (73.9%) spice samples were contaminated with aflatoxins. The observed differences can be attributed to variations in climatic conditions and handling practices.

However, the prevalence reported in this study (24.2%) was in concurrence with the observation made in Brazil, where spices were reported to be highly susceptible to aflatoxins contamination as a result had high *Aspergillus* species contamination (Garcia *et al.,* 2018). This indicates the global nature of the problem and might be due to the observed high moisture content (Table 3) caused by inadequate drying of spices as well as poor handling practices which favour the growth of fungus and aflatoxins production.

Turmeric had low percentage of aflatoxins contamination (3.3%) and was only contaminated with AFB2 (Table 4). Probably turmeric has antimicrobial activity inhibiting fungal growth and mycotoxins production. Turmeric has been reported to have a wide range of medicinal properties and the essential oils from turmeric exhibited significant inhibition of fungal growth as well as

production of aflatoxins B1 and G1 (Sindhu *et al.*, 2011).

Findings from this study reveal that cloves have high aflatoxins contamination in which 15 (50%) of the samples were contaminated with aflatoxins compared to the other three types of spices. Among the contaminated samples, seven (7) samples (23.3%) exceeded the maximum limit for aflatoxins levels set by TBS (Table 4 and Table 5). The highest aflatoxin concentration was 147.5 ppb being higher than the levels of aflatoxin in cloves reported in Serbia (31.5 ppb) (Škrinjar *et al.*, 2012) and in Kenya (7 ppb) (Mwangi, 2014). The higher aflatoxin contamination observed in cloves samples may be due to poor post-harvest handling practices such as storage conditions and inadequate drying (Nurtjahja, 2019).

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

Seven samples of cinnamon were contaminated with aflatoxins (23.3%) and maximum concentration was 109.85 ppb (Table 4 and 5). The concentration was higher than reported in Poland (1.79 ppb) (Waskiewicz *et al.*, 2013); in Iraq (20.8 ppb) (Farid and Nareen, 2013) and Italy (2.27 ppb) (Romagnoli *et al.*, 2007). Five samples (16.7%) of cinnamon exceeded Tanzania regulatory limit with concentration level of AFB1 ranging from 10.33 - 26.94 ppb. However, this was at lower compared with the findings in Turkey in which all samples of cinnamon (100%) exceeded EU maximum limit with concentration range of 49.4- 53 ppb (Tosun and Arslan, 2013). The concentration was lower than reported by Fundikira *et al.* (2020) in Tanzania which revealed higher percentage (43.3%) of cinnamon samples contaminated with Aflatoxin.

Although most of the spice samples (75.8%) were not contaminated by aflatoxins, spices are highly susceptible to aflatoxins as observed in this study where by maximum concentration of 164.86 ppb was obtained which is higher than the acceptable maximum limit of 5 ppb for AFB1, 10 ppb for Total aflatoxins according to TBS. This indicated that consumers may be at risk to aflatoxicosis although the risk depends on both consumption patterns and contamination levels (Ali *et al.*, 2015).

Conclusion

The study detected aflatoxins contamination in clove, turmeric, cinnamon and black pepper produced and traded in Morogoro region, Tanzania. Cloves had high prevalence of aflatoxins contamination compared to other three evaluated spices. Some samples exceeded the maximum limit for aflatoxins levels in spices according to Tanzania set standards. To prevent health risk to consumers, awareness on aflatoxin and its effects should be created to the spice's traders and general public. Also monitoring of aflatoxin contamination in spices is important.

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