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Impact of conservation farming practices on *Aspergillus* population density, peanut aflatoxin level, and exposure risk in Zambia

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Aflatoxin contamination in peanuts (*Arachis hypogaea* L.) by aflatoxin-producing *Aspergillus* spp. is a serious problem worldwide affecting human health and restricting trade. Recently, conservation agriculture practices such as minimum tillage, crop rotation, soil surface cover, intercropping, and planting in basins alongside trees that enhance soil fertility, have been introduced in Zambia as tools to conserve soil moisture, increase peanut yield, and reduce the risk of aflatoxin contamination. However, a limited understanding exists of the impact of conservation agriculture on *Aspergillus* population densities in the soil and aflatoxin concentrations in crops grown under conservation agriculture. This study assessed the impact of conservation agriculture on (i) *Aspergillus* communities profile in soil cropped to peanuts, (ii) peanut aflatoxin contamination levels, and (iii) influences of the practices on liver cancer risks as a result of consuming peanuts grown in basins, peanuts grown in basins in farms having *Gliricidia sepium* trees (agroforestry), and peanuts grown on ridges (conventional tillage). Densities of *Aspergillus* spp. in soils ranged from 10 to 7,400 CFU/g (mean = 295 CFU/g). The densities differed among farming techniques ($p = 0.0011$) with conventional tillage (357 CFU/g) supporting higher densities than agroforestry (296 CFU/g) and peanuts grown in basins (230 CFU/g). *Aspergillus* communities were dominated by fungi with S morphology (55%; which is composed of several morphologically similar species), followed by *A. parasiticus* (34%), and the L morphotype of *A. flavus* (11%). Over 60% of the peanut samples were contaminated but there were no differences among farming techniques. Mean aflatoxin contents in peanuts were 110, 99, and 87, $\mu\text{g}/\text{kg}$ for agroforestry, conventional tillage, and peanuts grown in basins, respectively. Probable daily aflatoxin intake ranged from 12.7 to 767.1 ng/kg body weight/d, depending on the age of consumers. The estimated liver cancer risk due to consumption of raw peanuts (0.334 to 20.128 cases/100,000 persons/year) was highest among children below 5 years and least for young adults. The results showed that growing peanuts in basins has the potential to reduce *Aspergillus* populations in soil. Possibly, planting in basins and agroforestry had minimal effect on water runoff and did not support sufficient moisture retention in the soil to reduce *Aspergillus* spp. densities and aflatoxin accumulation in the peanuts. Thus, more research is needed to improve the effectiveness of peanut

cultivation in basins in shaping the *Aspergillus* species profiles for ultimately reducing aflatoxin in peanuts to safe levels. Given the high exposure of Zambia's population, especially children, and that contaminated food is the major source of exposure, it is essential that appropriate technical, policy and institutional measures are promoted for aflatoxin mitigation in Zambia.

KEYWORDS

mycotoxin, agroforestry trees, conservation agriculture, exposure assessment, liver cancer

1 Introduction

In many regions of the world, peanut (*Arachis hypogaea* L.) is an essential oil and food security crop. In Zambia, peanut is considered a “woman's crop”, ranks second in cultivation after maize, and is highly valued because it helps meet the nutritional needs of people in rural areas (Mofya-Mukuka and Shipekesa, 2013; Tembo and Sitko, 2013; Mofya-Mukuka and Chisanga, 2014). Smallholder peanut production in Zambia is rain-fed with the largest production taking place in Eastern (31%), Northern (16%), and Southern (12%) provinces [Ross and de Klerk, 2012; MAL (Ministry of Agriculture and Livestock), 2013; Sichoongwe et al., 2014].

Despite its strategic food and nutrition security roles, peanut yields in Zambia are low with average yields ranging from 220 to 1,500 kg/ha (ZARI, 2018, 2019). Erratic rainfall patterns, prolonged dry spells and/or drought along with pest and disease pressures, poor soils and inaccessibility to high quality, improved seeds are factors limiting peanut production in Zambia (Mofya-Mukuka and Chisanga, 2014; Desmae and Sones, 2017). Furthermore, the quality, nutritional value, and safety of peanuts produced in Zambia are lowered by molds that often produce toxic metabolites, such as aflatoxins, in infected kernels (Bumbangi et al., 2016; Njoroge et al., 2017; Kachapulula et al., 2017a).

Aflatoxins, which are cancer-causing, growth-retardant and immune-suppressive toxic substances produced mainly by *Aspergillus flavus* (Link) and *A. parasiticus* (Speare), are the most common mycotoxins found in Zambian peanuts. About 12–25% of raw peanut grains or powder and 53–80% of processed peanut butter obtained at farmgate or markets contain aflatoxin exceeding levels considered safe for human consumption (10 µg/kg) (Bumbangi et al., 2016; Njoroge et al., 2017; Kachapulula et al., 2017a). High aflatoxin levels can worsen food insecurity and malnutrition. Coincidentally, in Zambia, high malnutrition rates occur in high peanut-producing provinces (Mofya-Mukuka and Shipekesa, 2013; Mofya-Mukuka and Kuhlitz, 2016), where unacceptable aflatoxin levels have been found in children's weaning and complementary foods (Alamu et al., 2020). Unfortunately, most farmers in Zambia have limited knowledge of aflatoxin health-related risks and do not employ effective aflatoxin management strategy to limit human exposure (Tembo et al., 2023).

Good agronomic practices (GAP), biocontrol using atoxigenic isolates of *A. flavus*, resistant crop varieties, food diversification, and the adoption of improved storage technologies can curtail aflatoxin in foodstuffs and prevent exposure among consumers (Ortega-Beltran and Bandyopadhyay, 2021). However, there are several other practices/techniques that may aid in reducing aflatoxin in peanuts that remain

relatively unexplored. Conservation Agriculture (CA), which entails techniques that support minimal soil disturbance, permanent soil cover, and diversification of crop species, is currently being promoted across Southern Africa, including Zambia, as a climate-smart GAP for managing agroecosystems and preserving the environment for sustainable agricultural productivity, increased profits, and food security (Komarek et al., 2021; Ngoma et al., 2021; Mhlanga et al., 2022; Tufa et al., 2023).

Recently, CA practices (e.g., early planting, crop rotation, intercropping and planting in basins, alongside planting soil fertility enhancing trees) have been introduced in Zambia as tools for conserving soil moisture, increasing peanut yield, and reducing risks of aflatoxin (Kortei et al., 2022). However, CA may have both beneficial effects and some drawbacks (Andersson and D'Souza, 2014; Palm et al., 2014). For instance, mulching and using green manure as a CA practice may facilitate the build-up of soil- and crop residue-borne plant pathogens (Thomas et al., 2007). On the other hand, cultural practices such as manure application, mulching and tied ridges were reported as effective in enhancing peanut safety by reducing aflatoxin levels (Njoroge, 2018).

A recent study revealed that CA practices like tillage regimes had variable effects on soil microbiome without causing any significant change to fungal diversity (Li et al., 2020). In another study, different CA tillage regimes were found to alter soil fungal alpha diversity (Wang et al., 2016). However, limited literature exists on the effect of CA on the fungal component of soil microbial community structure under peanut cropping system. Furthermore, CA effects on *Aspergillus* species and subsequent aflatoxin levels in peanuts remain to be investigated. The objectives of the current study were to determine the impact of CA on (i) aflatoxin-producing *Aspergillus* species composition in soil cropped to peanuts, (ii) peanut aflatoxin contamination levels, and (iii) aflatoxin exposure risks. We hypothesized that CA practices in peanuts support more diverse fungal communities with reduced densities of aflatoxin-producing fungi leading to reduced aflatoxin contamination. The results can aid in enhancing peanut productivity and quality in different agro-ecological zones (AEZs) of Zambia using CA approaches.

2 Methodology

2.1 Survey sites

To assess the impact of the CA farming methods on aflatoxin-producing fungi and aflatoxin levels, a two-year survey was conducted

in AEZs II and III of Zambia. AEZ II has the most fertile soils in Zambia with a growing period of 100–140 days and receives moderate rainfall (800–1,000 mm *per annum*). AEZ III receives the highest amount of rainfall (>1,000 mm *per annum*) and has a growing period of 120–150 days (Bunyolo et al., 1995). AEZ III has highly weathered, leached, and often extremely acidic soils that may be toxic to crops (Muimba-Kankolongo, 2018; Muimba-Kankolongo et al., 2021). In AEZ II, soil and crop samples were collected from seven districts in Eastern Province (i.e., Lundazi, Chipata, Katete, Mambwe, Sinda, Petauke, and Nyimba), while in AEZ III, samples were collected from Mpika and Chama districts of Muchinga Province (Figure 1). Usually, farmers in these districts sow peanuts from mid-November to late January and crop harvesting period stretches from May to July. The survey was carried out immediately after farmers harvested their crops between June and July 2017 and 2018 for the 2016/2017 and 2017/2018 cropping seasons, respectively.

2.2 Crop and soil sampling

Overall, 270 in-shell peanut samples were collected from female-managed and owned peanut fields. Farmers grew their crops in three types of cropping systems: (1) in basins without (CAB) or (2) with 2–5 year-old *Gliricidia sepium* agroforestry trees (AGF), and (3) on ridges in fields lacking *G. sepium* (CT). In each cropping season, five samples per farming technique were collected from each of the nine districts,

giving a total of 135 samples (i.e., AGF=45, CAB=45, and CT=45). Every sample consisted of 5 kg in-shell peanuts. The samples were labeled, geo-referenced, and transported to the pathology laboratory at Zambia Agriculture Research Institute (ZARI), Mt. Makulu, Chilanga for processing. The pods of each sample were hand-shelled and kernels ground using a laboratory blender (Waring Commercial, Springfield, MO) that was cleaned between samples with 70% methanol to prevent cross-contamination of aflatoxins and microorganisms.

For the soil samples, three peanut fields per cropping system (i.e., AGF, CAB, and CT) were randomly selected and sampled in each district in October 2018. From each field, a sample consisted of 30 subsamples of soil (~10 to 20 g each) collected from the surface to a depth of 5 cm at different sites across the entire field using a diagonal sampling framework. The 30 subsamples were mixed to make one composite sample. A total of 81 soil samples (3 fields × 3 cropping systems × 9 districts) were collected, placed in plastic Ziplock bags, stored in a cooler box, and transported to the laboratory. Samples were sieved to remove stones and debris. Grain and soil samples were stored at 4°C prior to *Aspergillus* isolation and aflatoxin quantification (only for kernels).

2.3 *Aspergillus* isolation and characterization

Aspergillus fungi were isolated from the 81 soil and 270 peanut samples on Modified Rose Bengal Agar (MRBA) using the dilution

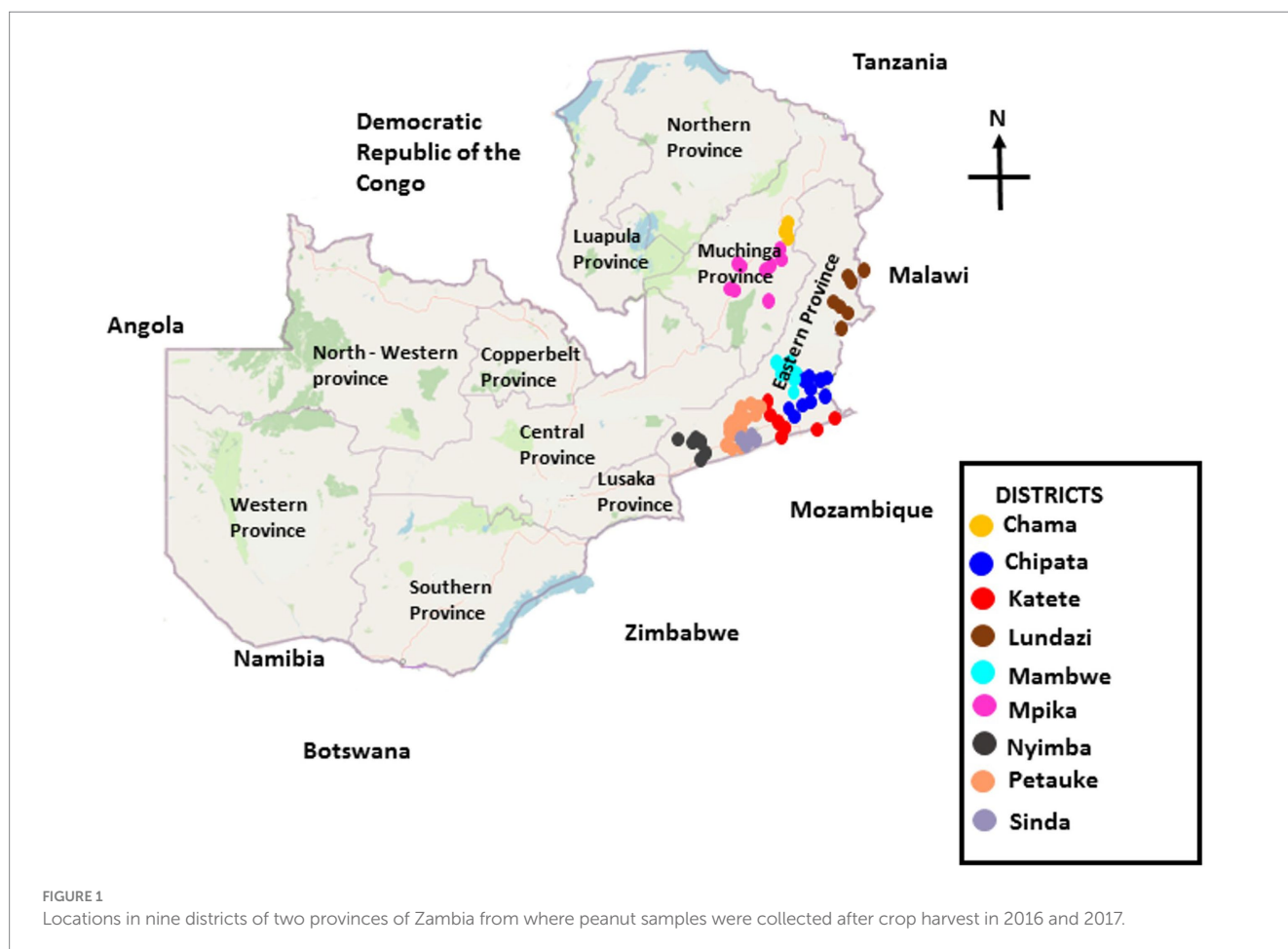


plate technique (Cotty, 1994; Probst et al., 2007). One liter of MRBA comprised of 3 g Sucrose, 3 g NaNO₃, 0.75 g KH₂PO₄, 0.25 g K₂HPO₄, 0.5 g MgSO₄·7H₂O, 0.5 g KCl, 1 mL micronutrients, 5 mL Rose Bengal, 5 mL of chloramphenicol and 20 g agar. After sterilizing at 121°C for 20 min, the media was cooled, supplemented with 10 mL/L dichloran and 5 mL/L streptomycin sulfate, and then dispensed into 9 cm Petri dishes. One to five gram of soil or peanut flour sample was weighed and suspended in 10 mL sterile distilled water. Thereafter, 50–200 μL of the sample suspension was spread on MRBA plates and then incubated (dark, 31°C, 3 days). The number of *Aspergillus* colonies was counted and recorded before transferring 15 colonies onto 5/2 agar [5% V8 Juice (Campbell Soup Company, Camden, NJ, United States); 2% agar, pH 6.0] in 5.5 cm Petri dishes and incubated (dark, 31°C, 5 days). Species characterization was based on morphological features, colony pigmentation, and conidial size and appearance (Klich and Pitt, 1988; Cotty, 1989). On 5/2 agar, *A. parasiticus* colonies were dark green, while *A. flavus* and fungi with S-morphotype were light green and the latter produced numerous sclerotia (Cotty, 1994). Isolates with abundant small and smooth sclerotia (average < 400 μm) were classified as fungi with S-morphotype (Cotty and Cardwell, 1999). Isolates with smooth conidia and large sclerotia (average diameter > 400 μm) were classified as *A. flavus* L morphotype. Isolates with rough conidial surfaces were classified as *A. parasiticus* (Klich and Pitt, 1988).

2.4 Aflatoxin quantification in peanut

Total aflatoxin (AF) in each peanut sample was determined using the GIPSA-approved Neogen Reveal Q+ kit (Reveal Q+ for aflatoxin, Neogen Corporation, Lansing, United States). Ground peanut (50 g) was weighed and transferred to a 500 mL Pyrex bottle. Then, 250 mL 65% ethanol was added to the bottle, which was capped and shaken at 500 rpm for 3 min on a MaxQ 2000 orbital shaker (Thermo Fisher Scientific Inc., Bartlesville, United States). The extract was allowed to settle, the supernatant filtered through a Whatman No. 1 filter paper (Whatman Intl. Ltd., Maidstone, United Kingdom), and the AF in the extract was quantified using a Neogen AccuScan Reader. The reader can quantify AF from 2 μg/kg to 150 μg/kg. The extracts of samples containing AF levels exceeding the upper limit were diluted with 65% ethanol and re-assayed. Aflatoxin reference material (Neogen Corporation, Lansing, United States) with a predetermined AF level was included in the analyses after every 20 sample runs to serve as a quality control check.

2.5 Estimation of daily food consumption and body weight of consumers

A one-time survey was undertaken in August 2018 to determine the quantity of peanuts consumed by individuals within farming households of Chipata, Lundazi, and Katete districts of Eastern Province. The study focused on raw peanut grain, peanut butter, roasted peanuts, and peanut powder, the most typical forms of peanuts consumed in the province. Dietary intake was based on the weighing method for each selected peanut product (Alamu et al., 2020) following the farmers' estimation of quantities consumed per day. The estimated quantity for consumption indicated by each farmer was weighed and recorded. For children, caretakers and/or mothers were asked to estimate the quantity given to each child per

day. Overall, 322 respondents participated in the study, among which 138 respondents were from Chipata, 113 from Katete, and 71 from Lundazi. The body weight (bw) of each respondent was measured. Ethical approval was issued by the University of Zambia Biomedical Research Ethics Committee.

2.6 Risk assessment: dietary exposure and potential impact on health

Aflatoxin dietary exposure risk was assessed only for the three districts (i.e., Chipata, Lundazi, and Katete) in which peanut consumption data were collected. The average peanut consumption data for raw grain and peanut powder and the average bw for the different age groups used in the analyses are presented in the Results section. The AFB1 value was obtained for each sample by halving the AF value. Dietary exposure to AFB1 was estimated using the probable daily intake (PDI) approach. For this, the product of mean toxin concentration and peanut consumption rate was divided by the bw as indicated in Equation (1). The margin of exposure (MOE) was then calculated using a Benchmark Dose Lower Limit (BMDL) of 170 ng/kg/bw/day [European Food Safety Authority (EFSA), 2007] (Equation 2). The risk of public health concern was considered when the MOE was lower than 10,000 and/or aflatoxin exposure exceeded 0.017 ng/kg/bw/day.

$$PDI (\mu g / kg \text{ bw} / \text{day}) = (\mu \times C) / B_w \quad (1)$$

Where;

PDI = probable daily intake for AFB1; μ = mean AFB1 level; C = quantity of peanuts consumed in each district; and B_w = average body weight for the different age groups.

$$MOE = BMDL / \text{mean PDI} \quad (2)$$

The risk of developing liver cancer (i.e., aflatoxin-induced hepatocellular carcinoma (HCC) rates in the population) attributed to peanut consumption in the three districts across the different age groups was estimated for AFB1 by obtaining the product of AF potency and mean PDI. The average potency (AP) was computed using (Equation 3) from individual potencies of hepatitis B virus surface antigen (HBsAg)-positive and HBsAg-negative groups. In the analysis, the AFB1 carcinogen potency prior value of 0.02624 cases per year per 100,000 persons in ng/kg bw/d for Zambians aged 15–59 years was considered based on an estimated hepatitis B virus (HBV) prevalence of 5.6% (for this age group) and HBV negative groups of 94.4% (Zambian Ministry of Health, 2018; Nshimbi and Ngoma, 2019).

$$AP = (0.3 \times \text{proportion of HBsAg – positive prevalence rate}) + (0.01 \times \text{proportion of HBsAg – negative prevalence}) \quad (3)$$

2.7 Data analysis

Aspergillus population densities were computed as colony-forming units (CFU)/g of sample using the formula (CFU = [dilution

factor \times number of colonies] divided by sample weight). The dilution factor was computed by dividing the suspension volume by the plated volume. For a species composition, the frequency was computed as the number of given species divided by the total number of isolated species \times 100. All samples in the dataset containing AF values below 2 $\mu\text{g}/\text{kg}$ were replaced with zero. The CFU and *Aspergillus* species characterization datasets were pooled for the two seasons, while that of aflatoxin level was not pooled between the two seasons before conducting statistical analyses. The datasets were then subjected to normality and homogeneity of variance tests using the Shapiro–Wilk and Levene’s test, respectively. Thereafter, the AF and CFU/g datasets were log-transformed before conducting an analysis of variance (ANOVA). Means were separated using Tukey’s studentized range test ($\alpha = 0.05$). All the analyses were carried out using SAS v9.4. Descriptive analyses were also carried out to determine the mean (SD) for food consumption data. To assess the impact of AF on health (exposure potencies), Equations (1)–(3) were used.

3 Results

3.1 Effect of conservation agriculture practices on *Aspergillus* spp. population densities

Aspergillus spp. propagules were present in all soil and grain samples. Irrespective of the AEZ or farming technique, CFU/g of *Aspergillus* spp. in soil ranged from 10 to 7,400 (mean = 295 CFU/g) and 10 to 3,760,000 (mean = 67,726 CFU/g) in grain. Densities in the soil significantly varied by location ($F = 9.55$, $df = 8$; $p < 0.0001$), farming technique ($F = 7.01$, $df = 2$; $p = 0.0011$), and AEZ ($F = 16.33$, $df = 1$; $p < 0.0001$).

Overall, AEZ II soils contained significantly higher *Aspergillus* spp. densities than AEZ III, while conventional tillage systems on ridges (CT) supported higher densities than soils from CAB or AGF farms (Table 1). The densities were influenced by the interaction between farming technique and location ($F = 3.86$, $df = 16$, $p < 0.0001$), but not between AEZ and farming technique ($p > 0.05$). Apart from Mpika and Sinda, in which no statistical difference occurred in fungal population density among farming techniques, significant differences ($p < 0.05$) existed in the fungal densities among the farming techniques for the other seven districts. Compared to CAB, fungal density was higher in the CT system in Chama, Katete, Mambwe, Nyimba and Petauke (data not shown). In Lundazi, AGF contained higher fungal densities than CA but was not significantly different from CT (data not shown).

Equally, fungal densities in grain depended on AEZ ($F = 38.32$, $df = 1$; $p < 0.0001$), location ($F = 8.35$, $df = 8$; $p < 0.0001$) but not on farming techniques ($p > 0.05$). Peanuts from AEZ II had significantly higher densities than peanuts from AEZ III. Mambwe peanuts had the highest densities, while Chipata peanuts had the least (Table 1). The densities in kernels were also affected by the interaction between AEZ and farming technique ($F = 5.37$, $df = 2$; $p = 0.0049$) and location and farming technique ($F = 3.43$, $df = 16$, $p < 0.0001$). In AEZ III, fungal densities were similar among farming techniques. In AEZ II, fungal densities were higher in CT soils compared with CAB farms (data not shown).

TABLE 1 Densities of *Aspergillus* spp. in soil samples cropped to peanuts and in harvested peanut grain samples during the 2017/2018 season expressed as colony forming units (CFU)/g.

Category		<i>Aspergillus</i> propagules (CFU/g)			
		Soil ^y		Crop ^y	
District	Chama ^a	189	dce	171,481	b
	Chipata	339	bcd	1,246	e
	Katete	983	a	16,870	d
	Lundazi	128	de	13,402	d
	Mambwe	132	de	245,810	a
	Mpika ^a	95	e	25,063	cd
	Nyimba	136	de	41,812	c
	Petauke	439	abc	25,802	cd
	Sinda	666	ab	17,277	d
Agroecological zone (AEZ) ^y	II	355	a	45,398	a
	III	138	b	124,280	b
Farming technique ^z	AGF	296	b	55,618	a
	CAB	230	b	59,647	a
	CT	357	a	88,463	a

^aRepresents districts located in Muchinga Province (AEZ III), while the rest are found in the Eastern Province (AEZ II).

^yWithin each category (i.e., location, AEZ, and farming technique), similar letters in columns of each sample type (soil/grain) are not significantly different ($\alpha = 0.05$) in Turkey’s studentized range test.

^zCAB represents peanut cultivated using conservation agriculture practices (i.e., peanuts planted in basins); AGF represent peanuts cultivated in farms containing 2–5 year-old agroforestry trees (*Gliricidia sepium*), and CT represent peanuts cultivated using the farmer-preferred practice of plowing and planting on ridges.

3.2 Effect of conservation agriculture on *Aspergillus* spp. profile

All soil and grain samples were associated with at least one of the main aflatoxin-producing *Aspergillus* spp. Among these were *Aspergillus flavus* L morphotype, *A. parasiticus*, and fungi with S morphology. Overall, *A. flavus* was recovered from 49% of soil samples. In contrast, 89 and 88% of soil samples contained *A. parasiticus* and fungi with S morphology, respectively. Irrespective of farming technique, *Aspergillus* species composition was dominated by fungi with S morphology (55%), followed by *A. parasiticus* (34%) and *A. flavus* L morphotype (11%) (Figure 2).

In soil, *Aspergillus* spp. distribution depended on the fungal type ($F = 18.36$, $df = 2$; $p < 0.0001$) but not on location ($F = 0.76$, $df = 8$; $p = 0.63$) or farming technique ($F = 0.94$, $df = 2$; $p = 0.39$). The occurrence and distribution of fungi with S morphology and *A. parasiticus* were similar but significantly higher than that of *A. flavus* L morphotype ($p < 0.0001$) (Figure 3).

Although there was no interaction between location and farming technique ($F = 0.62$, $df = 16$; $p = 0.86$), *Aspergillus* spp. distribution in soil was influenced by the interaction between location and fungal type ($F = 4.44$, $df = 16$; $p < 0.0001$) as well as between fungal type and farming technique ($F = 2.63$, $df = 4$; $p = 0.0376$). In CT and CAB farms, the densities of *A. parasiticus* and fungi with S morphology were similar but statistically higher than that of *A. flavus* L morphotype. In AGF farms, the frequency of *A. flavus* was similar

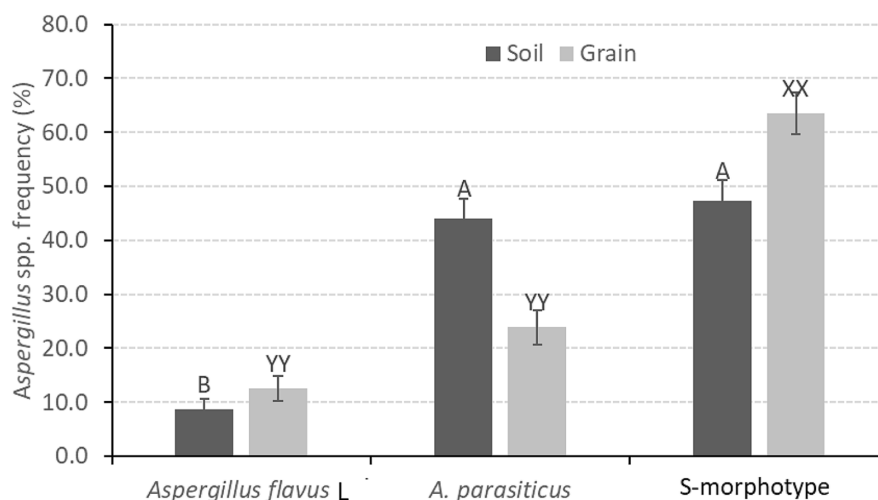


FIGURE 2

The distribution of *Aspergillus* spp. in soil and peanut grain samples collected from Eastern and Muchinga provinces of Zambia following the 2017/2018 cropping season. Bars followed by the same letter for the two sample types are not significantly different at $p = 0.05$, Tukey's studentized range test.

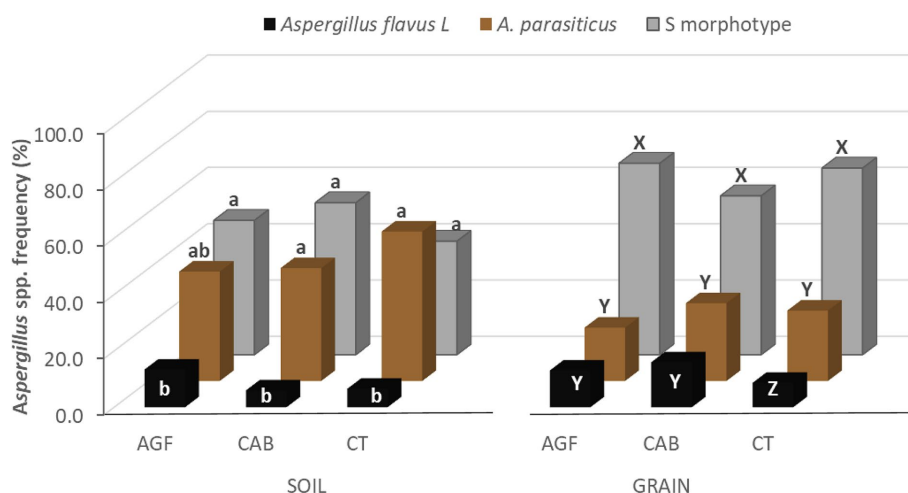


FIGURE 3

The effect of the farming technique on *Aspergillus* profile in soil and peanut samples collected from Eastern and Muchinga provinces of Zambia. The CAB represents peanuts cultivated using a conservation agriculture practice (i.e., peanuts planted in basins); AGF represents peanuts cultivated in farms containing 2–5-year-old agroforestry trees (*Gliricidia sepium*), and CT represents peanuts cultivated using the farmer-preferred practice of plowing and planting on ridges. For each sample type and farming technique, bars with the same letters are not significantly different at $p = 0.05$, Tukey's studentized range test.

to *A. parasiticus* but significantly lower than fungi with S morphology (Figure 3).

Across districts, the occurrence and distribution of the three *Aspergillus* types in soil samples from Chama, Katete, Chipata, Lundazi, and Mambwe were dominated by fungi with S morphology and significantly higher than *A. flavus* L morphotype ($p < 0.0001$). In Mpika, Nyimba, Petauke, and Sinda, *A. parasiticus* was the dominant *Aspergillus* spp. (data not shown).

Aspergillus flavus L morphotype was not recovered from 45% of the peanut samples (i.e., 10 CAB, 11 AGE, and 16 CT samples). Fungi

with S morphology were detected in all samples except in 2 AGE, 2 CAB, and 3 CT kernel samples. *Aspergillus parasiticus* was not recovered from 22% of kernel samples (i.e., 9 AGE, 7 CAB, and 4 CT samples). Although statistically lower than the frequency of fungi with S morphology, the frequencies of *A. parasiticus* and *A. flavus* in kernels were not significantly different (Figure 3).

Equally, *Aspergillus* spp. distribution in kernel samples depended on the fungal type ($F = 111.13$, $df = 2$; $p < 0.0001$) but not on AEZ ($F = 0.04$, $df = 1$; $p = 0.84$), location ($F = 1.06$, $df = 2$; $p = 0.38$) or farming technique ($F = 0.54$, $df = 2$; $p = 0.58$). *Aspergillus* spp.

distribution, however, depended on the interaction between location and fungal type ($F=12.57$, $df=16$; $p<0.0001$), location and farming technique ($F=13.89$, $df=16$; $p<0.0001$) and fungal type with farming technique ($F=7.75$, $df=4$; $p<0.0001$). In peanuts from CT farms, the frequency of fungi with S morphology was significantly higher than that of *A. parasiticus*, which in turn was higher than that of *A. flavus* L morphotype ($p<0.0001$). For the other AGF and CAB kernel samples, *A. parasiticus* and *A. flavus* frequencies were similar but statistically lower than fungi with S morphology (Figure 3). Across districts, the frequency of *A. flavus* L morphotype and *A. parasiticus* in peanuts were similar but significantly lower than that of the fungi with S morphology in Chama, Katete, Mambwe, and Mpika ($p<0.0001$). In Chipata, Lundazi, and Petauke, the frequency of fungi with S morphology was significantly higher than that of *A. parasiticus*, which in turn was higher than the *A. flavus* L morphotype. In Nyimba, no significant difference was observed in fungal type distribution. In Sinda, the population of the S morphology fungi was similar to the *A. flavus* L morphotype but higher than *A. parasiticus* (data not shown).

3.3 Effect of CA on peanut aflatoxin levels

Aflatoxin incidence and contamination levels varied between the two sampling periods, with low incidence and severity recorded in the 2016/2017 cropping season (incidence = 37.8%) compared to the 2017/2018 cropping season (incidence = 83.0%) ($F=31.87$, $df=1$; $p<0.0001$). Peanuts harvested following the 2017/2018 cropping season contained at least three times higher aflatoxin levels (range = ND–5,350 $\mu\text{g kg}^{-1}$; mean = 165.2 $\mu\text{g kg}^{-1}$) than those harvested in 2016/2017 (range = ND–1,308 $\mu\text{g kg}^{-1}$; mean = 32.4 $\mu\text{g kg}^{-1}$). The proportion of non-compliant peanut samples as per the European Union (EU; 4 $\mu\text{g kg}^{-1}$), Common Market for Eastern and Southern Africa (COMESA (provisional) = 10 $\mu\text{g kg}^{-1}$), and the US-Federal Drugs Administration (US-FDA; 20 $\mu\text{g kg}^{-1}$) regulatory limits was moderate across the districts but depended on the season. Peanuts from 2016/2017 had less probability of rejection than those from 2017/2018 (Table 2).

Aflatoxin levels also depended on planting time ($F=31.6$, $df=1$; $p<0.0001$), peanut variety ($F=2.69$, $df=7$, $p=0.0113$), drying technique ($F=5.07$, $df=3$, $p=0.0018$) and location ($F=2.68$, $df=8$; $p=0.0079$), but not on the farming technique ($p>0.05$). Late planting from 20th December onwards (mean AF = 98.3 \pm 27.7 $\mu\text{g kg}^{-1}$) supported higher AF than planting before mid-December (mean AF = 71.8 \pm 9.6 $\mu\text{g kg}^{-1}$) or in November (mean AF = 66.9 \pm 13.6 $\mu\text{g kg}^{-1}$). Across districts, peanuts from Chama and Katete had higher AF than peanuts from Mambwe (Table 2).

Though AF levels depended on the cropping season, no interaction was observed between farming technique and season ($p>0.05$). Analysis of pooled data showed that farming techniques did not affect AF levels ($p=0.97$). Irrespective of the season, 32% (AGF), 31% (CAB), and 29% (CT) of peanuts had AF exceeding the 20 $\mu\text{g kg}^{-1}$ US-FDA acceptable limit (Figure 4). About 15% of the samples had high AF levels, i.e., >100 $\mu\text{g kg}^{-1}$. In the two years, there were 33–43%, 26–33% and 18–25% of samples not meeting EU, COMESA, and US-FDA regulatory limits, respectively (Table 3).

3.4 Body weight of consumers

Of the 322 respondents persons, 138 were from Chipata, 113 from Katete, and 71 from Lundazi. There were 201 female and 121 male respondents. Their age ranged from 9 months to 87 years, with 13.7% children under 5 years of age, 4.3% children aged 6–9 year, 23% adolescents (10–19 year), 18.9% young adults (20–35 year), 24.2% adults (36–55 year), and 15.8% seniors (> 55 year). The average bw of respondents belonging to each category is presented in Figure 5.

3.5 Consumption pattern of peanuts in Eastern Province

All respondents reported that peanut is part of their dietary and nutritional requirements. In the Eastern province, peanuts may be consumed every week in different forms (boiled, roasted, eaten fresh, pounded into paste and added to relish dishes or *samp*-breakfast dish made from pounded fresh maize), and occasionally when used to make *cimponde* (peanut butter). Most households utilize peanuts daily or 2–3 times a week at harvest periods when peanuts are in plenty. Peanut consumption patterns, however, varied with location, age group, and form. Table 4 shows the quantity of peanut-based products consumed by different age groups in the selected districts. In all the districts and age groups, peanut butter was the least consumed peanut-based product by all age groups, with a consumption rate of 56.4–116.9 g/d. In the Katete district, for under-five children, children, and adolescents, peanut powder was the most consumed product (154.3 to 180.9 g/d), while for young adults and senior adults, the highest consumption rate was with raw nuts. In this district, adults consumed roasted nuts more (Table 4).

3.6 Risk assessment: dietary exposure and potential health impact

AFB1 dietary exposure among consumers of peanut-based foods was in the range of 2 to 5,571 ng/kg bw/d but varied with the form of utilization, location, age group and season (Table 5). Generally, consumption of peanuts in its raw grain form resulted in lower exposure to AFB1 (avg. PDI = 12.7–724.4 ng/kg bw/d) than when utilized in powder form (PDI = 14.8–1,040.7 ng/kg bw/d) (Tables 5–7). In all districts and cropping seasons, children below five years of age exhibited the highest dietary exposure to aflatoxin, followed by those within the 5–9-year age bracket. On the other hand, the lowest dietary exposure in all districts within the two years was observed among those older than 55. Except for the Chipata district, consumer dietary exposure to aflatoxins was higher following the 2017/2018 cropping season than that of peanuts farmed during the 2016/2017 season (Tables 5–7).

The MOE (range = 0.222–13.348) exceeded the tolerable limit of 0.017 ng/kg bw/d for all age groups in all districts, greatly posing a hazardous health risk to the consumers. Thus, the consumption of peanuts in grain or powder form led to an estimated HCC rate of 0.387 to 27.308 cases/100,000 persons/yr (CPY) for powder and 0.334 to 20.128 CPY for kernels. Depending on the season and the form in which the peanut was consumed, children (1–9 years) with an estimated rate of up to 27.308 CPY were at the most significant risk of

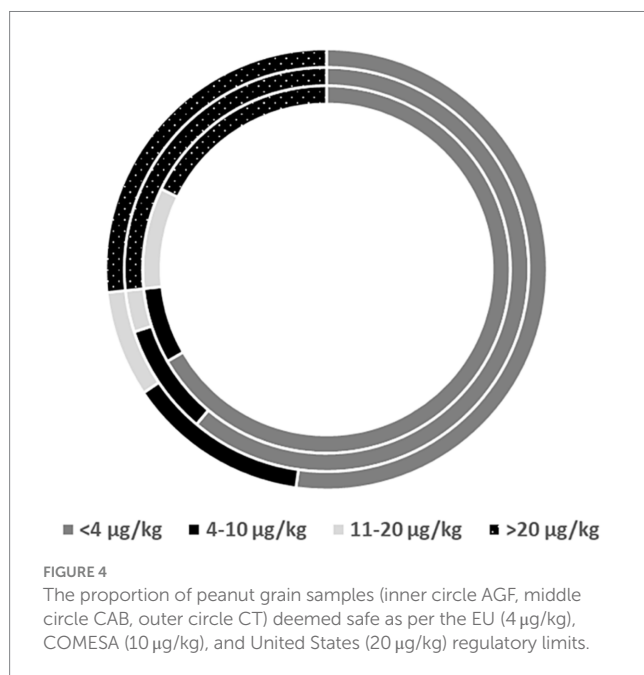
TABLE 2 Level of total aflatoxins detected in peanuts and the potential of rejection due to aflatoxin contamination in different districts of Zambia during the 2016/2017 and 2017/2018 cropping seasons.

Cropping season	District ^a	N	% P ^a	Total aflatoxin ($\mu\text{g kg}^{-1}$)		% rejections for human consumption ^b		
				Range	Mean	EU	COMESA	United States
016/2017	Chama	15	60.0	ND ^c -1,308	119.3	46.7	33.3	33.3
	Chipata	15	60.0	ND-628	75.3	60.0	46.7	33.3
	Katete	15	33.3	ND-138	18.7	33.3	13.3	13.3
	Lundazi	15	40.0	ND-51	12.1	33.3	26.7	6.7
	Mambwe	15	13.3	ND-76	7.1	13.3	6.7	6.7
	Mpika	15	20.0	ND-60	5.6	13.3	6.7	6.7
	Nyimba	15	46.7	ND-110	17.3	33.3	20.0	13.3
	Petauke	15	26.7	ND-136	16.3	26.7	20.0	13.3
	Sinda	15	40.0	ND-121	20.3	40.0	20.0	13.3
2017/2018	Chama	15	100	2–5,350	613.2	53.3	40.0	40.0
	Chipata	15	73.3	ND-213	26.9	40.0	26.7	20.0
	Katete	15	86.7	ND-1,169	282.4	53.3	53.3	46.7
	Lundazi	15	93.3	ND-1,250	125.8	60.0	60.0	40.0
	Mambwe	15	60.0	ND-63	6.3	6.7	6.7	6.7
	Mpika	15	73.3	ND-1,100	150.1	40.0	33.3	33.3
	Nyimba	15	73.3	ND-430	85.2	53.3	46.7	26.7
	Petauke	15	100	2–740	110.9	60.0	46.7	40.0
	Sinda	15	86.7	ND-431	85.7	53.3	53.3	46.7

^aProportion (%) of samples with toxin concentration above the limit of detection ($2\mu\text{g/kg}$).

^bProportion of samples with total aflatoxin concentration exceeding the regulatory limit of the European Union ($4\mu\text{g/kg}$), COMESA ($10\mu\text{g/kg}$), and United States Food and Drug Administration ($20\mu\text{g/kg}$).

^cNot detected; limit of detection $2\mu\text{g kg}^{-1}$.



developing LC, followed by adolescents (up to 15 CPY). Across districts, peanuts collected from Katete (0.541–27.308 CPY) posed higher health risks to the public when compared to Chipata (0.651–9.738 CPY) and Lundazi (0.334–14.711 CPY) (Tables 5–7).

4 Discussion

Peanut production, spurred by private sector industry demand, remains one of the most important economic activities of smallholder farmers, especially women, in the Eastern and Muchinga Provinces of Zambia. The propagules of toxigenic *Aspergillus* spp. were highly prevalent in soil (95 to 983 CFU/g) and peanut samples (e.g., 1,246 to 245,810 CFU/g of peanut powder) collected from the two provinces of Zambia. Generally, the toxigenic *Aspergillus* species are soil-dwelling ubiquitous molds known to exist as saprophytes in different ecosystems (e.g., agricultural croplands) or as decay-causing molds on food grains, wild fruits and insects (Wilson et al., 2002; Horn, 2003; Kachapulula et al., 2017b). In Zambia, *Aspergillus* spp. are common in maize and peanut farms as well as in uncultivated forest soils in various AEZs (Kachapulula et al., 2017b). Such findings confirm the diverse ecologies in which these fungi can thrive.

In our study, it was observed that soils and peanut samples collected from AEZ II supported higher *Aspergillus* propagules than AEZ III. This is in line with the findings of Kachapulula et al. (2017a), wherein aflatoxin prevalence and severity were lower (probably due to low *Aspergillus* load in that environment) in the high-rainfall belt of Zambia when compared to the other AEZs. A study by Monyo et al. (2012) also showed that populations of aflatoxigenic fungi were higher in the hotter regions of neighboring Malawi. Contrary to our findings, Njoroge et al. (2016) reported higher *Aspergilli* populations from the cooler plateau regions compared to the hot Luangwa Valley; however,

TABLE 3 The effect of conservation agricultural practices on aflatoxin levels in peanuts collected between 2017 and 2018 and the potential of rejection due to aflatoxin contamination.

Farming technique ^u	% P ^v	Total aflatoxin ($\mu\text{g kg}^{-1}$)				% Rejections for human consumption ^y		
		Range	Median	Mean \pm SD ^w	P95 ^x	EU	COMESA	United States
AGF	57.8	ND ^z -5,350	2.4	110.4 \pm 61.2	335.0	33.3	26.7	17.8
CAB	70.0	ND-1,260	2.9	87.0 \pm 24.7	395.5	34.0	26.0	24.0
CT	64.4	ND-1,880	5.7	99.0 \pm 30.7	368.0	43.0	33.0	25.0

^uAGF represents peanuts cultivated in farms containing 2–5-year-old agroforestry trees (*Gliricidia sepium*), CAB represents peanuts cultivated using conservation agriculture practices (i.e., peanuts planted in basins); and CT represent peanuts cultivated using the farmer-preferred practice of plowing and planting on ridges.

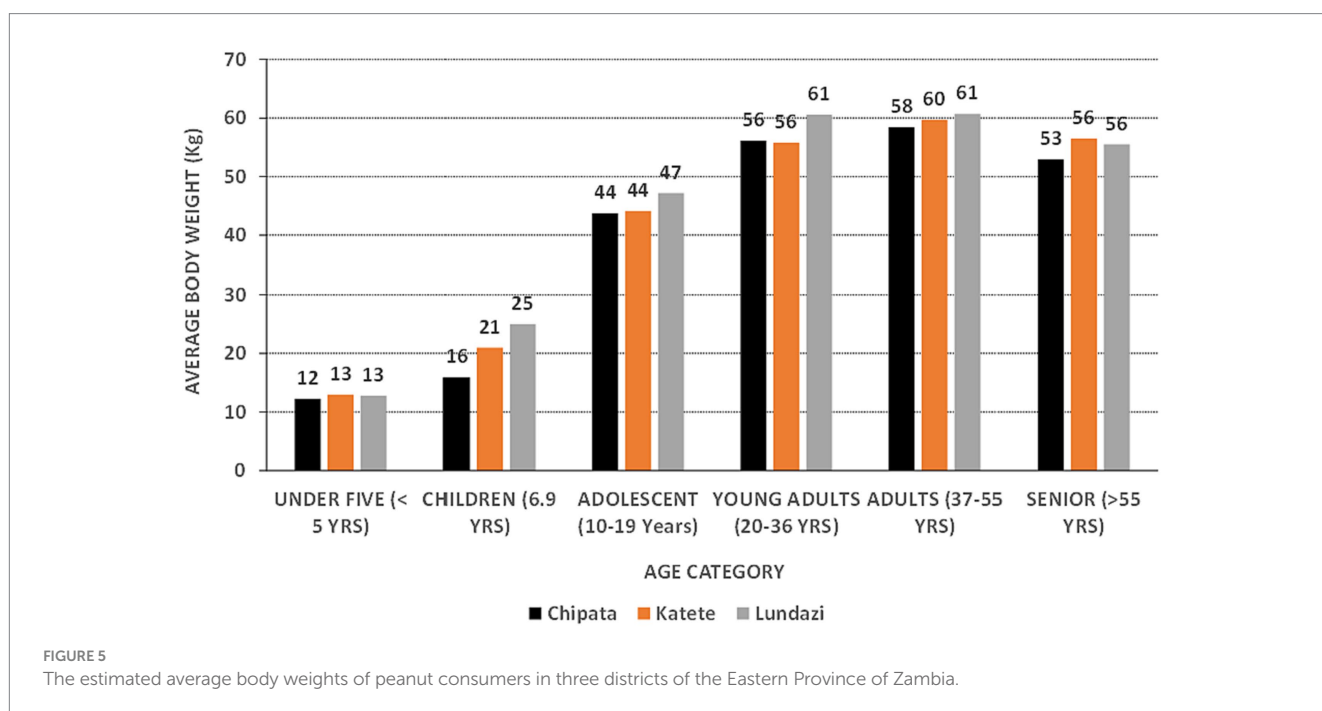
^vProportion (%) of samples with toxin concentration above limit of detection ($2 \mu\text{g/kg}$).

^wSD = standard deviation.

^x95th percentile.

^yProportion of samples with total aflatoxin concentration exceeding the regulatory limit of the European Union ($4 \mu\text{g/kg}$), COMESA ($10 \mu\text{g/kg}$), and United States Food and Drug Administration ($20 \mu\text{g/kg}$).

^zNot detected; limit of detection $2 \mu\text{g kg}^{-1}$.



in that study, flooding had occurred in that Valley before soil sampling, which may have affected the fungal population densities in the area. *Aspergillus* spp. exist as aerobic fungi in the soil's upper profiles. Environmental factors like waterlogging or deposition of alluvial soils from floods can significantly reduce the population in the soil (Horn, 2003). Soils collected from the conventional tillage of growing on ridges (CT) supported a higher *Aspergillus* population than those obtained from CAB or AGF farms (i.e., those containing *G. sepium* trees). Soil fertility factors such as organic matter content, nitrate and extractable phosphorus are generally known to affect *Aspergillus* spp., *Fusarium* spp., and the soil's total fungal density. For instance, in Mississippi, Zablotowicz et al. (2007) observed the highest *A. flavus* populations in high organic matter plots that were under zero-tillage management systems.

The *Aspergillus* species profiling revealed that the soils and peanuts collected from the two provinces comprised *A. flavus* L-strain, *A. parasiticus*, and the S-morphotype, with *A. flavus* being the least encountered species. According to Kortei et al. (2021), peanut kernels harbor a diversity of toxigenic and pathogenic fungal species including

mycotoxigenic *Aspergillus* spp. which supports our current findings. The occurrence and distribution of the three *Aspergillus* spp. in soil samples were location-dependent. However, there was no effect of the farming technique on *Aspergillus* species occurrence and distribution, with all the tillage systems supporting relatively lower densities of *A. flavus*. This may suggest that under the current farming system, using agroforestry trees in combination with conservation practices like digging basins may have minimal effect on the *Aspergillus* population density or its composition within the soil environment.

Depending on the location, aflatoxin prevalence was observed to vary from 20 to 60% and 60 to 100% during the 2016/2017 and 2017/2018 cropping seasons, respectively. However, seasonal variability played a significant role in shaping peanut aflatoxin contamination levels, with peanuts cultivated during the drier farming season (i.e., 2017/2018) exhibiting higher incidence and contamination severity than the 2016/2017 cropping season-farmed peanuts that received between normal to above-average rainfall. Such seasonal effects, primarily attributed to climatic variability during the cropping season, are not unique and have been observed both in

TABLE 4 Quantity of peanut-based products consumed by people of various age categories in farming households of three Eastern Province districts, Zambia.

District	Peanut products	Quantity consumed (g/d; mean \pm standard deviation)					
		Under five children (≤ 5 years)	Children (6–9 years)	Adolescent (10–19 years)	Young adults (20–36 years)	Adult (37–55 years)	Senior (>55 years)
Chipata	Peanut powder ^w	120.5 \pm 57.5	125.8 \pm 10.1	211.2 \pm 15.2	181.2 \pm 19.4	202.8 \pm 14.8	96.6 \pm 9.1
	Raw nuts ^x	72.5 \pm 17.5	77.3 \pm 6.2	140.8 \pm 7.8	144.2 \pm 16.1	130.8 \pm 9.8	112.4 \pm 20.6
	Peanut butter ^y	61.0 \pm 10.0	76.6 \pm 7.9	90.8 \pm 6.4	109.9 \pm 11.8	81.6 \pm 6.9	72.6 \pm 7.9
	Roasted nuts ^z	68.0 \pm 11.0	85.0 \pm 6.2	146.6 \pm 7.4	182.3 \pm 20.1	123.5 \pm 7.7	84.6 \pm 10.7
Lundazi	Peanut powder	114.0 \pm 24.6	118.2 \pm 24.2	161.3 \pm 65.4	158.0 \pm 51.1	282.3 \pm 49.7	129.7 \pm 14.8
	Raw nuts	67.0 \pm 10.0	59.5 \pm 12.7	126.2 \pm 23.	137.1 \pm 25.3	156.5 \pm 34.9	111.9 \pm 12.3
	Peanut butter	69.3 \pm 9.2	56.4 \pm 15.6	74.4 \pm 24.0	110.4 \pm 17.5	116.0 \pm 16.8	88.8 \pm 8.7
	Roasted nuts	55.3 \pm 10.3	87.8 \pm 23.7	111.0 \pm 20.3	149.1 \pm 18.2	192.7 \pm 35.6	117.6 \pm 12.2
Katete	Peanut powder	154.3 \pm 35.3	154.3 \pm 35.3	180.9 \pm 27.3	152.2 \pm 6.6	146.3 \pm 13.6	121.4 \pm 19.7
	Raw nuts	107.4 \pm 11.1	70.3 \pm 16.3	148.4 \pm 11.3	181.3 \pm 34.3	127.0 \pm 14.2	145.9 \pm 28.0
	Peanut butter	102.4 \pm 21.7	52.4 \pm 16.5	96.9 \pm 8.4	116.9 \pm 10.2	113.4 \pm 12.0	109.0 \pm 22.0
	Roasted nuts	130.8 \pm 13.4	74.9 \pm 16.2	164.2 \pm 14.0	161.6 \pm 16.1	155.2 \pm 16.4	128.8 \pm 18.4

^wPeanut flour prepared by pounding using traditional mortar and pestle or milled using a mechanical grinder. The powder was often incorporated in maize-based porridge or vegetable relish before eating.

^xPeanut grain consumed as a snack in its raw form.

^yPeanut paste made by roasting dry peanut grains followed by grinding or pounding. The peanut butter was eaten as a bread spread or with “Nshima”.

^zDry-roasted peanut grain that was consumed as a snack.

TABLE 5 Exposure risk assessment: probable daily intake (PDI), average PDI (APDI), and maximum PDI (MPDI) of aflatoxin B1 in peanuts produced and consumed as flour and grain by farming households in Chipata district of Eastern province of Zambia during 2017 and 2018.

Cropping season	Age group ^t	Mean AFB1 ($\mu\text{g kg}^{-1}$) ^u	Flour ^v					Grain ^v				
			Dietary exposure (ng/kg bw/d) ^w			MOE ^x	Pop. risk ^y	Dietary exposure (ng/kg bw/d)			MOE	Pop. risk
			PDI (Range)	APDI (Mean)	MPDI (P95 ^z)			PDI (Range)	APDI (Mean)	MPDI (P95)		
2017/2018	Under five	37.8	10–3,084	371.1	728.3	0.458	9.738	6–1,885	223.3	438.2	0.761	5.859
	Children		8–2,484	299.0	586.7	0.569	7.845	5–1,527	183.7	360.5	0.925	4.82
	Adolescents		5–1,516	182.5	358.1	0.932	4.789	3–1,011	121.7	238.7	1.397	3.192
	Young adults		3–1,014	122.0	239.4	1.394	3.201	3–807	97.1	190.5	1.751	2.547
	Adults		3–1,090	131.2	257.4	1.296	3.442	2–703	84.6	166.0	2.009	2.22
	Seniors		2–573	68.9	135.3	2.466	1.809	2–668	80.4	157.7	2.116	2.109
2018/2019	Under five	13.6	10–1,046	133.6	387.0	1.273	3.505	6–629	80.4	232.8	2.115	2.109
	Children		8–843	107.6	311.7	1.58	2.823	5–518	66.1	191.5	2.571	1.735
	Adolescents		5–514	65.7	190.3	2.588	1.723	3–343	43.8	126.9	3.882	1.149
	Young adults		3–344	43.9	127.2	3.872	1.152	3–274	34.9	101.2	4.865	0.917
	Adults		3–370	47.2	136.8	3.601	1.239	2–238	30.4	88.2	5.583	0.799
	Seniors		2–194	24.8	71.9	6.851	0.651	2–226	28.9	83.8	5.878	0.759

^tRange of age for different groups are children ≤ 5 years old, 6–9 years for children, 10–19 years for adolescents, 20–36 years for young adults, 37–55 years for adults, and > 55 years for senior citizens.

^uAFB1 was estimated by halving the total aflatoxins detected in each sample.

^vPeanut grain was consumed as a snack in raw form. Flour was prepared by pounding/milling and mixing with porridge/vegetable relish.

^wThe average body weights for age groups used in the analyses were: under five = 12.3 kg; children = 15.9 kg; adolescents = 43.7 kg; young adults = 56.1 kg; adults = 58.4 kg; and seniors = 52.9 kg.

^xMargin of exposure.

^yNumber of persons within the population at risk of developing liver cancer per year (cases/100,000 population/year); exposure risks are computed using calculated AFB1 data.

^z95th percentile for each category.

Zambia and elsewhere in the region (Mukanga et al., 2019; Akello et al., 2021). For instance, a study conducted in Zimbabwe showed that aflatoxin levels in maize harvested following a wet season were

significantly lower than in crops harvested following a suboptimal rainfall season (Akello et al., 2021). Early planting of peanuts, i.e., before mid-December, supported less aflatoxin build-up in harvested

TABLE 6 Exposure risk assessment: probable daily intake (PDI), average PDI (APDI), and maximum PDI (MPDI) of aflatoxin B1 in peanuts produced and consumed as flour and grain by farming households in Lundazi district of the Eastern province of Zambia during 2017 and 2018.

Cropping season	Age group [†]	Mean AFB1 (µg kg ⁻¹) [‡]	Flour [‡]					Grain [‡]				
			Dietary exposure (ng/kg bw/d) [‡]			MOE ^x	Pop. risk ^y	Dietary exposure (ng/kg bw/d)			MOE	Pop. risk
			PDI (Range)	APDI (Mean)	MPDI (P95 ^z)			PDI (Range)	APDI (Mean)	MPDI (P95)		
2017/2018	Under five	6.3	9–227	56.3	172.5	3.019	1.478	5–134	33.1	101.4	5.137	0.868
	Children		5–121	29.9	91.5	5.692	0.784	2–61	15.0	46.1	11.308	0.394
	Adolescents		3–87	21.6	66.2	7.867	0.567	3–68	16.9	51.8	10.055	0.444
	Young adults		3–67	16.5	50.5	10.313	0.433	2–58	14.3	43.8	11.885	0.375
	Adults		5–119	29.4	90.0	5.786	0.771	3–66	16.3	49.9	10.436	0.427
	Seniors		2–60	14.8	45.2	11.516	0.387	2–51	12.7	39.0	13.348	0.334
2018/2019	Under five	62.9	9–5,571	133.6	387.0	0.303	14.711	5–3,274	329.5	523.9	0.516	8.646
	Children		5–2,955	107.6	311.7	0.572	7.803	2–1,488	149.7	238.0	1.136	3.928
	Adolescents		3–2,138	65.7	190.3	0.790	5.646	3–1,673	168.3	267.7	1.010	4.417
	Young adults		3–1,631	43.9	127.2	1.036	4.307	2–1,415	142.4	226.4	1.194	3.737
	Adults		5–2,907	47.2	136.8	0.581	7.677	3–1,612	162.2	257.9	1.048	4.256
	Seniors		2–1,461	24.8	71.9	1.157	3.857	2–1,260	126.8	201.6	1.341	3.327

[†]Range of age for different groups are children ≤ 5 years old, 6–9 years for children, 10–19 years for adolescents, 20–36 years for young adults, 37–55 years for adults, and > 55 years for senior citizens.

[‡]AFB1 was estimated by halving the total aflatoxins detected in each sample.

[‡]Peanut grain was consumed as a snack in raw form, while flour was prepared by pounding/milling and mixing with porridge or vegetable relish.

[‡]The average body weights for age groups used in the analyses were: under five = 12.8 kg; children = 25.0 kg; adolescents = 47.2 kg; young adults 60.5 kg; adults = 60.7 kg; and seniors = 55.5 kg.

^xMargin of exposure.

^yNumber of persons within the population at risk of developing liver cancer per year (cases/100,000 population/year); exposure risks are computed using calculated AFB1 data.

^z95th percentile for each category.

TABLE 7 Exposure risk assessment: probable daily intake (PDI), average PDI (APDI), and maximum PDI (MPDI) of aflatoxin B1 in peanuts produced and consumed as flour and grain by farming households in Katete district of the Eastern province of Zambia during 2017 and 2018.

Cropping season	Age group [†]	Mean AFB1 (µg kg ⁻¹) [‡]	Flour [‡]					Grain [‡]				
			Dietary exposure (ng/kg bw/d) [‡]			MOE ^x	Pop. risk ^y	Dietary exposure (ng/kg bw/d)			MOE	Pop. risk
			PDI (Range)	APDI (Mean)	MPDI (P95 ^z)			PDI (Range)	APDI (Mean)	MPDI (P95)		
2017/2018	Under five	9.7	7–480	67.4	316.0	2.522	1.769	5–375	52.6	246.5	3.232	1.380
	Children		7–508	71.4	334.5	2.382	1.873	5–354	49.7	232.8	3.422	1.303
	Adolescents		4–283	39.7	186.0	4.283	1.041	3–232	32.6	152.6	5.221	0.854
	Young adults		3–124	26.4	123.9	6.433	0.693	3–224	31.5	147.5	5.400	0.826
	Adults		2–148	20.8	97.6	8.161	0.547	3–178	25.0	117.3	6.790	0.657
	Seniors		2–169	23.7	111.3	7.160	0.623	2–97	20.6	96.6	8.248	0.541
2018/2019	Under five	141.3	7–4,068	983.1	3758.4	0.173	25.796	5–3,174	767.1	2932.5	0.222	20.128
	Children		7–4,306	1040.7	3978.5	0.163	27.308	5–2,997	724.4	2769.2	0.235	19.007
	Adolescents		4–2,395	578.7	2212.5	0.294	15.186	3–11,965	474.8	1815.0	0.358	12.458
	Young adults		3–1,595	385.3	1473.2	0.441	10.112	3–1,889	459.0	1754.9	0.370	12.045
	Adults		2–1,257	303.8	1161.3	0.560	7.971	3–1,511	365.1	1395.7	0.466	9.580
	Seniors		2–1,433	346.2	1323.7	0.491	9.085	2–1,244	300.6	1149.0	0.566	7.887

[†]Range of age for different groups are children ≤ 5 years old, 6–9 years for children, 10–19 years for adolescents, 20–36 years for young adults, 37–55 years for adults, and > 55 years for senior citizens.

[‡]AFB1 was estimated by halving the total aflatoxins detected in each sample.

[‡]Peanut grain was consumed as a snack in raw form, while flour was prepared by pounding/milling and mixing with porridge or vegetable relish.

[‡]The average body weights for age groups used in the analyses were: under five = 12.9 kg; children = 20.9 kg; adolescents = 44.2 kg; young adults 55.8 kg; adults = 59.7 kg; and seniors = 56.5 kg.

^xMargin of exposure.

^yNumber of persons within the population at risk of developing liver cancer per year (cases/100,000 population/year); exposure risks are computed using calculated AFB1 data.

^z95th percentile for each category.

peanuts, which was also earlier observed in the same province (Njoroge, 2018). An on-station experiment conducted in the Eastern Province of Zambia revealed that a delay in planting peanuts by about 2 weeks after the onset of rains significantly increased aflatoxin contamination levels in harvested peanuts (Njoroge, 2018). It has been postulated that the high risk of aflatoxin contamination in late-planted crops is attributed to erratic rains, especially if it ends before the crop has reached physiological maturity. Apart from aflatoxin contamination risks, the late-planted crop may also have reduced productivity attributed to drought stress. Generally, the smallholder farming communities of Malawi and Zambia prioritize the sowing of maize (a food security crop) and tobacco (a cash crop) over peanuts, which may be planted up to 2 weeks or more after the onset of the rainy season. Therefore, timely planting at the onset of the rains is important in peanut farming for maintaining crop productivity and food safety.

Even though higher densities of aflatoxin-producing *Aspergillus* were found in CT farms compared to the other techniques, planting in basins had no effect on peanut aflatoxin prevalence or severity. According to Mukanga et al. (2019), water harvesting techniques like tied ridges utilize “small ponds” between the rows for retaining moisture in the soil, especially during plant growth and pod development, thereby reducing drought stress and aflatoxin build-up in the harvested peanuts. In our study, planting in basins and incorporation of *Gliricidia* probably had minimal effect on water runoff nor supported sufficient moisture retention in the soil to reduce aflatoxin accumulation in the harvested peanuts.

For the first time, we document an estimated quantity of peanut-based products consumed in the Eastern province of Zambia, a parameter that is required for computing aflatoxin exposure risks attributed to the consumption of such products. Respondents reported consuming peanuts in their raw form or processed as powder and/or peanut butter. The high level of consumption of peanuts and their various forms agrees with a previous report (Mofya-Mukuka and Shipekesa, 2013).

Peanut plays an integral role in livelihood and is a vital food component in the healthy diets of most rural communities (Mofya-Mukuka and Shipekesa, 2013; Jung et al., 2020). Also, a significant increase in the nutritional quality of diets is expected with peanut consumption because peanuts are known to have cardio-protective, anti-inflammatory, and antioxidant properties that may influence blood lipid levels, endothelial function, and inflammatory biomarkers (Mahatma et al., 2016). However, variability in peanut consumption was observed across age groups and locations. Peanut powder, for instance, was observed as the most consumed peanut-based product in the three districts for under-five children (≤ 5 years), children (6–9 years), and adolescents (10–19 years). Zonal variability and consumption patterns of peanuts were also observed in Benin and Togo where peanut butter was least preferred (Gong et al., 2003) as in the Eastern provinces of Zambia. On the contrary, peanut butter is the most consumed peanut-based product in Ghana because the populace uses it for soup preparation (Jolly et al., 2008). In agreement with the findings of our study, He et al. (2013), Jolly et al. (2006), and Gama et al. (2018) observed that the age of the respondents significantly affects the rate of consumption of peanut butter as age groups younger than 22 or older than 35 are less likely to eat peanut butter.

The study revealed that the consumption of peanuts in various forms would lead to high exposure to AFB1 among consumers in the

studied districts. With an estimated PDI ranging from 12.7 to 1040.7 ng/kg bw/d and a MOE of 0.222 to 13.348 ng/kg bw/d, consumption of peanuts in their raw or processed form posed a high health hazard to the different age groups. Similarly, a study by Kortei et al. (2021) reported that consumption of raw peanuts sourced from local markets could result in high exposure to aflatoxin B1 with risks of causing adverse health effects to Ghanaian consumers belonging to different age groups (i.e., infants (0–52 months), children (5–11 years), adolescents (12–18 years), and adults (18–60 years)). According to several studies, exposure to aflatoxins at levels greater than 1 $\mu\text{g}/\text{kg}$ bw/d through consumption of aflatoxin-contaminated food may have adverse health effects on consumers and lead to aflatoxin-related illnesses (Wu and Khlangwiset, 2010; Hove et al., 2016; Adetunji et al., 2017). This was evident in the current study in which a high risk of chronic exposure to aflatoxins was computed for all the different age groups in the sampled districts. During the two-year study period, cancer risks in the range of 0.334 to 27.308 cases/100,000 persons/yr. were calculated among consumers depending on cropping season, location and the different age groups. In a recent study, Ghanaian infants, children, adolescents and adults had an estimated population exposure risk of 1.19×10^{-3} , 6.34×10^{-4} , 3.56×10^{-4} , and 2.69×10^{-4} CPY, respectively. While consumption of peanut powder posed a higher health risk than in its raw grain form, children followed by the adolescent category were at higher risk of developing aflatoxin-related illnesses. It has been reported that among the mothers from Eastern and Southern Provinces of Zambia, the addition of peanut flour to both porridge and vegetable dishes was a common practice and thus may be the primary exposure form for the children (Alamu et al., 2020). Generally, children are known to be more susceptible to diseases, including aflatoxin-related ill effects, because of their undeveloped and/or weak immune systems. The high exposure risk among children and low exposure risk among senior citizens could be attributed to the quantity of peanut-based foods consumed. The children receiving complementary foods or in the weaning stage are offered peanut-based porridge several times a day, increasing the quantity consumed. On the other hand, the elderly generally appeared to consume less peanut-based products, signifying less exposure. Also, the consumption of peanuts in Katete district posed the most significant health hazard. These results confirm the possible health risks across age groups associated with the consumption of aflatoxin-contaminated foods.

5 Conclusion

The current study demonstrated that *Aspergillus* propagules in conventionally tilled land is generally higher than those in conservation agriculture-farmed land. Overall, fungi with S morphology were the dominant *Aspergillus* fungal type followed by *A. parasiticus* and *A. flavus* L morphotype. We found no effect of farming technique on *Aspergillus* spp. occurrence and distribution in all tillage systems. Equally, there was no effect of the farming technique on aflatoxin level. For the first time, we document an estimated quantity of peanut-based products consumed in the Eastern province of Zambia, a critical parameter in computing exposure risks attributed to the consumption of such a product. Consumption of peanuts in various forms can lead to high exposure to AFB1 and cancer risks in Eastern and Muchinga Provinces. Given the high exposure of Zambia's

population, especially children, and that contaminated food is the major source of exposure, it is essential that appropriate technical, policy and institutional measures are promoted for aflatoxin mitigation in Zambia. Aflatoxin contamination is a multi-sectoral issue involving the agriculture, trade and health sectors. These three sectors must work together, using a Food Convergence Innovation concept (Ortega-Beltran and Bandyopadhyay, 2021), to reduce the high aflatoxin exposure and attendant cancer risks among children and other vulnerable populations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by University of Zambia Biomedical Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants and/or their legal guardians/next of kin.

Author contributions

JA: Writing – review & editing, Conceptualization, Funding acquisition, Project administration, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Data curation. EA: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Data curation. CM: Investigation, Writing – original draft, Data curation. PK: Investigation, Writing – review & editing, Writing – original draft. MM: Writing – review & editing, Conceptualization, Methodology. HN: Conceptualization, Methodology, Writing – review & editing. DC: Conceptualization, Funding acquisition, Methodology, Writing – review & editing, Investigation, Project administration, Supervision, Writing – original draft. AO-B: Writing – review & editing, Data curation, Formal analysis. RB: Conceptualization, Writing – review & editing, Funding

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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