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Mycotoxigenic Fungi and Aflatoxins Quantification in Groundnuts (*Arachis hypogaea L.*) from Southern Mozambique

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Abstract: The present study aimed to evaluate the levels of fungal contamination and total aflatoxins levels in groundnut (*Arachis hypogaea L.*) grains harvested in the Southern Mozambique. Moisture content, fungi infection and levels of total aflatoxins (TAflt) were assessed. Moisture content was determined by the low temperature electric oven method. Fungal rate was analyzed using the blotter test method. ELISA test was used for the quantification of total aflatoxins levels. The observed moisture content was within the limits considered safe for groundnut storage (10%). The identified fungi were of the genera Aspergillus, Alternaria, Cladosporium, Fusarium, Macrophomina, Penicillium, Rhizoctonia and Rhizopus. *Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer, Penicillium sp., Fusarium verticillioides and Fusarium oxysporum* were the most prevalent species. The fungi reported in this study are associated with the production of at least three important mycotoxins, namely: Aflatoxin, Fumonisin and Ochratoxin. About 83% of the analyzed samples were contaminated with TAflt, of which 38% below and 45% above the maximum allowable limit according to the Codex alimentarius (10 ppb). Gaza province registered the highest percentage of samples with TAflt above the codex maximum tolerable limit (10 ppb) ranging from 63% to 75%, while Inhambane province leaded the sample within the safety range (bellow 10 ppb). The risk factor was further elucidated with the average PDI values which ranged from 1.21 to 15.73 ng/kg Bw/day. The high prevalence of aflatoxigenic fungi detected suggests that if storage conditions deteriorate, aflatoxin levels may increase, leading to acute or chronic intoxication of the consumers.

Keywords: *Aspergillus flavus; Aflatoxin; Fungi; Groundnut; Probable daily intake; Total Aflatoxin*

1. Introduction

Groundnuts (*Arachis hipogaea L.*) are the fourth most cultivated oilseeds in the world, covering about 23 million hectares with a current world production of 49 million tons per year.[1-38] Groundnut production in Mozambique have been increasing from about 93 x 10^3 tones in 2015 to 151 x 10^3 tones in 2019, with cultivated area ranging from 382 x 10^3 ha in 2015 to 532 x 10^3 ha in 2019.[19] Cultivation and storage of groundnut under favourable conditions favour the occurrence of several pests and plant pathogens causing different types of deterioration which make them improper for human and animal consumption[12] leading to food insecurity. The climatic conditions in the tropics and subtropics promote the propagation of diverse pathogenic fungi capable of producing mycotoxins.[4] Aflatoxins are the main mycotoxins that represent the greatest danger associated with the groundnut production chain, produced mainly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* under favourable conditions of humidity and temperature.[32] In tropical and subtropical countries with less or lack of regulatory activities regarding the acceptable level of aflatoxin in food and feeds, the risk of human aflatoxicosis is huge.[1] About 4.5 billion people in developing countries are systematically exposed to uncontrolled amounts of aflatoxins.[33] Aflatoxins are primarily hepatotoxic causing liver damage in animals, are immunosuppressive and may lead to decreased production (milk, eggs, weight gains, etc.).[4] Aflatoxins can be present in milk of dairy cows, meat, chicken eggs if the animals consume sufficient amounts in their feed.[6] Most African countries, including Mozambique, lack basic facilities for routine assessment of the maximum tolerable mycotoxins levels in raw and processed foods, exposing the population to food intoxication. Aflatoxins exposures have been reported to be responsible for deaths resulting from liver cancer in about 26,000 Africans living in south of the Sahara annually.[36] A relationship between aflatoxin contamination of groundnuts and the high rate of liver cancer was reported in Inhambane province, southern Mozambique in seventies.[31] Even though there are some research activities going on in Mycotoxins related issues in Mozambique, which is public health concern, for several reasons most of them haven't yet reached publication status, hence not publicly available. The present study aims to assess the level of contamination by mycotoxigenic fungi and aflatoxins in groundnut grains from Inhambane and Gaza provinces in southern Mozambique. The study

Table 1. Number of samples collected in Inhambane and Gaza Provinces per District.

Province			
Inhambane		Gaza	
District	**N° of samples**	**District**	**N° of samples**
Maxixe	3	Manjacaze	12
Jangamo	7	Chokwe	7
Zavala	10	Xai-Xai	8
Total	**20**		**27**

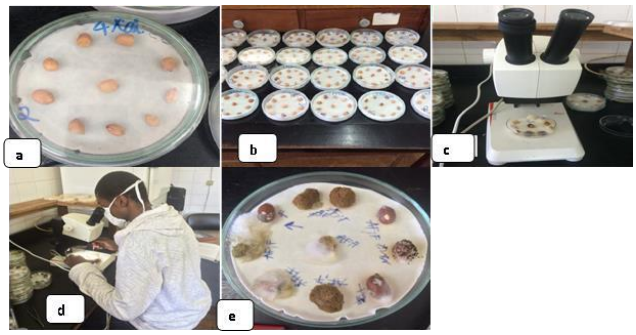

Fig. 1. Blotter test method. Equidistant layout of the grains in the petri dish (a), incubation at room temperature (b), stereo microscopy used for fungi identification (c), identification of the fungi with the help of stereo microscopy magnifying glass (d) and (e) fungi labelling on the petri dish after identification and record.

will generate knowledge about the current sanitary and health quality of groundnut, and raise the awareness about this neglected public health problem in Mozambique.

2. Material and Methods

2.1. Study Sites and Sampling

Fieldwork was conducted from December 2014 to January 2015 in Gaza and Inhambane provinces. The samples were collected from farmers store facilities in three districts from Inhambane province (Maxixe, Jangamo and Zavala) and three from Gaza province (Manjacaze, Chokwe and Xai-Xai). For the identification and quantification of fungi associated with groundnut grains 47 samples from previous season harvest, were collected (Table 1). About 1 Kg sample size were collected from each farmer storage facilities and placed in small paper bag. From each collected sample, several working subsamples were drawn in a specific amounts for each laboratory test.[24]

2.2. Identification of Fungi Associated with Groundnut Grains

The identification and quantification of fungi associated with groundnut grains was carried out in the Plant Pathology Laboratory, Department of Crop Protection, Faculty of Agronomy and Forestry Engineering (FAEF), Eduardo Mondlane University (UEM), from July to November 2015. For the identification of fungi the deep-freeze blotter test method was used.[9,24] A total of 200 grains of each sample were used, placing 10 grains in each petri dish on a filter paper layer (3 papers per plate) moistened with sterilized distilled water, and sealed with parafilm (Fig. 1a and 1b). The recovered isolates were purified by sub-culturing on PDA until axenic pure cultures were generated after which the fungal identity was confirmed on compound microscopy using identification keys.[22] The isolated fungal strains were identified combining macroscopic (Fig. 1c, 1d, and 1e) and microscopic characteristics, using slide culture technique.[24]

2.3. Quantification of Fungal Contamination Levels in Groundnut Grains

To quantify the levels of fungal contamination of the grains, the infected groundnuts were counted and the percentage of infection calculated using the quantitative method based on the incidence rate. The percentage of fungal occurrence was calculated by dividing the occurrence of individual isolates per sample with the total number of all kernels seeded per sample which was then expressed as a percentage using the following formula: [26]

$$Fungal\ Incidence\ (I) = \frac{Number\ of\ grains\ containing\ a\ particular\ fungal\ species}{\text{Total number of grains analyzed per sample}} \times 100$$

2.4. Determination of Groundnut grain Moisture Content

The determination of the moisture content (MC) of the groundnut grains was carried out using oven drying method, which consisted of submitting the sample to 130°C for 17 hours.[9] About 25g of groundnuts were ground per sample using cereal and legume grinder and 5g were used as working sample into aluminum capsule. After submitting the crushed grains to heating in the oven, the capsules were removed and placed in a desiccator for about 45 minutes to cool, followed by final weighing using a high precision scale (MP200A, Laboratory & Scientific Equipment CO, LASEC, Cape Town, South Africa)

Moisture content data was expressed in percentage, using the following equation:[25]

$$\mathrm{MC}\ (\%) = \frac{(\mathrm{M0}-\mathrm{M1})\ \mathrm{x100}}{\mathrm{M0}}$$

Where: MC – Moisture content (%); $\mathrm{M0}$ – initial weight, in grams of test portion; $\mathrm{M1}$ - final weight, in grams of dried test portion.

2.5. Detection and Quantification of total Aflatoxin

Detection and quantification of total-aflatoxin (TAflt) in groundnuts was carried out using competitive ELISA (Enzyme Immunoassay) assay, which consisted of two stages, Preparation of samples and run the ELISA test, according to the instructions of the Kit (AgraQuant® Total Aflatoxin ELISA Test). For reading the TAflt results, the plate was placed in the ELISA Reader (EL800, BIOTEK Instruments, Highland Park Box 998, Winooski, USA) connected to a desktop with Gen5TM Micro plate Reader software installed. The wavelength was then calibrated to 450 nm and the absorbance results were read using Gen5TM Micro plate Reader software. The absorbance values were saved in excels for later conversion into concentrations (ppb) and analysis.

Using the absorbance's values of the standard provided in the kit, the calibration curve was determined with the absorbance as a function of the concentration of Atlatoxins (0, 1, 2, 4, 10 and 20 ppb): $\mathrm{Y} = \mathrm{aX} + \mathrm{b}$; $\mathrm{Total\ Aflatoxin\ (ppb)} = \frac{Y-b}{a}$; where Y – Samples absorbance's values; X – Total Aflatoxin concentration in the samples (ppb).

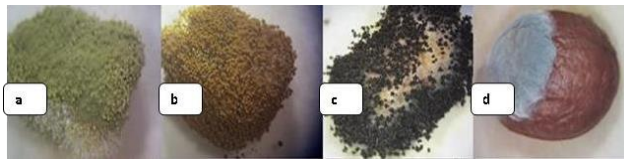

Fig. 2. Habit features of the main storage fungi detected: immature white heads and yellow-cream to green mature heads of *Aspergillus flavus* (a) under stereo microscopy, yellowish pinkish/orange color of *Aspergillus ochraceus* (b) under stereo microscopy, black conidial heads of *Aspergillus niger* (c), blue mold penicilium sp. mass (d) under magnifying glass.

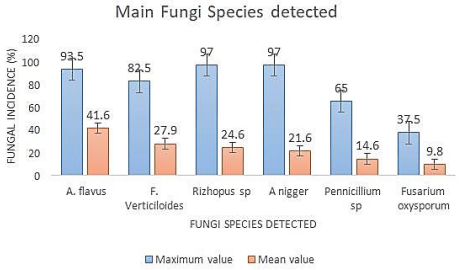

Fig. 3. Maximum and mean incidence values of the main fungal species detected.

2.6. Provisional Daily Intake for Total Aflatoxins Assessment

The provisional daily intake for total aflatoxins of the nut consumers were determined as described by Boni et al.[8] The mean value of aflatoxins in the groundnut samples per location was multiplied by the average consumption rate of the peanuts in Mozambique (g/person/day)[35,37] which was then divided by the average adult body weight (Bw) of 60 kg for adults. Furthermore, the average peanut per-capita consumption rate in Mozambique ranged from 0.4 to 1.0, with mean value of 0.7 kg per week.[35] The peanut average per-capita consumption rate was then estimated using both the minimal, maximal and mean figures. This figure was then divided by seven days to get the daily average per-capita consumption figure of 57, 100 and 142 g/person/day for minimal, mean and maximal figures respectively. The provisional daily intake (PDI) was thus calculated as illustrated below: PDI (ng/kg bw/day) = peanut intake (g/person/day) × levels of aflatoxins in the samples (µg/kg)/Bw (kg).

2.7. Data Analysis

Data were organized using the statistical package Microsoft Office Excel. For statistical analysis, specification tests (normal distribution and homogeneity of residues) were carried out, using the statistical package STATA10, followed by the analysis of variance (ANOVA) of the treatments. The comparison of means was done using the Turkey test at 5% significance level (α = 5%) and 95% confidence interval. The original data of the MC and *Pennicilium sp, Fusarium oxysporum and Rhizopus stolonifer* incidence were corrected to conform to the normal distribution criteria. In the analysis of variance, the sampling locations (districts) were considered as an independent variable and the parameters MC and fungal incidence as dependent variables. The correlation between the dependent variables was determined using person coefficients.

3. Results and Discussions

3.1. Identification of Fungal Species Detected on Groundnut Grains

The results reveal that 11 fungi species belonging to 8 genera were identified. The field fungi species found were: *Fusarium moniliforme (Fusarium verticillioides), Fusarium oxysporum, Rhizoctonia solani, Alternaria sp., cladosporium sp., and Macrophomina phaseolina*. On the other hand, the storage fungi identified were: *Aspergillus flavus, Aspergillus Niger, Aspergillus ochraceus, Penicillium sp., and Rhizopus stolonifer* (Fig. 2). Similar findings were reported in previous studies conducted in our lab using groundnut samples from Inhambane province, where 12 fungi species were detected.[35,37] Related findings were also reported in studies carried out in Brazil[7,28] and Argentina,[5] where most of the fungi reported in the present study were detected in groundnut grains. The presence of these fungi is an indicator of grain deterioration as they cause discoloration, change in flavour, nutritional changes, loss of dry matter, in addition to the possibility of producing mycotoxins.[26] The occurrence of *A. Flavus, F. verticillioides, Aspergillus ochraceus, Aspergillus niger and Penicillium sp*. are of great concern due to its ability to produce the main mycotoxins harmful to humans and animals.[27,33]

3.2. Prevalence of Fungal Species Detected

The districts of Maxixe, Jangamo, Zavala, Xai-Xai, Manjacaze and Chokwe did not differ statistically from each other ($p < 0.05$) in the incidence of the *Penicillium sp., F. verticillioides, F. oxysporum, A. ochraceus, M. phaseolina, Cladosporium sp. and Rhizoctonia solani* (Table 2). However, *A. flavus, A. niger and R. stolonifer* species had significantly different incidences among the districts ($p<0.05$). The relative difference on the fungal incidence may be associated with groundnut variety, farming and harvest practices, drying practices and conditions, post-harvest handling, storage practices and facilities, weather conditions, pest infestation, etc.[1,13,18]

Regarding *A. flavus*, the district of Chokwe differed statistically only from the districts of Maxixe and Xai-Xai. The districts of Maxixe had the highest percentage of incidence (66.5%) while the district of Chokwe had the lowest percentage of incidence (13.3%). For *A. niger*, the districts of Gaza province were statistically equal and lower to those of Inhambane province. These findings suggest that factors other than cropping and agricultural practices influence the distribution of *A. niger* populations.

The fungi *Rhizoctonia solani, Alternaria sp., cladosporium sp., and Macrophomina phaseolina* were of low occurrence with an average incidence percentage close to 1% (Table 2). This fact may be associated with the loss of viability of some field fungi during the storage period.

The most frequent storage species detected in the present study were *A. flavus* (41.61%), *Rhizopus stolonifer* (24.64%) and *A. niger* (21.62%) (Fig. 3). Fungi of the genera *Aspergillus* and *Penicullium* have been classified as fungi that grow during grain storage.[11] In the previous studies conducted in our lab using groundnut samples from Inhambane province *A. flavus*, *Penicillium sp*. and *A. niger* were the most common storage fungi while a low occurrence of the *R. stolonifer* was reported.[20,30]

Table 2. Average percentage of fungi incidence among districts.

Fungi species	Fungal mean incidence value (%)					
	Districts of Inhambane Province			Districts of Gaza Province		
	Maxixe	Jangamo	Zavala	Xai-Xai	Manjacazec	Chokwe
Alternaria sp.	0.83 a	0.33 a	0.9 a	0.8 a	1.17 a	0.8 a
Aspergillus flavus	66.5 a	27.7 ab	47.6 ab	56.2 a	39.9 ab	13.3 b
Aspergillus niger	63.5 a	43 a	24.55ab	10.38 b	9.87 b	10. 79 b
Aspergillus ochraceus	1 a	2.25 a	3.5 a	2.5 a	2.66 a	3.5 a
Cladosporium sp.	1.5 a	0.6 a	1.3 a	0.6 a	1.6 a	1.7 a
Fusarium moniliforme	21.5 a	36.92 a	14.8 a	27.46 a	34.25 a	33.86 a
Fusarium oxysporum	2.67 a	13.42 a	8.8 a	13.88 a	8 a	6 a
Macrophomina phas.	0.5 a	0.6 a	0.7 a	0.4 a	0.3 a	1.64 a
Penicillium sp.,	0.22 a	0.54 a	0.38 a	0.34 a	0.48 a	0.60 a
Rhizoctonia solani	0.33 a	0.44 a	0.65 a	0.41 a	1.5 a	0.43 a
Rhizopus stolonifer	2.72 ab	7.44 a	3.87 ab	2.39 b	4.37 ab	4.38 ab

** Mean values followed by different letters in the same line are statistically different from each other based on Turkey (p<0.05) test*

Table 3. Total aflatoxin levels detected in each Province per district.

Province	District	Number of samples	Mean value	Minimum value	MaximumValue	Standard Deviation
Inhambane	Maxixe	3	1.43 a	0	2.22	0.72
	Jangamo	7	3.09 a	0	10.93	1.52
	Zavala	10	3.13 a	0	17.42	1.63
Gaza	Manjacaze	12	10.85 b	2.75	13.0	1.37
	Xai – Xai	8	10.48 b	0	16.99	2.23
	Chokwe	7	9.59 ab	0	16.46	2.19

** Mean values followed by different letters in the same column are statistically different from each other based on Turkey (p<0.05) test*

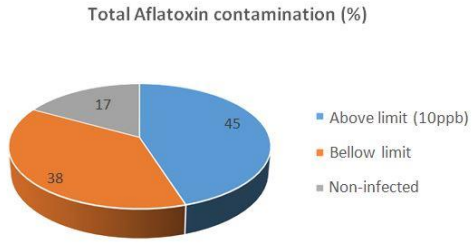

Fig. 4. Total Aflatoxin Levels detected in all Samples tested.

In the present study, three species of the genus Aspergillus were detected (*A. flavus, A. niger* and *A. Ochraceus*), all associated with the production of two important mycotoxins, aflatoxin and ochratoxin.[15,27] *A. flavus* was detected in all samples at percentages ranging from 2.5 to 93.5% (Fig. 3), observed in the districts of Chokwe and Manjacaze respectively. *A. niger* was also detected in all samples ranging from 0.5 (in Xai-Xai) to 97% in Zavala district. *A. niger* are not aflatoxignic but are associated with production of ochratoxin A other highly toxic mycotoxin.[27] *A. ochraceus* was detected in 34% of the tested samples, but in a lower incidence with an average percentage of incidence close to 1%. Searching for the biodiversity of aflatoxigenic *Aspergillus* species in diary feeds in Zimbabwe revealed that *A. ochraceus* were only detected in one out of 142 isolates (0.7%).[27] Despite the low incidence of this fungus, its occurrence is somewhat worrying due to its potential to produce ochratoxin.[11,15] *Pennicilium sp*. was observed in 89% of the tested samples, in percentages ranging from 0 to 65% with an average percentage of 14.61% (Fig. 3). The higher incidence of mycotoxigenic fungi detected in this study suggests that if storage conditions deteriorate, aflatoxin levels may increase; contributing to chronic or acute poisoning due to the consumption of contaminated groundnuts or it's derivate, negatively affecting the consumer's public health. Aflatoxins exposures have been reported to be responsible for deaths resulting from liver cancer in about 26,000 Africans living in south of the Sahara annual.[37] Hence not all *A. flavi* sections are able to produce aflatoxins.[3,27]

3.3. Total Aflatoxin Concentrations Detected in Groundnut Grains

About 39 (83%) tested sample were contaminated with total aflatoxins (TAflt) and of these, 21 (45%) were above the maximum limit accepted (Fig. 4) in Mozambique (10 µg/kg for total aflatoxins).[19] Previous studies conducted in our laboratory with groundnut samples from Inhambane revealed that 97% of the samples were contaminated with TAflt, of which 81% below and 16% above the maximum allowable limit in Mozambique and only 3% were free from TAflt.[20] This growing trend of samples above the tolerable limit may be associated with the weakening of the best practices recommended for the cultivation and storage of grains. Maximum production of aflatoxin usually happens at temperatures range of 24 - 25 degrees and grain humidity above 15%.[23] Comparison of different groundnut harvesting period in Mozambique found that the higher aflatoxin contamination levels were associated with higher pod and kernel moisture content which provided a conductive environment for both fungal growth and aflatoxin production.[38] The districts of Gaza Province had a higher percentage of samples contaminated with TAflt, ranging from 85% to 100%, while in Inhambane the contamination ranged from 53 to 80% (Fig. 5). Furthermore, Gaza province registered the highest percentage of samples with TAflt above the codex tolerable limit (10 ppb) ranging from 63% to 75%, while Inhambane province led the sample within the safety range (bellow 10 ppb), ranging from 43% to 70% (Fig. 5). The district of Jangamo, in Inhambane province had the highest (43%) percentage of samples free from TAflt, while in Gaza, Manjacaze had the highest (100%) percentage of contaminated samples (Fig. 5).

Table 4. Average Provisional Maximum Tolerable Daily Intake (ng Total Aflatoxin kg^{-1} Bw) based on peanut daily per-capita consumption estimates.

Province	District	Number of samples	Mean Aflatoxin (ppb)	Provisional Tolerable Daily Intake (ng Total Aflatoxin kg^{-1} Bw)			
				Estimated daily consumption in Mozambique Minimal (11g)*	Estimated daily consumption in Mozambique Minimal (57g)**	Estimated daily consumption in Mozambique Average (100g)**	Estimated daily consumption in Mozambique Maximum (143g)**
Inhambane	Maxixe	3	1.44	0.26	1.36	2.39	3.42
	Jangamo	7	3.10	0.57	2.94	5.16	7.38
	Zavala	10	4.13	0.75	3.93	6.89	9.85
Gaza	Manjacaze	12	10.86	2.0	10.32	18.10	25.88
	Xai – Xai	8	10.49	1.92	9.96	17.48	25.0
	Chòkwé	7	9.59	1.75	9.11	16.0	22.87
Mean Value				1.20	6.27	11.00	15.73

**WHO, 2006; **USDA, 2019.*

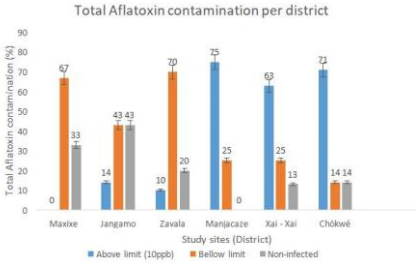

Fig. 5. Total aflatoxin detected in samples tested per district.

The mean TAflt levels ranged from 1.43 ppb to 10.85 ppb, and were statistically different between districts ($p < 0.05$) (Table 3). The districts of Gaza Province were statistically equal to each other and above to those of Inhambane Province (table 3). Although there was no significant correlation between TAflt levels and moisture content (MC), the high levels of aflatoxin recorded in Manjacaze and Xai-Xai, in Gaza Province, may be associated with the high levels of grain MC observed whose maximum levels ranged between 9.2 and 13.3 (Table 5). Groundnut kernel should be stored with a water content ranging between 8% and 10%, which can slow down the speed and intensity of spoilage.[26] The higher grain MC recorded in Manjacaze and Xai-Xai can probably be explained by insufficient drying, or storage in places where there are fluctuations in temperature and/or relative air humidity.[25] The physical (aeration, cold storage, rapid drying, and radiation) and chemical (food preservatives and pesticides) treatments, are commonly used methods to prevent the growth of aflatoxin producing fungi in foods and feeds.[33]

With the exception of two (33%) districts, namely Manjacaze and Xai-Xai, the mean levels of TAflt observed in the other four districts were below the tolerable limit according to the codex alimentarius (10 ppb) (Table 3). On the other hand, at least one sample from 5 out of 6 districts recorded maximum levels of TAflt above the accepted limit (10 ppb), ranging from 10.93 ppb to 17.42 ppb) (Table 3). These results suggest a worrying scenario considering the importance of groundnuts in the diet of the population in the study site, and in the country in general.

To elucidate the safe status of the peanut samples tested in this study, the Provisional Daily Intake (PDI) was estimated. The PDI for adults in all peanut samples contaminated with TAflt ranged from 0.26 to 26 TAflt ng/kg Bw/day to Maxixe and Manjazace districts respectively (Table 4). Furthermore, the average PDI values ranged from 1.21 to 15.73 ng/kg Bw/day, above the maximum PDI of AFB1 for adults (1 ng/kg body weight/day),[8] raising health concerns for the Mozambican peanut consumers. These results indicate that even if the peanut consumption is not on daily basis, it may contribute for daily exposure leading to chronic intoxication. Assessment of the quality of peanut in Tanzania reported PDI exposure to total aflatoxin range from 0.02 to 15.12 ng/kg Bw/day, with an average of 2.39 ng/kg Bw/day.[8] Among other factors, the exposure to aflatoxin is a function of the level of contamination, the amount consumed and the frequency of consumption. Peanut per-capita consumption in Mozambique vary depending on dietary habits, purchasing power parity, market prices, locations (peanut growing or selling regions), period of the year (growing, harvest or storage), consumer's preferences, etc. The peanut average per-capita consumption rate in Mozambique is 0.7 kg per week, with majority of Mozambicans eating peanut related food at least once a week.[35] Therefore, even the slightly lower levels of aflatoxins observed in Inhambane province may represents a potential health hazard, given the high rates of peanut consumption in that region. This may apply for the majority of Mozambicans that may have choice limitations due to poverty. Food contaminated by Aflatoxin poses a serious risk when greater part of the population is poor and have fewer choices on the type of food consumed.[21]

There are several studies that associate the consumption of aflatoxin contaminated food with the incidence of liver cancer. Liver cancer was traditionally considered to be frequent in Eastern and Southern Africa, including Mozambique. Seventeen years (1991-2008) trend in Cancer incidence in Maputo (Mozambique capital city) study revealed that liver cancer remains of serious health concern and showed a moderate increase in woman and decrease in man.[22] Maputo liver cancer figures may roughly represent a picture from the whole country since in most places of Mozambique we may lack facilities for liver cancer detection and diagnose. The association between liver cancer and consumption of aflatoxin-contaminated food in Mozambique date back to seventies. A relationship between aflatoxin contamination of groundnuts and the high rate of liver cancer in Inhambane province has been reported in mid-seventies.[31] In line with food related cancer the esophageal cancer, which was uncommon in 1950s showed a marked increase during 1991-2008 cancer trend study, conducted in Maputo.[22] Among other foodstuff, esophageal cancer is linked to consumption of food contaminated with fumonisin.[38] In the present study fumonisin test was not conducted, but the higher prevalence of *F. moniliforme* (*F. verticillioides*) detected may pose a great risk for uncontrolled fumonisin contamination.

Table 5. Moisture content (%) of the groundnut grains detected in each Province per district.

Province	District	Number of samples	Mean	Minimum	Maximum	Standard Deviation
Inhambane	Maxixe	3	5.9 a	5.6	6.3	0.72
	Jangamo	7	5.9 a	4.3	8.3	1.52
	Zavala	10	5.88 a	4.9	8.2	1.63
Gaza	Manjacaze	12	5.87 a	4.8	9.2	1.37
	Xai – Xai	8	7.41 a	5	13.3	2.23
	Chokwe	7	5.72 a	4.9	6.10	2.20

**Mean values followed by different letters in the same column are statistically different from each other based on Turkey ($p<0.05$) test*

3.4. Moisture Content of the Groundnut Grains

Moisture content ranged from 4.3 to 13.3%, observed in the districts of Jangamo, and Xai-Xai respectively (Table 5). The mean MC of the six districts was statistically equal ranging from 5.9% to 7.41%, values considered safe for groundnut storage.[23] The maximum values observed in each district ranged from 6.3 to 13.3 (Table 5, below the 14% considered as a safety limit for the conservation of groundnuts.[23] WHO and FAO, recommend that for storage peanut kernels should be dried for safety ≤ 10% MC level.[32] However, several authors claim that fungi growth may happen when they are already attached to the grains, even if the humidity is less than favourable for their development.[17] Hence, should be noted that drying nuts to acceptable moisture levels are constrained in many tropical countries including Mozambique, due naturally prevailing high relative humidity, making drying ineffective, thus increasing the risk of fungal and aflatoxin contamination.[25]

The results of the correlation test showed no association between aflatoxin concentration and MC. Likewise, there was no association between the incidences of *A. Flavus* with the concentration of aflatoxins. In line with this finding, the MC results (Table 5) detected in this study were statistically equal among all samples, and within the safety range for preserving groundnuts. These results are in agreement with those obtained in other studies where the growth of mycotoxigenic fungi didn't relate to the production of the respective mycotoxin.[14,20] Recent study tested 46 *A. flavi* isolates and found that some lacked one or more of the aflatoxin cluster genes.[27] Furthermore, Biosynthesis of aflatoxins comprises several enzymes and regulatory proteins whose genes are located in a single cluster.[27] Other researchers in Brazil also found no association between corn grain MC (which was <14%) and the presence of aflatoxins.[2]

4. Conclusions

Eleven species and 8 genera of fungi associated with groundnut were identified. The field fungi found were *F. verticillioides, F. oxysporum, R. solani, Alternaria sp., Cladosporium sp., and M. phaseolina* while the storage fungi identified were *A. flavus, A. ochraceus, A. niger, Penicillium sp. and R. stolonifer*. The fungi reported in this study are associated with the production of at least three important mycotoxins, namely Aflatoxin, Fumonisin and Ochratoxin. About 83% of the tested samples were contaminated with total TAfl, 38% below and 45% above the maximum acceptable limit in Mozambique (10 ppb). Furthermore, Gaza province registered the highest percentage of samples with TAflt above the codex tolerable limit (10 ppb) while Inhambane province leaded the sample within the safety range (below 10ppb). The provisional daily intake for adults ranged from 1.4 to 26 ng TAfl kg^{-1} BW/day. These results indicate that even if the peanut consumption is not on daily basis, it may contribute for daily exposure leading to chronic intoxication. There was no association between the incidences of *A. flavus* species with the concentration of aflatoxins. However, the high prevalence of mycotoxigenic fungi suggests that if the storage conditions deteriorate, aflatoxin levels may increase, posing serious risk for public health.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1 Adetunji M.C.; Aroyeun S.O.; Osho M.B.; Sulyok M.; Krska R.; Mwanza M. Fungal Metabolite and Mycotoxins Profile of Cashew Nut from Selected Locations in Two African Countries. *Food Addit. Contam.: Part A*, 2019, **36**, 1847-1859. [CrossRef]
2 Almeida A.V.A.F.; Botura M.B.; Abreu R.D.; Bittencourt T.C.C.; Batatinha M.J.M. Ocorrencia de Aflatoxinas em Milho Destinado a Alimentaçao de Aves no Estado da Bahia. *Arq. Inst. Biol.*, 2021, **76**, 353-358. [CrossRef]
3 Atanda O.; Ogunrinu M.; Olorunfemi F. A Neutral Red Desiccated Coconut Agar for Rapid Detection of Aflatoxigenic Fungi and Visual Determination of Aflatoxins. *World Mycotoxin J.*, 2011, **4**, 147-155. [CrossRef]
4 Atanda S.A.; Pessu P.O.; Aina J.A.; Agoda S.; Adekalu O.A.; Ihionu G.C. Mycotoxin Management in Agriculture. *Greener J. Agric. Sci.*, 2013, **3**, 176-184. [Link]
5 Barros D.I.; Nunes H.V.; Dias D.C.F.S.; Bhering M.C. Comparacao Entre Testes de Vigor para Avaliacao da Qualidade Fisiologica de Sementes de Tomate. *Revista Brasileira de sementes*, 2002, **24**, 12-16. [CrossRef]
6 Bbosa G.S.; Kitya D.; Ogwal-Okeng J. Aflatoxins Metabolism, Effects on Epigenetic Mechanisms and their Role in Carcinogenesis. 2013. [Link]
7 Bellettini N.M.T.; Endo R.M.; Miglioranza E.; Santiago D.C. Patogenicidade de Fungos Associados as Sementes e Plantulas de Amendoim cv. Tatu. *Semina: Cienc. Agrar.*, 2005, **26**, 167-172. [Link]
8 Boni S.B.; Beed F.; Kimanya M.E.; Koyano E.; Mponda O.; Mamiro D.; Kaoneka B.; Bandyopadhyay R.; Korie S.; Mahuku G. Aflatoxin Contamination in Tanzania: Quantifying the Problem in Maize and

Groundnuts from Rural Households. *World Mycotoxin J*., 2021, **14**, 553-564. [CrossRef]
9 Brasilia. Regra Para Analise de Sementes. 1ª Edicao, Ministerio da Agricultura e Reforma Agraria. Brasilia, DF: MARA, 2009. 1-365. [Link]
10 Bueno D.J.; Silva J.O.; Oliver G. Fungal Isolation and Enumeration in Foods. *In Public Health Microbiology*, 2004, 127-131. Humana Press. [Link]
11 Bueno D.; Munoz R.; Marty J.L. Low Cost and Optical Detection from Fluorescence of Ochratoxin A. In *2017 Global Medical Engineering Physics Exchanges/Pan American Health Care Exchanges (GMEPE/PAHCE)*. IEEE, 2017, 1-4. [CrossRef]
12 Bulaong S.S. Fungal Population, Aflatoxin and Free Fatty Acid Contents of Peanuts Packed in Different Bag Types. *BIOTROPIA-The Southeast Asian Journal of Tropical Biology*, 2002, **19**, 1-25. [Link]
13 Cardoso F.C.; Calvet R.M.; Pereyra C.M.; Pereira M.M.G.; Rosa C.A.R.; Torres A.M.; Muratori M.C.S. Ocorrencia de Aspergillus SPP., Penicillium SPP. E Aflatoxinas em Amostras de Farinha de Milho Utilizadas no Consumo Humano, Piaui, Brasil. 2011. [Link]
14 Chalfoun Y.; Chalfoun S.; Perreira M. Análises microbiológicas e de aflatoxinas no controle de qualidade de rações para cães. *Revista Ciência e Vida*., 2008, **28**, 25-27.
15 Chebil S.; Rjiba-Bahri W.; Oueslati S.; Ben Ismail H.; Ben-Amar A.; Natskoulis P. Ochratoxigenic Fungi and Ochratoxin A Determination in Dried Grapes Marketed in Tunisia. *Ann. Microbiol*., 2020, **70**, 1-9. [Link]
16 Codex Alimentarius Commission. General Standard for Contaminants and Toxins in Food and Feed. FAO & WHO, 2019, 1-66. [Link]
17 Eltariki F.E.M.; Tiwari K.; Ariffin I.A.; Alhoot M.A. Genetic Diversity of Fungi Producing Mycotoxins in Stored Crops. *J. Pure Appl. Microbiol*., 2018, **12**, 1815-1823. [CrossRef]
18 Hermanns G., Pinto F.T.; Kitazawa S.E.; Noll I.B. Fungos e Fumonisinas no Periodo Pre-Colheita do Milho. *Food Sci. Technol*., 2006, **26**, 7-10. [CrossRef].
19 Instituto Nacional de Estatística. INE. Indicadores Básicos de Agricultura e Alimentação 2015-2019. 2020. [Link]
20 Leão, G. Microflora e Aflatoxinas em grãos de Amendoim (*Arachis hypogaea* L.) Provenientes da Província de Inhambane. Degree-Universidade Eduardo Mondlane, Maputo, Mozambique, 2011.
21 Liu Y.; Wu F. Global Burden of Aflatoxin-Induced Hepatocellular Carcinoma: A Risk Assessment. *Environ. Health Perspect*., 2010, **118**, 818-824. [CrossRef]
22 Lorenzoni C.; Vilajeliu A.; Carrilho C.; Ismail M.R.; Castillo P.; Augusto O.; Garcia-Basteiro A.L.; Sidat M.; De Sanjose S.; Menendez C.; Ordi J. Trends in Cancer Incidence in Maputo, Mozambique, 1991–2008. *PLoS One*, 2015, **10**, 0130469. [CrossRef]
23 Marcos Filho J. Fisiologia de Sementes de Plantas Cultivadas. 2015. [Link]
24 Mathur S.B.; Kongsdal O. Common Laboratory Seed Health Testing Methods for Detecting Fungi. *International Seed Testing Association*. 2003. [Link]
25 Mutegi C.K.; Wagacha J.M.; Christie M.E.; Kimani J.; Karanja L. Effect of Storage Conditions on Quality and Aflatoxin Contamination of Peanuts (*Arachis Hypogaea L*). 2013, **3**, 746-75. [Link]
26 Nakagawa J; Rosolem C.A. O Amendoim: Tecnologia de Producao, Edition, FEPAF, 2011, 1-325. [Link]
27 Nleya N.; Ngoma L.; Adetunji M.C.; Mwanza M. Biodiversity of Aflatoxigenic Aspergillus Species in Dairy Feeds in Bulawayo, Zimbabwe. *Front. Microbiol*., 2021, **11**, 599605. [CrossRef]
28 Amaral F.V.N.; Dias N.S. Analise Sanitaria de Sementes de Amendoim (*Arachis Hypogaea L*.) Armazenadasem Algumas Areas do Estado da Paraiba. *Revista de Biologia e Ciencias da Terra*, 2004, **4**. [Link]
29 Pathak N.; Zaidi R.K. Studies on Seed-Borne Fungi of Wheat in Seed Health Testing Programme. *Arch. Phytopathol. Plant Prot*., 2013, **46**, 389-401. [Link]
30 Rebocho, D. Avaliação da Microflora e de Aflatoxinas nos Grãos/Semente deAmendoim Armazenados na Província de Inhambane. Degree-Universidade Eduardo Mondlane Maputo, Mozambique, 2010.
31 Van Rensburg SJ.; Kirsipuu A.; Pereira Coutinho L.; Van der Watt J. Circumstances Associated with the Contamination of Food by Aflatoxin in a High Primary Liver Cancer Area. *S. Afr. Med. J*., 1975, **49**, 877-883. [Link]
32 Rodriguez-Amaya D.B.; Sabino M. Mycotoxin Research in Brazil: The Last Decade in Review. *Braz. J. Microbiol*., 2002, **33**, 1-11. [CrossRef]
33 Thippeswamy S.; Mohana D.C.; Abhishek R.U.; Manjunath K. Inhibitory Activity of Plant Extracts on Aflatoxin B1 Biosynthesis by Aspergillus flavus. *J. Agric. Sci. Technol*., 2014, **16**, 1123-1132. [Link]
34 United State Department of Agriculture. USDA. Foreign Agricultural Services. World Production: Groundnut. U.S. International Production Assessment Division (IPAD). PSD estimates updated on: 01/2022; 2020. Accessed in February 4th 2022. [Link]
35 United State Department of Agriculture. USDA. Foreign Agricultural Services. FEED THE FUTURE. The US Government's Global Hunger & Food Security Initiative. Feed the Future Mozambique Agricultural Innovations Activity (FTF Inova) Final Technical Report on the Assessment of Consumer Demand for Raw Peanuts; 2019, 21. [Link]
36 World Health Organization/Food and Agriculture Organization of the United Nations. WHO/FAO. Prevention and Reduction of food and Feed Contamination, 2012, 180. Accessed on March 1st 2022 [Link]
37 WHO. Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme (GEMS/Food), 2006. Accessed on March 1st 2022.
38 Zuza E.; Mondjana A.; Muitia A.; Amane M. October. Effects of Harvesting Date on Aflatoxin Contamination in Groundnuts in Northern Mozambique. In *The Fifth RUFORUM Biennial Conference and African Higher Education Week; Nampala MP.; Egeru A.; Tusiime G.; Osiru M.; Mensah S.; Adipala E. Eds*, 2016, 167-172. [Link]