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Smallholder farmer's awareness and determination of aflatoxins in sesame seeds of southern Tanzania

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ABSTRACT

Consumption of aflatoxins contaminated foods has led to detrimental health effects worldwide, with even more severe cases in African countries including Tanzania. A cross-sectional study was conducted to assess awareness and aflatoxins contamination of sesame seeds in Lindi and Mtwara regions. Subsequently, a total of 70 sesame seed samples were randomly purchased from local markets for aflatoxin determination using High Performance Liquid Chromatography (HPLC). Qualitative data were analyzed using SPSS 20 for descriptive and correlation analysis. Results show that 82.4% of the respondents were not aware of aflatoxin contamination of agricultural produce. Awareness was negatively correlated to the levels of education ($p=-0.309$) and positively correlated with gender whereby men were more aware than women ($p=0.03$). On the other hand, 37 out of 70 sesame seed samples were contaminated with total aflatoxins at a range of 0.009 ng/g to 5.557 ng/g. Although none of these samples exceeded the Tanzania maximum limits of 10 ng/g for total aflatoxins, 2 samples exceeded the maximum limit of 4 ng/g set by the European Union. Furthermore, Aflatoxin B1 was detected in 13 samples moreover the concentration was below the Tanzania and EU maximum limits of 5 ng/g and 2 ng/g respectively. Though the contamination was below the national maximum limits and limited to one agro-ecological zone and season, these findings provide useful insights on aflatoxins contamination of sesame seeds from the two main growing regions in Tanzania.

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1. Introduction

Sesame (*Sesamum indicum* L.) is one among the most cultivated crop in Africa and Asia. The crop is said to originate from Asia specifically India, but it has been transported to Africa at the early times of its occurrence (1). For this reason, many researchers regard the later as the origin of sesame seeds. Sesame seeds are known with different names such as benniseed, till, simsim, *ufuta* to mention few (2).

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High quality oil and protein contents are the factors that makes sesame seeds the third most grown oilseed in the world (3). Presence of the medicinal components such as sesamin and sesamolin make sesame oil unique; hence it is termed as "queen of oils" (4). Tanzania is currently the world's best producer of sesame yielding about 940,221 metric tons per year (5). Sesame is best grown in Southern part of Tanzania in Lindi and Mtwara regions and accounts for about 35% which is about 400,000 tons of all sesame seeds exported to the world market annually (6).

Nutritionally, sesame seeds contain oil (45.6 – 46.1%), protein (21.9 – 23.6%), carbohydrate (10.8 – 17.0%), moisture (4.18 – 5.41%), ash (6.16 – 7.34%) and fiber (4.7 – 7.15%) (7). The seeds have plenty of uses as ingredient to bakery and confectionary products (8,9). Presence of phytonutrients in sesame oil makes it a useful antioxidant, antitumor, anti-aging and anticancer (10). In African and Asian countries, sesame seeds are used as an ingredient in porridge flour for children of all ages (11). It is also an ingredient in most of African indigenous vegetables dishes, similar to peanut and cashew nut butter (12).

However, like cereals and other oily seeds and nuts such as maize and groundnuts, sesame seeds are vulnerable to fungal infestation and mycotoxins contamination such as aflatoxins (13) which are known to affect human and animal health (14-16). A study conducted in Senegal revealed that, sesame is vulnerable to aflatoxin contamination (17). A notable mycotoxigenic potential of *Aspergillus* genera in oilseed crops including sesame seeds, almonds and pistachio has been reported (18).

Aflatoxins are secondary metabolites produced by fungi particularly *Aspergillus flavus* (18) and *A. parasiticus. Aspergillus flavus* is an opportunistic pathogen with a cosmopolitan occurrence and has an ability to attack agricultural crops at any stage from planting to consumption. Plant stress, crop damage by insects, improper harvesting procedures or moisture caused by heavy rainfall during or after harvest are among the channels that the fungi uses to enter the crop (19). There are four main types of aflatoxin known; aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Of all, AFB1 is known to be the most dangerous and toxic (20). The International Agency for Research on Cancer (IARC) has classified aflatoxins as the primary human carcinogen (Group 1) that contributes to liver cancer cases worldwide (21) with the highest incidences occurring in Africa (22). Aflatoxin's exposure suppresses immune, digestive system, and nervous system functioning particularly central nervous system, debilitated fertility, and growth impairment in children (19, 23, 24). Growth retardation is the result of nutritional interference within the body as aflatoxin blinds DNA to reduce protein synthesis as well as interfering with vitamin A absorption in the body (25). Several aflatoxin outbreaks due to acute aflatoxin consumption resulting to hundreds of deaths have been reported worldwide. A major outbreak of aflatoxicosis occurred in India resulted to 106 deaths from consumption of aflatoxins contaminated maize (26). In 2014, aflatoxicosis case was reported in Kenya, with 125 deaths and 317 hospitalized (23). Tanzania has also faced re-ocurrence of aflatoxicosis in some parts of Dodoma and Manyara regions in 2016 with 65 cases and 20 deaths (27) and in 2019, an outbreak was reported again in Dodoma (Chemba, Kondoa and Kiteto districts) with 53 cases and 8 deaths (28).

Despite all health effects caused by aflatoxins contamination, less is known by farmers and the general population. Over 70% of the farmers in Meru (29) and 97% in Kilosa districts (30) were unaware that mycotoxins affect crops. Notably, 66.7% of the respondents were unaware of the health effects following mycotoxins exposure. A similar study in Nigeria showed that, 85% of the peanut consumers were unaware of aflatoxins contamination (31). Lack of awareness of aflatoxins contamination of crops is among the factors that decelerate its management, accelerate exposure and its related health effects.

Many studies on aflatoxins contamination of crops in Tanzania have focused on staple cereals and nuts mainly maize and groundnuts (27,32,33). The two crops have been ranked as the priority crops (34) in Tanzania due to the fact that, maize is consumed by majority of the population at a higher frequency in a day, weeks and months; sesame is common to some population and is used as ingredient in some meals for taste or in placement of cooking oil. Furthermore, sesame is an important food and cash crop with social-economic returns to the farmers and country in general. Nevertheless, sesame has been given little attention despite being susceptible to aflatoxins contamination. Therefore, this study aimed at assessing farmers' awareness and aflatoxins contamination on sesame seeds grown in Mtwara and Lindi regions.

2. Materials and Methods

2.1. Description of study sites

This study was conducted in Mtwara and Lindi regions of Tanzania. The regions are the leading producers of sesame seeds in the country (6). Along with sesame seeds, other main food and cash crops grown in the regions include cassava, millet, sorghum, groundnuts, and cashew nuts. Figure 1 shows a map of Tanzania with the main sesame producing regions; and districts where sesame seed samples were collected.

2.2. Study design

The study employed a cross sectional design in which a semi structured questionnaire was used for data collection. Information collected includes demographics, postharvest storage practices of sesame seeds, awareness, and management of aflatoxin contamination.

Purposive sampling method was used to select districts, wards and villages considering the highest number of sesame seeds farmers. Therefore, four districts namely Masasi (Mtwara) Nachingwea, Kilwa and Ruangwa (Lindi) were selected (Figure. 1). Subsequently, two wards were selected in each district except in Kilwa where only one ward cultivates sesame seeds. From the wards, a total of 15 villages were selected for this study. From each village, an average of 30 sesame seeds farmers/traders were selected randomly from the lists of farmers/traders given by the ward's Agricultural Extension officer to make a total of 454 farmers/traders who were interviewed. In addition, a total of 70 sesame seeds were collected from traders who were part of the interviewed farmers. The sesame seeds samples were well packed in paper bags labeled and transported to the laboratory stored at 27°C prior further analysis.

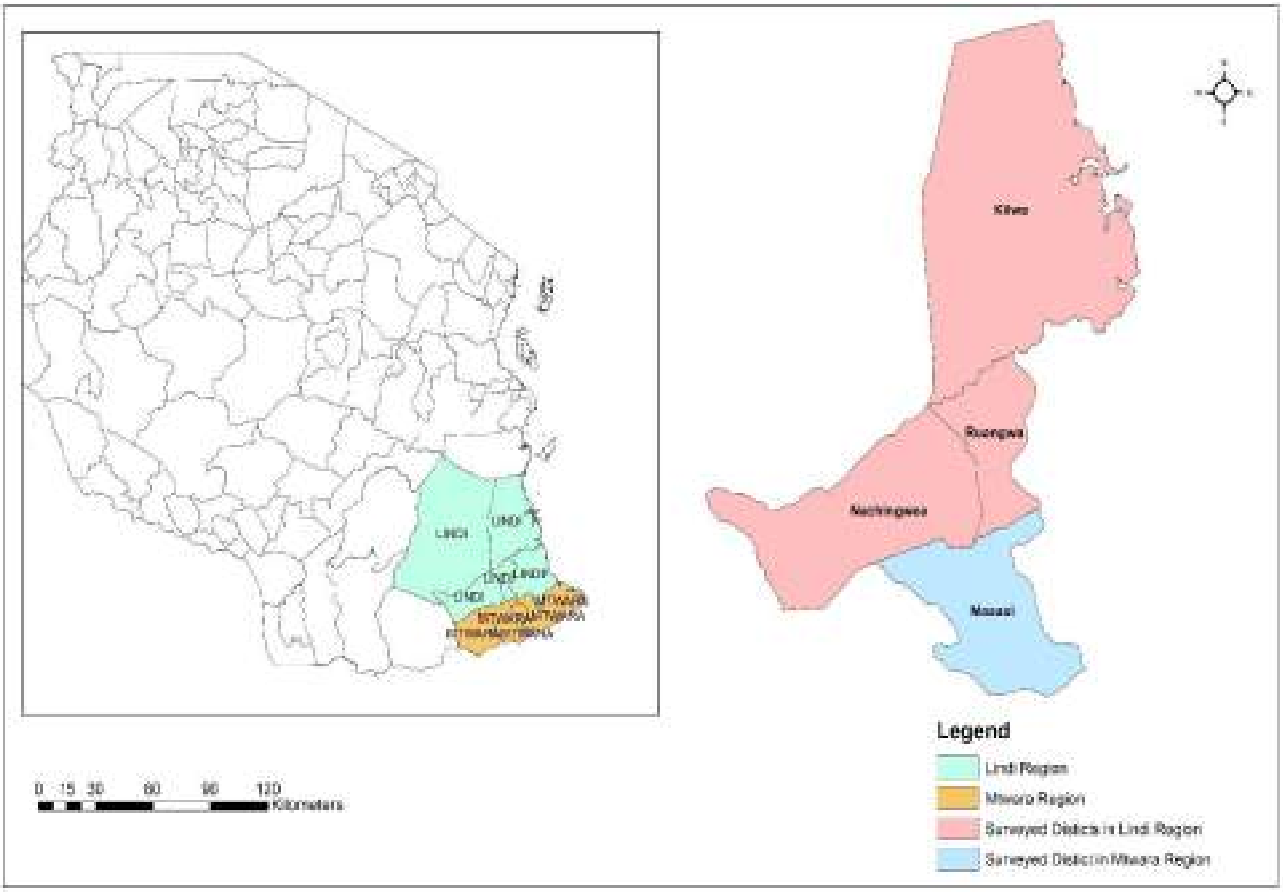

Figure 1. A map showing study districts in Mtwara and Lindi regions

2.3. Extraction of aflatoxins in Sesame seeds

The sesame seed samples were sorted to remove non-seed materials such as sands and plant stalks. Then the seeds were blended to make flour. Aflatoxin extraction was conducted as described by (35) with minor modifications. Briefly, 5 g of sesame seeds flour was extracted with 20 mL of Methanol: water, (80:20 v/v) and 20 mL of hexane (Loba Chemie, India) on a vortex mixer (Fischer Scientific, UK) for 3 min. The mixture was allowed to settle for the layers' separate and afterwards, the hexane layer containing fat was discarded. The extract was then filtered using a Whatman filter paper No. 1 (Sigma Aldrich, China).

Then, 4 mL of the filtrate was diluted with the 8 mL phosphate buffer saline (PBS) (Loba Chemie, India). The diluted extract was cleaned through immunoaffinity column (Aflatest VICAM, USA) fitted on a vacuum Manfold. The flow rate was set not to exceed 3 mL/min. The column was rinsed twice with 10 mL of HPLC water (Loba Chemie, India) to remove impurities. Then the bound aflatoxins were eluted using 1 mL of HPLC grade acetonitrile (Loba Chemie, India) by passing it through the column for about 1 min and at a gradient flow to allow intensive contact with the bound toxins.

Thereafter, 400 µL of the eluent was derivatized with 600 µL of derivatizing reagent (H_2O: Triflouroacetic acid (TFA): Acetic acid; 70:20:10 v/v/v) at 65 °C for 15 min. The mixture was allowed to cool before HPLC analysis.

2.4. HPLC analysis of aflatoxins in Sesame seeds

A total of 20 µL of the elute was injected into the HPLC (Shimadzu, Japan) equipped with fluorescence detector RF-20A set at 450 nm and 365 nm emission and excitation respectively. Mobile phase consisted of water: methanol: acetonitrile (60:30:10 v/v/v) at a flow rate of 1 mL/min. The column was Luna C18, 5µm C18 (2) 100A, 250*4.6 mm (made in USA) set at 40°C, and the running time was 20 min, calibration curve of R= 0.997 and retention time of 6.63 min, 8.78 min, 11.37 min, and 16.26 min was obtained for aflatoxins G2, G1, B2 and B1 respectively. Limit of Detection (LOD) and percentage recovery were determined as described by and were found to be 0.143, 0.050, 0.089, 0.412 and 64.9%, 70.2%, 102.1% and 157.7%; for aflatoxins G2, G1, B2 and B1 respectively. According to the criteria set by European Commission Regulation (EC) No. 401/2006, the acceptable recovery for concentration <1 ng/g is 50 - 120% while for the 1-10 ng/g is 70 - 110% (36).

2.5. Statistical analysis

The survey data were coded and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software for descriptive statistics such as frequency and percent distribution of the variables. Correlation tests were also performed to find out if there is any association between the demographic characteristics and awareness and aflatoxin contamination of sesame seeds.

A *p*-value less than 5% was considered significant. The data on laboratory analyses were subjected to a combined analysis of variance (ANOVA) using GenStat (15^{th} edition) statistical software for computing the grand mean for aflatoxin contamination.

3. Results

3.1. Demographic characteristics and awareness on aflatoxin contamination

The effects of different demographic characteristics such as gender, age, and education level on awareness of aflatoxin contamination to crops are presented in Table 1.

3.2. Aflatoxins contamination of sesame seeds

For years, several studies have reported aflatoxin contamination of sesame seeds around the world, particularly in Africa however, this is the first study to document aflatoxin contamination in Tanzania. The mean concentration and the occurrence of aflatoxin in sesame seeds are presented in Table 2.

Table 1. Demographic characteristics of the Sesame farmers from both Lindi and Mtwara regions (n=454)

Demographic category		**Masasi** (n=135)	**Nachingwea** (n=113)	**Ruangwa** (n=112)	**Kilwa** (n=94)	**Total** (n=454)
Gender	Male	85(62.9)	72(63.7%)	75(67%)	61(64.9%)	293(64.5%)
	Female	50(37.1%)	41(36.3%)	37(33%)	33(35.1%)	161(35.5%)
Age (years)	16-30	22(16.3%)	34(30.1%)	21(18.75%)	20(21.3%)	97(21.4%)
	31-45	69(51.1%)	50(44.2%)	45(40.17%)	45(47.9%)	209(46.0%)
	46-60	36(26.7%)	23(20.4%)	28(25%)	21(22.3%)	108(23.8%)
	>60	8(5.9%)	6(5.3%)	18(16.07%)	8(8.5%)	40(8.8%)
Education level	No formal education	6(4.4%)	17(15.0%)	17(15.17%)	2(2.1%)	42(9.2%)
	Standard 1-7	116(85.9%)	85(75.2%)	85(75.89%)	87(92.6%)	373(82.2%)
	Form 1-6	10(7.4%)	8(7.1%)	10(8.92%)	4(4.2%)	32(7.0%)
	College	3(2.2%)	3(2.6%)	0(0.0%)	1(1.0%)	7(1.5%)
Awareness status	Yes	42(31.11)	16(14.16%)	16(14.29%)	6(6.39%)	80(17.62%)
	No	93(68.89%)	97(85.84%)	96(85.71%)	88(93.61%)	374(82.38%)

Table 2. Occurrence of aflatoxins in sesame seeds (n=70)

Aflatoxins	Positive samples	Contamination range (ng/g)	Mean (ng/g)	Mean (ng/g) ± SD per region		> 5 ng/g for B1 and 10 ng/g for total AF	> 2 ng/g for B1 and 4 ng/g for total AF
				Lindi	Mtwara		
AFB1	13	0.011 - 0.779	0.041 ± 0.184	0.035 ± 0.134	0.047 ± 0.223	-	-
AFB2	15	0.076 - 0.744	0.049 ± 0.181	0.042 ±0.150	0.157 ± 0.927	-	-
AFG1	16	0.002 - 1.294	0.083 ± 0.347	0.043 ± 0.20	0.123 ± 0.446	-	-
AFG2	21	0.108 - 4.964	0.276 ± 0.849	0.257 ± 0.656	0.295 ± 1.011	-	-
Total AF	37	0.009 - 5.557	0.451 ± 1.014	0.379 ± 0.735	0.620 ± 1.529	-	2

Tanzania Maximum Limits; 5 ng/g for AFB1 and 10 ng/g for total aflatoxins, EU Maximum Limits; 2ng/g for AFB1 and 4 ng/g for total aflatoxins

4. Discussion

4.1. Demographic characteristics and awareness of aflatoxin contamination

Most respondents in this study were males, this may be contributed to the ongoing cropping season where most of the women were at the farms.

The phenomenon is common in African countries and especially in Tanzania whereby women are usually working on the farms to feed their families. About 54% of the labor force in the agricultural sector in Tanzania is made up of women (37). Ogunlela and Mukhtar (38) reported that women contribute up to 87% of the labor force used for growing foods consumed in Tanzania's households. On the other hand, Jolly and colleagues (39) indicated lower participation of women in the awareness of mycotoxins compared to men. There was a significant positive relationship between awareness of aflatoxin contamination and the gender of the respondents, whereas men were found to be more informed than women (Table 1). Similarly, Lee (40) found that men were more aware of aflatoxin contamination than women. On contrary, women were found to be more informative than men (39).

The literacy rate of the farmers was observed to be moderately low. According to Tanzania's census of 2012, Mtwara and Lindi regions are among the regions with an illiteracy rate of 34% and 37% respectively (41). Moreover, this study was conducted in the rural parts of the regions where most of their residents are moderately educated. A correlation test was performed to find if there was any relationship between education level and awareness of aflatoxin contamination. From the results, education was shown to be negatively correlated with the knowledge of aflatoxin contamination (correlation coefficient of -0.309**). Since a majority of the participants were local farmers who have not attained higher levels of formal education, the knowledge of aflatoxin contamination was shown to decrease with the increase in education level. Previous studies have also shown that awareness of aflatoxin contamination tends to increase with an increase in education level (31,39,42), which is in contrast with the current study. Likewise, education level has been observed to positively affect awareness level on aflatoxin, knowledge, and management strategies in combating aflatoxin contamination (39).

Most of the participants in this study were young people. Nonetheless, participants in this age group were also found to be more informed about aflatoxin contamination than the rest of the age groups. Dosman (42) showed that people at the age of 35 and above are highly informed about mold infestation and its effects.

The results on the awareness of aflatoxin contamination of agricultural produce including sesame seeds are also shown in Table 1. Although mycotoxins contamination and its effects are not the subjects of concern, especially in Tanzania's formal education system, illiteracy may suggest the observed results. Several studies conducted in Tanzania and other parts of the world revealed that education plays a greater role in creating awareness and reducing aflatoxins contamination of crops (29,39).

4.2. Aflatoxins contamination of sesame seeds

More than half of the tested samples were found to be contaminated with total aflatoxin of which 48.7% were from Mtwara and 51.3% from Lindi region. Among the contaminated samples, only 5.4% were found to have a concentration higher than 4 ng/g and 10ng/g which is above the maximum limit for the total aflatoxin set by EU and EAC respectively. These findings are in line with Hosseininia (43) whereby 50% of 269 analyzed sesame seeds were contaminated by aflatoxin although the concentrations were at low levels. Similarly, more than half of the sesame seeds analyzed in the study conducted by Kollia were found to be contaminated (44).

On the other hand, AFB1 was observed in the contaminated sesame seeds samples with the concentration ranging as illustrated in Table 2. From the findings, the occurrence of AFG2 and AFG1 was higher compared to AFB2 and AFB1. The levels of AFB1 in sesame seeds were low as none among them has exceeded the EU maximum limit of 2 ng/g for food and feeds (2). Of all aflatoxins that affect humans and animals, AFB1 is considered to be the most potent and it is of public concern in Tanzania and the world in general (20). It has been found to be the most produced aflatoxin by the aflatoxin-producing strains (44). AFB1 in sesame seeds has been reported in several studies conducted in different countries; 30% of sesame seeds were found to be contaminated by aflatoxins in the study which involved several food commodities (14), it was also detected in 18.1% of the sesame seeds in the study conducted in Iran (45).

Findings from this study are in contrast with several studies that analyzed aflatoxin concentration levels in sesame seeds. Sesame seeds were shown to be the least susceptible to A. flavus compared to maize in Senegal (17). Furthermore, none were contaminated by aflatoxin from Platue State Nigeria (46). Thus, these findings are essential to the sesame seeds domestic and export market.

Some limitations were encountered while undertaking this study. The study was conducted in rural areas where there were challenges of transport from one area to another. It was also difficult to find some village leaders in some villages as they were going to the farms or on their other activities. Some farmers did not agree to participate in the study. In some areas, it was also difficult to get sesame samples from farmers.

5. Conclusion

Lack of awareness on aflatoxins contamination of sesame seeds was observed, with aflatoxins contamination levels below the Tanzania and EU limits for both total aflatoxins and AFB1. Moreover, this level should not be underscored due to the exposure risks of the aflatoxins and through consumption of sesame seeds as well as its potential as a cash crop.

Conflict of interest

Authors declare to have no conflict of interest.

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