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Smallholder farmers’ awareness and aflatoxins contamination of sesame seeds grown in Southern Tanzania

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SMALLHOLDER FARMERS' AWARENESS AND AFLATOXINS CONTAMINATION OF SESAME SEEDS GROWN IN SOUTHERN TANZANIA

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A Dissertation Submitted in Partial Fulfillment of the Requirement for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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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 in Lindi and Mtwara regions to assess awareness and aflatoxins contamination of sesame seeds. Subsequently, a total of 70 sesame seed samples were randomly purchased from the local markets for assessing aflatoxin levels using HighPerformance Liquid Chromatography (HPLC). Qualitative data were analyzed using SPSS version 20 for descriptive and correlation analysis. Results show that the majority of the respondents (82.4%) 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 seeds 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 East Africa's (EAS) maximumlimit of 10 ng/gfor total aflatoxins. Furthermore, Aflatoxin AFB1 was detected in 13 samples, moreover, the concentration was below the EAS maximum limit of 5 ng/g. Though the contamination was below the 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.

DECLARATION

I, Catherine Gidabedi do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

03/07/2023

Catherine M. Gidabedi — Date

The above declaration is confirmed by:

28/07/2023

Prof. Athanasia Matemu — Date

28/08/23

Dr. Neema Kassim — Date

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology the dissertation entitled "*Smallholder Farmers' Awareness and Aflatoxins Contamination of Sesame Seeds Grown in Southern Tanzania*" in partial fulfillment of the requirements for the award of a Master's Degree in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Prof. Athanasia Matemu — Date

Dr. Neema Kassim — 28/08/23 — Date

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
AFB1	Aflatoxin Blue 1
AFB2	Aflatoxin Blue 2
AFG1	Aflatoxin Green 1
AFG2	Aflatoxin Green 2
ANOVA	Analysis of Variance
EAS	East African Standards
EU	European Union
G	Gram
HPLC	High Performance Liquid Chromatography
Kg	Kilogram
LOD	Limit of Detection
LOQ	limit of Quantification
ng/g	Nanogram/Gram
P	Probability Value in Statistics
PBS	Buffer Solution
SPSS	Statistical Package for Social Sciences
TFA	Trifluoroacetic Acid

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Sesame (*Sesamum indicum* L.) is a widely cultivated crop in Africa and Asia due to its ability to yield high-quality oil with high protein contents (Kadkhodaie *et al.*, 2014). Sesame oil is famously known as the “queen of oils” due to its tenderness, pleasant taste, and high protein content (Pusadkar *et al.,* 2016). Nutritionally sesame seeds contain; oil (45.6 to 46.1%), protein (21.9 to 23.6%), carbohydrate (10.8 to 17.0%), moisture (4.18 to 5.41%), ash (6.16 to 7.34%) and fiber (4.7 to 7.15%) (Makinde & Akinoso, 2013). They are also known to be rich in calcium, copper, zinc, phosphorous, magnesium manganese, and vitamin AFB1 (Deme *et al.,* 2017). Functional fatty acids such as oleic, linoleic, palmitic, and stearic are also found in sesame seeds with a trace amount of linolenic acids (Gharby *et al.,* 2017). Due to itsbenefit to food and nutrition security, sesame is an economically important crop.

Sesame seeds have plenty of uses; as an ingredient in many foods such as bread, cookies, chocolates, icecream, hamburger bans, spices, and confectionaries such as candy. Moreover, in Arab countries, sesame is dried to make tahini which is a very good source of energy, or milled to make sesame flour which is a good source of protein and calcium; sesame flour has a three times higher amount of calcium than milk (Morris, 2002).

However, like cereals and other oily seeds, sesame seeds are prone to mycotoxins contamination, especially aflatoxin (Wu *et al.,* 2011). Aflatoxins are the secondary metabolites produced by fungi particularly *Aspergillus flavus and A parasiticus.* These fungiare opportunistic pathogens with a cosmopolitan distribution andthe ability to attack crops before and after harvest (Hell *et al.,* 2008). Among the factors that favor *fungal* attack include stress or crop damage by insects, poor harvesting timing, or wet condition caused by heavy rainfall after harvest (Lizárraga-Paulín *et al.,* 2011). Aflatoxins have been associated with different health problems both in animals and humans. These include immune suppression, diseases of the digestive system, interference of the nervous system particularly the central nervous system, impaired fertility, and stunted growth (Lizárraga-Paulín *et al.,* 2011; Wu *et al.,* 2011; Yentür *et al.,* 2006). In addition, aflatoxins contamination has led to unwelcoming consequences in trade globally (Wu, 2015). Many countries have set aflatoxin concentration limits in the crops that are consumed by animals and people (WHO, 2018). The crops that have higher concentration than the permissible limit are rejected. Presence of untorelatable concentration of aflatoxin in crops have led into rejection of crops in international markets and eventually loss of income (PACA, 2015). The rejection can occur in the border or during distribution. Sometimes, the crops may be assigned to a reduced price or subjected to a non-human use. Similary, the same loses may occur in domestic markets where consumers are aware of aflatoxin contamination (PACA, 2015).

Despite these adverse effects caused by aflatoxin contamination of cereal and oily seeds, there is little information on the incidence of aflatoxins on sesame. Several studies on mycotoxins contamination of crops have focused mainly on cereals such as maize, sorghum, millet and their products (Magembe *et al.,* 2016; Sasamalo *et al.,* 2018) groundnuts and their products (Kuhumba *et al.,* 2018), and composite flour for complementary feeding (Ayo *et al.,* 2018). Little is known about aflatoxins contamination of sesame in Tanzania. Even though relatively researched, studies have reported low awareness of aflatoxins contamination of these crops and their products (Ayo *et al.,* 2018; Ezekiel *et al.,* 2013). Therefore, this study aimed at assessing awareness of aflatoxin contamination of sesame seeds among smallholder farmers, and quantifying aflatoxins in sesame seeds grown in Southern Tanzania.

1.2 Statement of the Problem

According to WHO (2018), aflatoxin contamination to crops threaten million lives of people world wide and mostly in African countries. This is due to its impacts in human and animal health as well as in trade. The dosage of aflatoxin consumed can lead into acute or chronic effects to an individual. The WHO reported that about 25% of the crops worldwide are destroyed by aflatoxins causing a significant economic burden which is much bigger in African countries than developed countries (WHO, 2018). Lack of awareness on aflatoxin contamination has been mentioned by different studies as one of the causes that accelerate aflatoxin contamination and ultimately causes mismanagement. Over 70% of the farmers who participated in the study conducted by Ayo *et al.* (2018) in Meru district, Arusha region had never heard of aflatoxin. About 97% of respondents in the study conducted by Magembe *et al.* (2016) were unaware of mycotoxins that affect crops. The same observation was found in the study carried out by Experience (2014). Although groundnuts and cereals including maize, sorghum and millet are relatively studied, there are limited or no studies of aflatoxins contamination of sesame. Tanzania is currently among the leading producers of sesame worldwide, however, there are limited of studies which assess awareness of sesame farmers on aflatoxin as well as detecting and quantifying aflatoxin in sesame grown in Southern Tanzania. Therefore, this study aimed at assessing awareness of aflatoxin contamination to sesame seed farmers as well as detecting and quantifying aflatoxin concentration in sesame seeds grown in southern Tanzania.

1.3 Rationale of the Study

Aflatoxin contaminates wider range of crops including cereals and oily seeds like sesame, causing not only public health but also trade barriers. According to WHO (2018), aflatoxin contamination to crops threaten million lives of people world wide and mostly in African countries. This is due to its impacts in human and animal health as well as in trade. The dosage of aflatoxin consumed can lead into acute or chronic effects to an individual. Acute exposure leads into nausea, vomiting, and abdominal pain while chronic exposure can lead into hepatoxicity, immunotoxicity and teratogenicity and even death (Dhakal & Sbar, 2020; Kamala *et al.,* 2018; Nabwire *et al.,* 2020). Aflatoxin consumption is considered to be one of the main cause of hepatocellular carcinoma especially in African countries (Dhakal & Sbar, 2020). Moreover, it is well linked with stunting of children and suppression of immune system (Mmongoyo *et al.,* 2017). In business perspective, presence of untorelatable concentration of aflatoxin in crops have led into rejection of crops in international markets and finally loss of huge amount of foreign exchange (Umar *et al.,* 2015). The WHO reported that about 25% of the crops worldwide are destroyed by aflatoxins causing a significant economic burden which is much bigger in African countries than developed countries (WHO, 2018). Sesame is one of the oil seed crops that ranks the third in yielding oil (Pusadkar *et al.,* 2015). Apart from non-culinary application such as ingredient of soap, detergents, cosmetics and medicines, sesame has two important components known as sesamin and sesamolin. The two are important in human health as they have cholesterol lowering effects thus preventing high blood pressure (Anilakumar *et al.,* 2010), act as an antioxidant (Dar *et al.,* 2015) and they control inflammatory responses (Jeng *et al.,* 2005). Also, Tanzania export about 35% of sesame that are grown in Lindi and Mtwara in the international market (Makinde & Akinoso, 2013). Yet, sesame seeds are prone to fungal infestation and mycotoxin contamination. Thus, knowing the aflatoxin contamination status is vital for the trade since contaminated crops are rejected in international market.

1.4 Resaerch Objectives

1.4.1 General Objective

The main objective of this study is to assess awareness of aflatoxin contamination of crops, detect and quantify aflatoxin in sesame seeds sold in the local markets in Mtwara and Lindi regions.

1.4.2 Specific Objectives

(i) To assess awareness of aflatoxin and management strategies among small-holderfarmers in Mtwara and Lindi regions.

(ii) To detect and quantify aflatoxins in sesame seeds sold in the local markets in Mtwara and Lindi regions.

1.5 Research Questions

(i) Are small-holder farmers in Mtwara and Lindi regions aware of aflatoxin contamination and their management stratergies?

(ii) What are the quantities of aflatoxin concentration in sesame seeds sold in the local markets in Lindi and Mtwara regions?

1.6 Significance of the Study

This study documents levels of awareness of aflatoxins contamination of crops among sesame farmers' from the Lindi and Mtwara regions. It provides baseline dataon the situation of aflatoxins contamination in sesame seeds from local markets in the Lindi and Mtwara regions. This information serves as literature and may be useful to sesame stakeholders including government and non-government agencies who are involved in the production, consumption, and trading of sesame, which might ultimately promote expanding the market. The findings of this study also will give insights on the levels of aflatoxins in the sesame seeds which has implications in monitoring the aflatoxin levels and setting maximum acceptable limits by the respective agencies such as Tanzania Bureau of Standard (TBS).

1.7 Delineation of the Study

This study aimed at assessing awareness of aflatoxin contamination of sesame seeds among smallholder farmers, and quantifying aflatoxins in sesame seeds grown in Southern Tanzania. It provides baseline dataon the situation of aflatoxins contamination in sesame seeds from local markets in the Lindi and Mtwara regions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Sesame Seeds Production in Tanzania

Tanzania, is currently the world's best producer of sesame, yielding about 940 221 metric tons per year (FAOSTAT, 2016). Sesame is best grown in the Southern part of Tanzania; Lindi and Mtwara accounts for about 35% of all sesame seeds exported from Tanzania (Mashindano & Kihenzile, 2013). These two regions export about 400 000 tonnes to the world market annually (Mashindano & Kihenzile, 2013). Other regions that cultivate sesame seeds in Tanzania include Dodoma, Singida, and Tabora. In 2019, Tanzania earned an average of 189.94 million USD by exporting sesame seeds to the world market. Among the countries that buy sesame seeds from Tanzania include China, Japan, South Korea, Turkey, and India (https:// www. selinawamucii. com/ insights/ market/tanzania/sesame-seeds/).

2.2 Nutritional Benefits of Sesame Seeds

Sesame (*Sesamum indicum* L) is an oilseed crop belonging to the family Pedaliaceae and order Tubiflorae of the Kingdom Plantae. The genus *Sesamum* has a total of 37 species; however, *Sesamum indicum* is the only dominant cultivated specie (Pusadkar *et al.,* 2016). It is believed that the crop originated from Africa since most of the *Sesamum* species are found in Africa and taken to India at very early times of its occurrence, hence regardedit originated from India (Alegbejo *et al.,* 2003). Sesame seeds are also known as benniseed, gingelly, til, simsim, sesame, and ajonjoli in different cultures around the world. It occupies the sixth position among oilseed crops produced in the world with high stability in drought, yielding quality oil, and ease of extraction (Mohammed, *et al.,* 2018).

Sesame is famously called the "Queen of Oilseed", simply because it produces high quality oil with antioxidants sesamolin and sesamin which can resist oxidation and rancidity (Bhat *et al.,* 2014). The presence of sesamin and sesamolin, in sesame oil has medicinal properties hence the ability to prevent cancers, tumors, and protecting the heart and liver (Bhat *et al.,* 2014; Harikumar *et al.,* 2010).

The sesame plant is very beneficial as a whole; its oil is used for cooking, making pharmaceuticals, insecticides, paints, and soap (Gharby *et al*., 2017; Bhat *et al*., 2014). The leftover after pressing oil is a feed with a good source of protein for poultry, the young leaves are consumed as vegetable soup in West Africa (Mohammed *et al*., 2018).

2.3 Aflatoxin Production

Aflatoxins are the toxic secondary metabolites that are produced by different species of fungi but the main two species are of economic importance; *Aspergillus flavus* and *Aspergillus parasiticus* (Narayan *et al*., 2015). During *Aspergillus flavus* normal metabolism, it releases metabolites to the host crop, that ismycotoxins which are toxic to humans, other animals, and plants (Wu *et al*., 2011). Different mycotoxins are released by *Aspergillus flavus* such as aflatoxins, citrinin, patulin, penicillic acid, tenuazonic acid, ochratoxin A, cytochalasins, deoxynivalenol, fumonisins, fusarin C, fusaric acid, and zearalenone (Ismaiel & Papenbrock, 2015). More than 16 types of aflatoxins exist in the world but AFB1, AFB2, AFG1, and AFG2 are the most common aflatoxins and the most dangerous (Okoth, 2016; WHO, 2018). However, AFB1 is the most potent carcinogen that accounts for a large number of liver cancer cases in the world (Kollia *et al.*, 2016a). The AFB1 and AFB2 are converted to aflatoxin M1 and M2, respectively by animals when they consume aflatoxin-contaminated crops (Obesity, 2015; Obesity, 2015; Liu & Wu, 2010) these forms are found in milk. *Aspergillus spp* can colonize more than 40 crops including sesame (John *et al.*, 2015). *Aspergillus spp* attacks crops under favorable conditions such as high temperature (between 26°C and 38°C and high humidity of about 85% (Narayan *et al.*, 2015; Negash, 2018). These species are soil-borne, so they can infect crops even before the harvesting activities however post-harvest activities such as drying, winnowing, bagging, and storing may favor the attack of these fungi on crops. The mycotoxins produced by fungi affect human beings when consuming contaminated crops (Shephard, 2003). Moreover, animals can be affected when given feeds that have already been contaminated by fungi (John *et al.*, 2015).

2.4 Health Effects of Aflatoxin

Aflatoxicosis may result in different health effects depending on the dosage of aflatoxin consumed; acute aflatoxicosis results from consuming high dosage of aflatoxin and this usually leads to liver toxicity (Lizárraga-Paulín *et al*., 2011) while chronic aflatoxicosis which develops as one consumes small to moderate amount of aflatoxin for a long period and it usually leads into liver cancer, immunosuppression and stunted to children (Okoth, 2016; WHO, 2018; Strosnider *et al.*, 2006; Wu *et al.*, 2011). Chronic aflatoxicosis is mostly occurring in developing countries where people consume aflatoxin-contaminated foods unknowingly from home-grown foods and feed for a long period. The toxins accumulate in the body slowly resulting in chronic aflatoxicosis.

2.5 Aflatoxin Contamination in Sesame

Aflatoxin contamination has raised major global concerns about food safety and security. The report produced by WHO (2018), stated that aflatoxin contamination threatensthe lives of millions of people worldwide. Different studies showed that its exposure to human and animals occur mainly through maize and groundnuts consumptions (Mmongoyo *et al*., 2017). However, it has been noted that aflatoxin contamination can also occur in sesame seeds. The study carried out by Apen *et al*. (2016) showed that the aflatoxin in sesame was high compared to sorghum and millet. Also, a study by Idris *et al*. (2010) in Sudanese edible oils detected a high and leading incidence of aflatoxin contamination in sesame. Aflatoxin contamination in sesame seeds was also observed in Greece where thirty samples of sesame varieties were examined of which 77.6% of all samples were found to be contaminated (Kollia *et al.*, 2016a). Further, a study that aimed at creating awareness among stakeholders on the dangers of aflatoxin in sesame seeds was carried out by Umar *et al*. (2015), and it seemed that aflatoxin contamination was thought to destroy the sesame industry in Nigeria. Therefore, a driving force of this study is the fact that there is insufficient data on the presence of mycotoxins especially aflatoxins in sesame seeds that are grown in Southern Tanzania.

2.6 Social and Economic Impacts of Aflatoxins Contamination in Sesame Seeds

From a business perspective, aflatoxin contamination affects the volume of the crops and hence the price. Tanzania exports sesame seeds to the international market, although aflatoxin contamination of sesame seeds leads toa fall in-demand, rejection by the international market hence market loss (Narayan *et al*., 2015; Umar *et al.*, 2015). Aflatoxin contamination in sesame seeds has an impact on the domestic and international market. According to IITA (2013) aflatoxin contamination leads to a reduction in the sesame seeds market which can result in revenue loss by domestic producers.

2.7 Awareness of Farmers on Aflatoxin and its Management

Bringing awareness to the community about aflatoxin contamination and its effects is the solution that is proposed by some researchers. Creating awareness among farmers has been mentioned by WHO (2018) in the aflatoxin report as the way to reduce contamination to prevent health effects caused by aflatoxin contamination. According to this report, creating personal awareness of aflatoxin contamination and its prevention can be done by responsible national authorities. The same recommendation has been put forward by Negash (2018) so as help to reduce or eliminate aflatoxin contamination in crops.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of Study Sites

This study was conducted in Mtwara and Lindi regions inTanzania. These regions have been selected because they are the main producers of sesame seeds in Tanzania (Mashindano & Kihenzile, 2013). Along with sesame seeds, other main food and cash crops grown in the regions include cassava, millet, sorghum, groundnuts, and cashew nuts. Mtwara is the southernmost region in Tanzania lying between longitudes 380 and 400 30" E and latitudes 100 05" and 110 25" S. It borders the Lindi region to the north, the Indian Ocean to the East, and is separated from Mozambique by the Ruvuma River in the South. To the West, it borders the Ruvuma region. On the other hand, Lindi is found at South-Eastern part of Tanzania at latitude 9°23′31.20″ South, longitude 38°23′38.40″ East. The region borders the Morogoro region in the West, the Ruvuma region in the North, Mtwara in the South, and the Indian Ocean in the East. Figure 1 shows a map of Tanzania with the main sesame producing regions; and districts where sesame seed samples were collected.

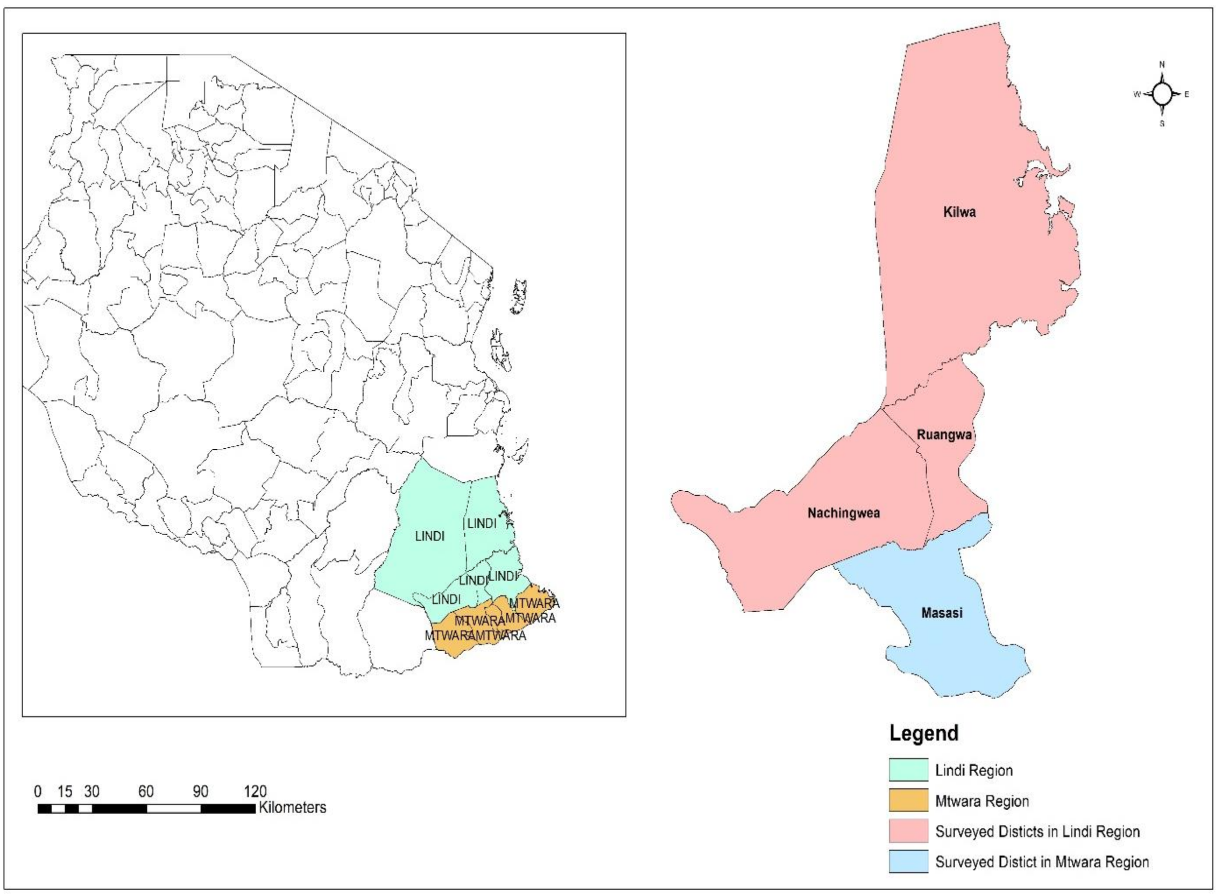

Figure 1: A map showing the main sesame producing study districts in Mtwara and Lindi regions

3.2 Study Design

The study employed a cross-sectional design in which a semi-structured questionnaire was used for data collection. The purposive sampling method was used to select districts, wards, and villages considering the highest number of sesame seeds farmers. The sample size was determined using the binomial formula established by Cochran (1977) for cross sectional study design;

$$n = \frac{z^2 p(1-p)}{d^2}$$

where n= minimum sample size (for population >10000)

z = level of significance, 1.96 at 95% confidence level

p = estimated prevelance of aflstoxin contamination to sesame seeds which is 50%

d = degree of precision required (usually as aproportion, 0.05 at 5%)

For this study, a total of 454 participants were interviewed and the data were recorded where by a minimum number of 384 participants were needed to make the sample.

Therefore, four districts namely Masasi (Mtwara) Nachingwea, Kilwa, and Ruangwa (Lindi) were selected (Fig. 1). Subsequently, two wards were selected in each district except in Kilwa where only one ward cultivates sesame seeds. For all 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 384 farmers and 70 sesame seeds traders who were interviewed.

3.3 Assessment of Farmers' Awareness of Aflatoxins Contamination of Crops and its Management Strategies

Assessment of farmers' awareness of aflatoxin and their management strategies were carried out using a structured questionnaire (Appendix 1). The questionnaire was adapted from Ezekiel *et al.* (2013) with minor modifications. This questionnaire was prepared in English language and later translated to the Swahili language prior to the interview.

3.4 Sample Collection and Analysis of Aflatoxins

3.4.1 Purchase of Sesame Seed Samples

A total of 70 sesame seed samples of ¼ kg each were purchased from traders and farmers in four districts of Lindi and Mtwara regions; Masasi, Nachingwea, Ruangwa and Kilwa. The samples were collected around May to June 2019 and most of the samples stayed for about 6 to 8 months from the time of harvest. The samples were packed in paper bags, labeled and transported to the Nelson Mandela African Institution of Science and Technology laboratory, stored at 4°C for 1-2 weeks beforeaflatoxinanalysis.

3.4.2 Validation of the High Performance Liquid Chromatography

(i) Method

Limit of detection (LOD) and limit of quantification (LOQ) were determined as per Heshmati *et al.* (2017) procedures. The stock solution of the mixed aflatoxin standards (AFAFB1, AFAFB2, and AFAFG1and AFAFG2) was diluted with acetonitrile to obtain 40 ng/mL. From this intermediate solution, a serial dilution was performed to obtain working standards of 1 ng/mL, 2 ng/mL, 5 ng/mL, 10 ng/mL, and 15 ng/mL. The standard solution was run through the HPLC machine made from Shimadzu, Japan and the calibration curve was obtained. Ten blank samples were spiked with different concentrations of AFs to assess recovery, the limit of detection (LOD), and the limit of quantification (LOQ). The results are presented in the results section.

(ii) Determination of Aflatoxin Concentrationin Sesame Seeds

The sesame seed were sorted to remove non-seed materials such as sands and plant stalks. Then the seeds were blended using a Kenwood blender (manufactured by Kenwood Limited, United Kingdom) to make sesame flour. Aflatoxin determination was carried out as described by Tahavori *et al.* (2012) with minor modifications. The process includes four stages; extraction, dilution, clean up, elution, and HPLC analysis using fluorescence detection.

Sesame seeds flour sample was weighed to obtain 5 g using OHAUS Explorer ® Version 2.01 weigh balance. The weighed sample was put into a 50 mL falcon tube and 20 mL of extraction solution (60:40 Methanol: Water) and 20 mL of hexane (Loba Chemie, India) were added. Methanol was purchased from Fischer Scientific, UK while HPLC water was bought from LOBA Chemie, India. The mixture was then well mixed using vortex (Fischer Scientific, UK) for 3 minutes to allow mixing of the sample and the extraction solution. The mixture was allowed to settle for the layers to separate and afterward, the hexane layer containing fat was discarded. The extract was then filtered using a Whatman filter paper No. 1 (125 mm) manufactured by Sigma-Aldrich Company China.

Then 4 mL of the filtrate was diluted with the 8 mL phosphate buffer solution (PBS). The immunoaffinity column (Aflatest Vicam, USA) was placed into the vacuum manifold (Welch, USA) for cleanup of the extract. The diluted extract was allowed to flow through the immunoaffinity column appended to the syringe barrel, and fitted on a vacuum manifold. The flow rate was set not to exceed 3 mL/min. Aflatoxin was bound on the column surfaces while debriswas allowed to pass through. The column was rinsed twice with 10 mL of HPLC grade water to ensure maximum trapping of the aflatoxin.

Vials were placed under the column for the collection of the eluent. The bounded aflatoxins were eluted using 1 mL of Acetonitrile HPLC grade (LOBA Chemie, India) by passing it through the column for 3 secondsand at a gradient flow to allow intensive contact with the surface. Thereafter, 400 µL of the eluent was taken and mixed with 600 µL of derivatizing reagent (70:20:10 H_2O: Trifluoroacetic acid (TFA): Acetic acid). All the chemicals used to make derivatizing reagents were from LOBA Chemie, India. The mixture was put in an oven (Memmert brand, Germany) conditioned at 65°C for 15 min to support derivatization, then allowed to cool before being injected into the HPLC.

The HPLC analysis procedures were adopted from (Anthony *et al.*, 2014; Anthony *et al.*, 2014) with minor modifications. For detection and quantification of aflatoxin, a total of 1 mL of the mixture was placed into the HPLC connected to a fluorescence detector (RF-20A) all made by SHIMADZU, Japan. The stationary phase was Luna C18, 5 µm, 250*4.6 mm (USA) set at 40°C. The mobile phase consisted of 60:30:10 (Water: Methanol: Acetonitrile), at the flow rate of 1mL/min. The injection volume was 20 µL and the running time was 20 min. The fluorescence detector's emission and excitation were set at 450 nm and 365 nm, respectively.

3.5 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 an association between the demographic characteristics and awareness and aflatoxin contamination of sesame seeds. The 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 (15th Edition) statistical software for computing the grand mean aflatoxin contamination.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Participants' (Farmers and Traders) Demographic Characteristics

The survey was conducted in two regions of Lindi and Mtwara where a total of 454 participants were interviewed. It was found that more men (64.5%) participated than women (35.5%). This was contributed by the fact that; the survey was done during the cropping season when most of the women were at the farms. The phenomenon is common in African countries, especially in Tanzania where farming activities are dominated by women. The study carried out by Kanyeka *et al.* (2012) showed that about 54% of the labor force in the agricultural sector in Tanzania is made up of women. Similarly, Ogunlela and Mukhtar (2009) reported that women contribute up to 87% of the labor force for household food production in Tanzania. Therefore, the interview of this study was conducted to men and women farmers who were not at the farms as well as the sesame seeds traders whom mostly were men.

Of the four districts that were visited, Masasi had a greater number of participants (135) followed by Nachingwea (113), Ruangwa (112), and Kilwa (94). Masasi is the leading district that cultivates sesame seeds in the Mtwara region while in the Lindi region, almost all districts cultivate sesame seeds.

For the age group, there were more participants between the age of 31-45 (46%) followed by 46-60 (23.8%), then 16-30 (21.4%), and lastly elders aged 61 and above (8.8%). This is so because the age group of 31 to 50 is considered mature, energetic, and owns production land. Though they own land and are somewhat active, the age group between 50 to 60 are reducing their farm activities as theytransfersuchenergy-consuming activities to their children between the age of 16 to 30.

In this study, about 82.2% of the participants have attended only primary education (standard 1 to 7) according to the education system of Tanzania and most of them claimed not to complete standard 7. It was noted that only 7% of the participants attended secondary education (form 1 to 6) and some of them did not complete form 6. Only 1.5% of the participants had attained colleges and other tertiary education levels of which most of them were village leaders and agricultural extension officers in the respective villages. Among the participants, 9.2% had never attended formal education and they don't know how to read and write. According to Tanzania's census of 2012, Mtwara and Lindi regions are among the regions with a high illiteracy rate of 34% and 37%, respectively (Census, 2012). Moreover, this study was conducted in the rural parts of the regions where most of their residents are moderately educated.

4.2 Awareness Status of Aflatoxin Contamination and Management Strategies

Demographic characteristics of the participants are shown in Table1. It was found out that, the majority of the participants (82.38%) were not aware of aflatoxins contamination in sesame seeds, and had never heard about it. Very few participants (17.62%) were aware of aflatoxin contamination of crops and sesame seeds in particular. Those aware of aflatoxin contamination mentioned some sources from which the information circulated. Among the sources were village meetings (19.7%), agricultural seminars (25.4%), expert talks (50.7%), and 4.2% with no specific sources. Generally, the awareness of aflatoxin contamination of crops was very low among the participants of this study. In this study, a significant positive relationship between awareness of aflatoxin contamination and the gender of the respondents was observed ($p= 0.03$), where men were found to be more informed than women. The study conducted by Lee *et al.* (2017) and Lee *et al.* (2017) found that, men were more aware of aflatoxin contamination than women. In contrast, Jolly *et al.* (2009) and Jolly *et al.* (2009) showed that women were less aware of mycotoxins compared to men,further supplement the current study's findings.

The majority (63.8%) with awarenesson the aflatoxin contamination were also able to identify some of the health effectscaused by aflatoxin contamination such as liver cancer and stunting in childrenwhile the rest (36.2%) were unable. About 58.8% of the respondents who were familiar with aflatoxin declared to have experienced it through the fungal infestation to their crops and applied some preventive measuressuch as extensive drying and keeping crops in dry places after harvesting to avoid moisture. Preventive measures taken by farmers are encouraged, however, are insufficient considering the fact that aflatoxin contamination is the result of fungal infestation to crops. Crops are usually dried outside on-air, and may contact soil and moisture that encourage fungal growth and finally mycotoxin. The changes that occur when fungi attack the crops include, changes in color to the greenish and bitter taste of the seeds when consumed were also mentioned. Fungi can attack crops at any stage of the chain, however, many farmers experience it after harvesting. This further suggests poor storage practices of the seeds after harvesting.

Some farmers claimed not to experience fungal infestation to sesame seeds because of immediately selling sesame seeds to agricultural and marketing cooperatives societies responsible for marketing right after harvesting. The small portion is left for household use, which usually doesn’t exceed 5 kg is usually kept as seed for the next cropping season. Other farmers reported not recycling seeds but purchase from the agricultural research institutes such as TARI-Nalienedele.

A correlation test was performed to find if there was any relationship between education level and awareness of aflatoxin contamination. From the results, education level was shown to be negatively correlated with the knowledge of aflatoxin contamination (correlation coefficient of -0.309). Since the majority of the participants were local farmers 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 shown that awareness of aflatoxin contamination tends to increase with an increase in education level (Dosman *et al.*, 2001; Ezekiel *et al.*, 2013; Jolly *et al.*, 2009), which is contrary from the current study. The findings of this study may be contributed to the fact that;the majority of the participants are just mere uneducated farmers obtaining knowledge of aflatoxin contamination by not attending formal classes but rather from the field itself.

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%)
	>61	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 to 7	116 (85.9%)	85 (75.2%)	85 (75.89%)	87 (92.6%)	373 (82.2%)
	Form 1 to 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 to aflatoxins	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)

4.3 Aflatoxins Contamination of Sesame Seeds

4.3.1 Method Validation

The calibration curve (Fig. 2) was obtained from the standard concentrations of 10 ng/g, 5 ng/g, 2 ng/g, 1 ng/g, and 0.5 ng/g. The calibration curve showed to have good linearity with a coefficient of determination (R^2) of 0.999 at a retention time of 6.63 min, 8.78 min, 11.37 min, and 16.26 min for aflatoxins AFG2, AFG1, AFB2, and AFB1, respectively.

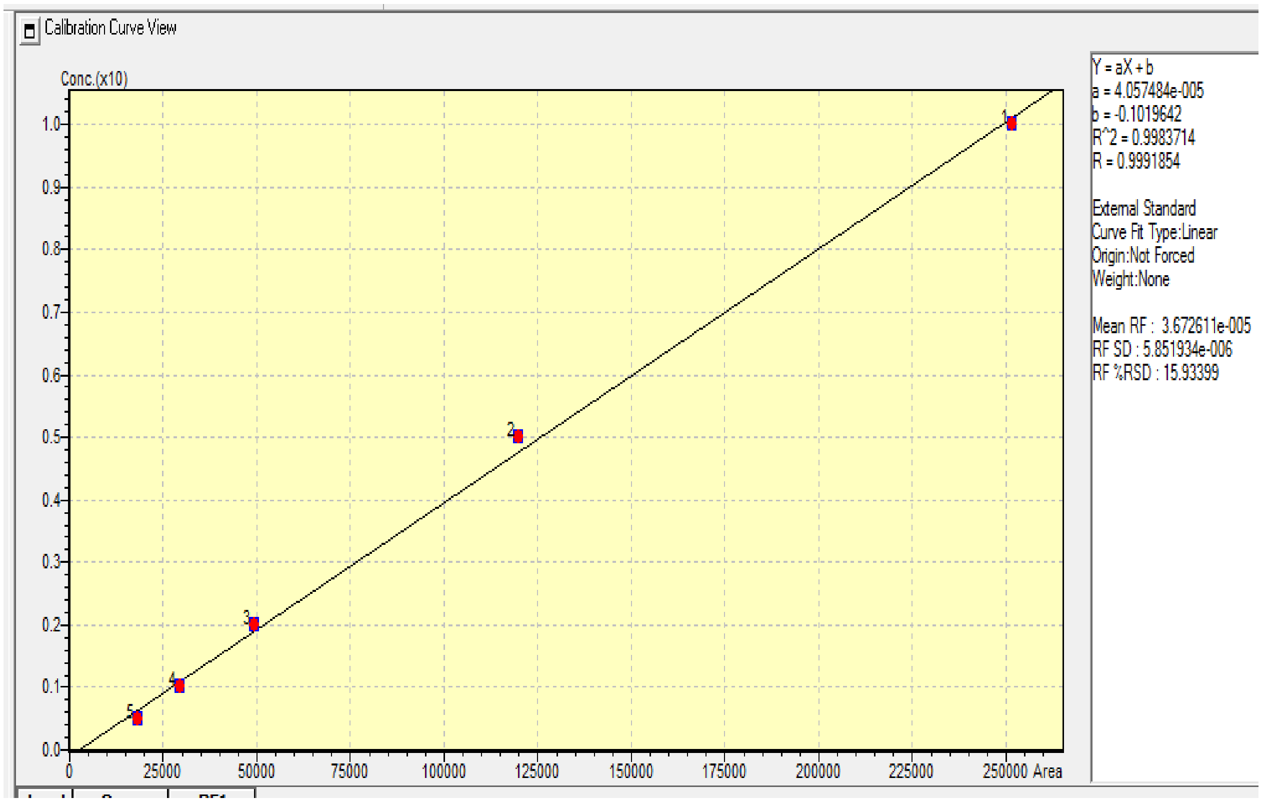

Figure 2: **Calibration curve for standard solution**

The results for standard recovery of the 2 ng/g, 1 ng/g and 0.5 ng/g aflatoxin standard are summarized in Table 2. The recoveries were observed to be 90.4%, 78.1%, and 85.2% for the spiked samples of 2 ng/g, 1 ng/g , and 0.5 ng/g, respectively. These results showed that the method was performing well.

The limit of detection (LOD) was found to be 0.10 ng/g for AFG2, 0.11 ng/g for AFG1, 0.23 ng/g for AFB2, and 0.26 ng/g for AFB1. On the other hand, the limit of quantification (LOQ) was found to be 0.30 ng/g, 0.18 ng/g, 0.31 ng/g and 0.36 ng/g for AFG2, AFG1, AFB2, and AFB, respectively.

Table 2: **Percentage recoveries of the spiked solution**

	Aflatoxins (ng/g)						
Conc (ng/g)	**AFG2**	**AFG1**	**AFB2**	**AFB1**	**Total AF (ng/g)**	**Average concentration (ng/g)**	**% Recovery**
2	1.92	1.60	1.48	2.12	7.14	7.23	90.4
	1.94	1.69	1.50	2.18	7.33		
1	0.98	0.65	0.52	1.04	3.20	3.12	78.1
	0.90	0.60	0.49	1.04	3.04		
0.5	0.58	0.33	0.18	0.65	1.75	1.67	85.2
	0.56	0.27	0.15	0.65	1.64		

4.3.2 Occurrence of Total Aflatoxin in Sesame Seeds

A total of 37 (52.9%) out of 70 samples tested were found to be contaminated with total aflatoxin of which 48.65% were from Mtwara and 51.35% from the Lindi region. The concentration levels of total aflatoxins ranged from 0.009 ng/g to 5.557 ng/g. None of the contaminated sesame seeds were found to have a concentration higher than 10 ng/g which is the maximum limit for the total aflatoxin set by East Africa Standard (EAS)(EAS 1006:2019). The highest total aflatoxin concentration for Mtwara and Lindi samples was 5.557 and 3.048 ng/g, respectively. These findings are in line with Hoseininia 2014 whereby 50% of 269 analyzed sesame seeds were contaminated by aflatoxin although the concentrations were at low levels. Similarly, more than half of sesame seeds (77.6%) in the study conducted by Kollia *et al.* (2016b) were found to be contaminated with aflatoxin at levels ranging from 0.02 ng/g to 14.49 ng/g.

The mean total aflatoxin concentrations are presented in Table 3. In both regions, aflatoxin AFG2 showed to have a higher concentration while aflatoxin AFB1 was observed to have the lowest concentration although none was above the East Africamaximum limit of 5 ng/g. The concentration of the total aflatoxin in the Mtwara region was almost two times that of the Lindi region.

Table 3: Individual and mean total aflatoxin concentrations in sesame seeds

Location	Concentration (ng/g)				Mean Total (ng/g)
	AFG2	AFG1	AFB2	AFB1	
Lindi	0.295	0.123	0.157	0.048	0.379
Mtwara	0.275	0.044	0.042	0.036	0.620

4.3.3 Occurrence of AFB1 in Analyzed Sesame Seeds

In this study, AFB1 was observed in 35.14% of the sesame seeds samples were contaminated with concentration ranging from 0.011 to 0.779 ng/g. The levels of AFB1 in sesame seeds were low as none among them exceeded the EAS acceptable maximum limit of 5 ng/g for sesame (EAS 1006:2019). Of all aflatoxins that affect humans and animals, AFB1 is considered to be the most potent and it is of public health concern in Tanzania and the world in general (Kimanya *et al.*, 2008). The AFB1 has been found to be the most produced aflatoxin by the aflatoxin producing strains Kollia 2016AFB1 in sesame seeds has been reportedin several studies conducted in different countries; 30% of sesame seeds samples were found to be contaminated by aflatoxins in the study which involved several food commodities (Anthony *et al.*, 2014), it was also detected in 18.1% of the sesame seeds samples in the study conducted in Iran (Asadi *et al.*, 2011).

The occurrenceof AFB2, AFG1, and AFG2was also considered wherebyAFG2 was found in 21 out of all 37 positive samples. The AFB2 and AFG1 had almost the same frequency (15 and 16, respectively). The AFG2 had a wide range of contamination (from 0.108 to 4.964 ng/g) followed by AFG1, AFB1, and lastly AFB2 (Table 4).

Table 4: Occurrence of aflatoxins in sesame seeds (n=70)

Aflatoxins	Positive samples	Contamination range (ng/g)	Mean (ng/g)	Mean (ng/g) ± SD per region	
				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

East Africa Maximum Limits; 5 ng/g forAFB1 and 10 ng/g for Total aflatoxins,

To the best of our knowledge, 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 sesame seeds in Tanzania. Therefore, despite thisbeing a cross-sectional study with a relatively small sample size, the findings give an insight and set a basis for information and future comprehensive studies.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

A total of 454 farmers from the Lindi and Mtwara regions participated in this study of assessing awareness and aflatoxins contamination of sesame seeds sold in local markets in the Mtwara and Lindi regions. Only 17.8% were found to be aware of aflatoxins contamination of crops. This implies that the majority of farmers and the public, in general, are unaware of aflatoxin contamination of crops.The lack of awareness may imply a low effort to their control strategy and may ultimately increase the risk of exposure to these toxins. Of the 70 samples tested for aflatoxins, 37 were contaminated at levels below the East Africa Standard limits for both total aflatoxins and AFB1 (10 ng/g and 5 ng/g, respectively). Although this is relieving, these low levels should not be underscored due to seasonality differences in the occurrence of aflatoxins and the risks associated with chronic exposure (exposure at low doses over a period of time), as well as the potential of this crop as food and cash crop. On the other hand, the study findings were limited to one agro-ecological zone and season, hence cannot be generalized and should be interpreted with care.

5.2 Recommendations

Considering the findings of this study, the following are recommended:

(i) Ministry of education and training institutionsto include mycotoxins subjects in Tanzania's formal syllabus from the primary level so that children are imparted with this knowledge early enough.

(ii) Provision of routine seminars on good agricultural practices and postharvest management to the actors in the whole food value chain that will equip them with skills on how to combat mycotoxins in their crops.

(iii) Routine monitoring of mycotoxins at local markets in Tanzania to ensure safety that may enhanceacceptance of these crops in the international markets. This will add value to our products that are sold in international markets.

(iv) Further studies are recommended to generate comprehensive data and document aflatoxin contamination of sesame seeds across agroecological zones and their products such as sesame oil.

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APPENDICES

Appendix 1: Sample questionnaire used for awareness assessment in Lindi and Mtwara regions

GENERAL INFORMATION			
District:	Ward:	Village:	Village Code:
Household No:			
Date of visit:			

A. DEMOGRAPHIC INFORMATION

S/N	Questions	Item Response	Tick the response
1.	Age of respondent		
2.	Sex	1. Male 2. Female	
3.	Marital status	1. Single 2. Married 3. Divorced 4. Widow	
4.	What is the level of your education?	1. No formal education 2. Standard 1-7 3. Form 1-6 4. Others (specify)	
5.	What is your occupation?	1. Farming 2. Livestock keeping 3. Farming and livestock keeping 4. Employed 5. Others (specify)	
6.	What is the size of your family? /how many people live in this family?	1. One 2. Two-five 3. More than five	
B. POSTHARVEST STORAGE PRACTISES OF SESAME SEEDS			
7.	What type of sesame seeds do you grow?		
8.	For how long have you been growing that type?	1. one year 2. more than one year	
9.	Why choosing that type in (7) above	1. High yield 2. Tolerance to diseases 3. Color 4. Early maturity	
10.	What are postharvest measure do you take before storing sesame seeds?	1. Drying 2. Winnowing 3. Packing	

11.	Where do you store sesame seeds?	1. Home 2. Warehouse/ Storehouse	
12.	What tools do you use to store your sesame seeds?	1. Sack bag (gunia) 2.Plastic gunny bag (mifuko ya salfeti) 3. Barrel/Drum (pipa) 4. Open space on the ground 5. Open space on tarpaulins	
C. AFLATOXIN AWARENESS			
13.	Have you heard of aflatoxin before?	1. Yes 2. No	
14.	Where have you heard it from?	1. Village meeting 2. Seminar 3. Expert talk 4. Other__________________	
15.	What is it according to what you have heard?	________________________	
16.	Have you experienced aflatoxin in your sesame seeds?	1. Yes 2. No	
17.	What do you do to prevent aflatoxin contamination?		
18	Do you know any health effect caused by consuming aflatoxin contaminated seeds? (mention)		

RESEARCH OUTPUTS

(i) Publication

Gidabedi, C., Kassim, N., & Matemu, A. (2022). Smallholder farmer's awareness and determination of aflatoxins in sesame seeds of southern Tanzania. *Journal of Food Safety and Hygiene,* 8(2), 94-104.

(ii) Poster Presentation