



Mycotoxin profile of staple grains in northern Uganda: Understanding the level of human exposure and potential risks

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ABSTRACT

Mycotoxins are toxic metabolites of fungi that contaminate food and feed. These toxins can cause acute and chronic health threats to both humans and animals. In sub-Saharan Africa, exposure to mycotoxins is chronic and under-reported. The study explores contamination of grains (sorghum, maize, groundnut, millet) with four mycotoxins (aflatoxins, fumonisins, ochratoxins, and deoxynivalenol) and dietary exposure to quantify associated health risks in northern Uganda. The results underscored the high prevalence of mycotoxins, only 7% of the samples were free from toxins. Sorghum grains seemed to be the most susceptible to toxin contamination, whereas in millet the toxin levels were, in general, the lowest. Besides, the results showed that the majority of grains were contaminated with more than one mycotoxin and that the toxin pattern was dependent on the grain type. Co-contamination with all four mycotoxins mainly occurred in sorghum grains. Besides the differences between grain types, there were also significant differences in toxins levels depending on the district where the grains came from. The estimated daily intakes for the mycotoxins were far above the recommended tolerable daily intake (TDI), especially for sorghum. So, it can be concluded that the majority of the people whose diet is mainly based on sorghum are exposed to multiple mycotoxins in a single diet and at a dose above the TDI. Such exposure to multiple mycotoxins elevates the associated health risks. Millet grains, which were the least contaminated, can provide an alternative to sorghum. However, to tackle the mycotoxin problem, other control and prevention mechanisms, e.g. good agricultural practices and optimized storage must be further explored and implemented in sub-Saharan Africa.

1. Introduction

Worldwide contamination of food with mycotoxins, produced by several fungi (e.g. *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp.), causes global health issues and negatively impacts the trade of food commodities. Among the mycotoxins; aflatoxins, fumonisins, ochratoxin A, and deoxynivalenol (DON) are of main concern. Aflatoxins and ochratoxin A are toxic metabolites from fungi belonging to the genus *Aspergillus* and *Penicillium* (Alshannaq & Yu, 2017; Wang et al., 2016). Fumonisin and deoxynivalenol are produced by *Fusarium* spp. (Yazar & Omurtag, 2008). Aflatoxin B1 induces liver cancer, immunosuppression, loss of appetite and acute exposure is fatal (International Agency for

Research on Cancer, 2016). Acute aflatoxicosis is a recurring problem in east Africa with a mortality rate of about 29%–33% (Azziz-Baumgartner et al., 2005; Kamala et al., 2018; Serck-Hanssen, 1970). The synergistic interaction between aflatoxin B1 and hepatitis B leads to the rapid development of hepatocellular carcinoma (Kew, 2003). Prevalence of hepatitis B in northern Uganda exceeds 25% (Ochola et al., 2013) which elevates the risk of hepatocellular carcinoma in the region. Therefore, understanding the dynamics of aflatoxins contamination of grain-based food in northern Uganda and estimating the overall health burden in communities is critical in guiding intervention in the region. Ochratoxin A belongs to group 2B human carcinogens and also has neurotoxic, nephrotoxic, and immunosuppressive effects on humans. Fumonisin B1

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and B2 are classified as possible group 2B human carcinogen based on evidence from experimental animals (International Agency for Research on Cancer, 2020). Deoxynivalenol causes ribotoxic stress, alteration of macrophage activation, growth impairment, and immune malfunction (Waché et al., 2009).

The presence of toxic fungal strains in combination with favourable climatic conditions (humid and hot climate) together with poor pre-harvest and post-harvest practices are the principal drivers of mycotoxin contamination of grain (Elias, 2016). As such 25% of the world's food gets contaminated with mycotoxins (Yard et al., 2013). This contamination is severe in sub-Saharan Africa, where nearly 100% of grains in hotspot areas get contaminated with mycotoxins (Udomkun, Wiredu, & Mutegi, 2017). Exposure to mycotoxins in sub-Saharan Africa is chronic as it begins at the infant in breast milk (Asiki et al., 2014; Kang'ethe et al., 2017; Turner, 2013) and worsens due to the consumption of grain-based food.

Many countries set stringent regulations to limit human and animal exposure to mycotoxins in food/feed. The limit for total aflatoxin (B1+B2+G1+G2) ranges from 4 to 20 µg/kg (EC (European Commission), 2006; FDA, 2011; UNBS, 2020). Fumonisin has a limit of 2–4 mg/kg in corn products set by US-FDA (US-FDA, 2001). Ochratoxin A has a limit of 5 µg/kg (Hajok, Kowalska, Piekut, & Ćwieląg-Drabek, 2019). One µg/kg. bw is the minimum quantity of fumonisins and DON that will not present a health risk when consumed daily for a lifetime (EC (European Commission), 2006; Knutsen et al., 2018). Similarly, 120 ng/kg. bw is the tolerable weekly intake (TWI) of ochratoxin A (EC (European Commission), 2006). The regulation standards for some mycotoxins are lacking for most countries in sub-Saharan Africa.

Nearly 80% of the population in Uganda rely on maize, sorghum, millet, and groundnut for their daily calories (Ssewanyana & Kasirye, 2010). The health risk associated with mycotoxins for the community of northern Uganda, whose diet primarily comprises maize, sorghum, and millet, is high (Echodu et al., 2018; Echodu, Maxwell Malinga, Moriku Kaducu, Ovuga, & Haesaert, 2019; Voss & Riley, 2013). Besides, tight regulation by international communities often limits farmers from poor countries' access to the international market (Dubey, Srivastava, & Kumar, 2008). Like other countries in East Africa, Uganda has often experienced its products being rejected to enter the international market because of mycotoxin contamination (Roseline, 2018). Economic loss in some parts of East Africa amounts up to 332 million dollars a year and is a recurring problem (Marechera, 2015). Such economic limitation slows down the national and united nation's sustainable development goal of poverty reduction and good health for all (Sasson, 2012).

To underline the risks to which people from sub-Saharan countries are exposed, this study determines the overall prevalence of four mycotoxins in northern Uganda, estimates the daily intake of the mycotoxins using an average body weight of children and adults in Uganda, and estimates the likelihood of contamination of grains with mycotoxins. Finally, grains most susceptible to mycotoxin contamination in northern Uganda were determined.

2. Materials and methods

2.1. Study area

Northern Uganda is bordered by South Sudan and the Democratic Republic of Congo (DRC) to the north. The region covers 30 districts with a total land area of 85,391.7 km² and a human population of 7,188,139 (Uganda Bureau of Statistics, 2014). It is structured into four sub-regions; Acholi, Lango, Karamoja, and West Nile. Acholi and Lango sub-regions were at the epicenter of 20 years of war (1986–2006) that lead to the displacement of people to internally displaced person camps, the disruption of agricultural activities, the disruption of social services, and the death of many people (Levine, 2016). As a result, the northern region continues to grapple with a high level of poverty compared to the other regions (FEWS NET, 2017). The main economic activity is

predominantly agriculture. Eighty percent of the population are peasant farmers who depend on subsistence agriculture to sustain their daily livelihood requirements (Levine, 2009). The climate has two seasons (wet and dry). Normally the wet season begins in April and extends until November. The rainfall pattern is unimodal with a peak occurring between May, June, July, and August. The average rainfall received is between 600 and 1000 mm per annum. The dry season begins in November and goes until March. In the cropping cycles, grain production normally starts around April at the onset of the rainy season. The temperature can reach 40 °C during the dry season and occasionally drops to a minimum of 20 °C in the wet season. The relative humidity is high during the wet season and low during the dry season.

2.2. Study design

A cross-sectional study was carried out in August 2018 and August and December 2019 from nine districts in northern Uganda. The districts were Gulu (2°47'N, 32°18'E), Nwoya (02°38'N, 32°00'E), Kole (02°24'N, 32°48'E), Oyam (02°14'N, 32°23'E), Omoro (02°35'N, 32°22'E), Lira (02°20'N, 33°06'E), Kitgum (3°13'N, 32°47'E), Pader (2°50'N, 33°05'E), and Lamwo (3°32'N 32°48'E). Two sub-counties that actively grow cereals were identified with the help of teams from the district Agriculture office. Per sub-counties two parishes were selected for collecting samples.

2.3. Household sampling

Each of the households sampled had stored grains from any of the following crops: maize (*Zea mays*), sorghum (*Sorghum bicolor*), millet (*Eleusine coracana*), and groundnut (*Arachis hypogaea*). Every household gave between 0.5 and 1 kg of grains for laboratory quantification of mycotoxins. The number of samples for each grain type that were collected and analysed were: groundnut (n = 66), maize (n = 126), millet (n = 78), and sorghum (n = 130). Samples taken from farmers were transferred to Ziploc bags containing sample identity code. All samples were stored at −20 °C before sample preparation and mycotoxin quantification.

2.4. Quantification of mycotoxins

Grains (0.5–1 kg) were ground into a fine flour using a motorized laboratory grinding mill (IKA, Model M20, Germany). Extraction of aflatoxins, fumonisins, and ochratoxins was done with 70% methanol. In brief, 100 mL of 70% methanol was added to 20g of finely ground samples in clean bottles and shaken for 3 min by hand. It was allowed to settle for 5 min and filtered with Whatman number 1 filter paper. The filtrate was kept for mycotoxin quantification in 2 mL tubes. The extraction of the DON was done using distilled water. Briefly, 20 g of the flour was mixed with 100 ml of distilled water in a clean glass bottle and shaken for 3 min by hand. The mixture was allowed to settle and then filtered using Whatman number 1 filter paper. Filtrates for DON were further diluted with distilled water in the ratio of 1 part of the filtrate to 3 parts of distilled water. Mycotoxin quantification was done with ELISA kits purchased from Romer Labs® Division Holding GmbH (Erber Campus 1, 3131 Getzersdorf, Austria). The plate was then read using a Multiskan ELISA reader with filters set at 630 nm and 450 nm wavelength. The results were calculated using the Romer Labs® spreadsheet. The spreadsheet applies the Log/Logit regression model to construct a calibration curve using the absorbance and concentration of the standard solutions. The mycotoxin concentration in the samples was calculated by interpolation with the calibration curve.

2.5. Specification of ELISA test kit

The AgraQuant® ELISA test kits used were designed for quantitative detection of respective mycotoxins in food and feed samples as indicated

by the manufacture. AgraQuant® Ochratoxin ELISA kits were designed for the quantitative analysis of ochratoxins (A and B) and had a limit of detection (LOD) of 1.9 µg/kg. AgraQuant® fumonisin ELISA test kit (LOD = 0.20 mg/kg) used was designed for the quantitative analysis of fumonisins (B1, B2, and B3). AgraQuant® Total aflatoxin ELISA kit (LOD = 3 µg/kg) used were designed for quantitative analysis of aflatoxins (B1, B2, G1, and G2). AgraQuant® deoxynivalenol ELISA test kit (LOD = 0.2 mg/kg) used were designed for quantitative analysis of deoxynivalenol in food and feed component samples.

2.6. Scoring of data

Samples with mycotoxin concentrations below the limit of detection (LOD) were scored as negative and those with a detectable concentration were scored as positive. The frequency of occurrence of mycotoxins in different grains was calculated by summing up all samples tested positive. The co-occurrence of the mycotoxins in a sample was summarised as binary co-occurrence when only two mycotoxins were detected in a sample, triple co-occurrence when three mycotoxins were detected in a sample, and quadruple co-occurrence when all the four mycotoxins were detected in a sample. The frequency of co-occurrence was computed for the different grain types. The concentration was compared with the set standards for east Africa and those used by the USA, EU, and FAO. The frequency of samples with mycotoxin concentration above the regulatory standard was calculated to assess the magnitude of the mycotoxin contamination that would translate to a non-tariff trade barrier of grains from northern Uganda.

2.7. Estimated daily intake (EDI)

Estimated daily intakes for the different mycotoxins were calculated using the mean daily intake of grains among women for all age groups. The average daily intake for the grains in northern Uganda was as follows: maize = 106 g/day, sorghum = 115 g/day, millet = 0.18 g/day (Harvey, Rambeloson, & Dary, 2010). The daily intake of groundnut among Uganda is 14.7 g/day (FAOSTAT, 2020). The average daily intake of the grains was converted to a kilogram per day (kg/day). The average body weight of an adult Ugandan was estimated at 61.6 kg (Kirunda, Fadnes, Wamani, Van Den Broeck, & Tylleskär, 2015). An average body weight of 11 kg was used for infants (Echodu et al., 2019). The Estimated daily intake (EDI) = Daily intake of a given food type (maize, sorghum, millet, groundnut) multiplied by the mean concentration of a given mycotoxin divided by average body weight. The daily intake was measured against the standard tolerable daily intake (TDI)/tolerable weekly intake (TWI). The value was as follows: Tolerable weekly intake for ochratoxin A is 120 ng/kg. bw, TDI for fumonisins (FB1+FB2+FB3+FB4) is 1 µg/kg. bw, TDI for DON is 1 µg/kg. bw (EC (European Commission), 2006; Knutsen et al., 2018). Ochratoxin A TWI was converted to TDI. As well the unit for ochratoxin A TDI was converted from ng/kg. bw to µg/kg. bw (0.017 µg/kg. bw) (European food safety authority, 2006).

2.8. Hazard quotient (HQ)

A hazard quotient (HQ) was used to quantify the risk of exposure to fumonisins, ochratoxins, and DON. The HQ was calculated as a ratio of EDI to TDI for a specific mycotoxin. An HQ with a value less or equal to one was interpreted as the dose intake level of the mycotoxin that will probably not present any harmful effect to individuals in northern Uganda. An HQ greater than one indicates that the average daily intake of the mycotoxin exceeds the tolerable daily intake and could potentially present a health risk (United States Environmental Protection Agency, 2014).

2.9. Risk characterization of total aflatoxin (B1+B2+G1+G2)

The risk characterization of aflatoxins exposure relies on determining the margin of exposure (MOE) as no safe level for aflatoxin exposure currently exists owing to its carcinogenic potential in humans (European food safety authority (EFSA) (2007b)). The margin of exposure in the study was calculated as a ratio of the dose that produces the specific effect (Benchmark dose) to the estimated daily intake of aflatoxin in northern Uganda. The benchmark dose for a 1% increase in cancer incidence (BMDL110) for studies conducted in Africa was 78 ng/kg. bw per day. For Europe, the lower confidence limit of the benchmark dose (BMDL109) for a 10% increase in cancer incidence of 170 ng/kg. bw per day was used for an estimate of the risk of exposure to aflatoxin (European food safety authority (EFSA) (2007a); Smith, Madec, Coton, & Hymery, 2016). BMDL10 derived from a study conducted in China was 870 ng/kg. bw per day (Bol, Araujo, Veras, & Welke, 2016). For this study, the margin of exposure was determined for total aflatoxin using both BMD derived from Africa and Europe for comparison. The margin of exposure (MOE) specifies a level of concern. MOE value < 10,000 indicate a high level of concern and MOE value > 10,000 is associated with a reduced risk of cancer development in humans (Al Jabir et al., 2019; European Food Safety Authority (EFSA), 2005; Gilbert Sandoval, Wesseling, & Rietjens, 2019).

2.10. Statistical analysis

The data were grouped according to grain types (maize, sorghum, millet, and groundnut). Second stage grouping was done according to the location where the samples were collected. Prevalence was used to determine grain types and districts with the highest mycotoxin contamination levels. Prevalence was calculated as the total number of grains positive for a mycotoxin divided by the total number of samples tested and then multiplied by 100. The normality of the mycotoxin concentrations was tested using the Shapiro-Wilk test at a significance level of 0.05. Since the normality assumption was not fulfilled, a Kruskal–Wallis test was used to test if the concentration of mycotoxins in the four-grain types (maize, sorghum, millet, and peanut) significantly differed. A post-hoc DunnTest was done to determine which grain types significantly differed in mycotoxin concentration. Wilcoxon–Mann–Whitney test was used to determine differences in concentrations of the mycotoxins between maize and sorghum grains that were collected from the same households. The level of significance was 5% ($p < 0.05$) for all the statistical tests performed. Statistical analysis was done in R-Studio v4.0 (<http://www.rstudio.com/>).

3. Results

3.1. Mycotoxin concentration in grains

Comparison of the mycotoxin concentration across the four-grain types showed significant differences ($p < 0.01$) in the concentration of mycotoxins. There was no significant difference in the concentration of aflatoxins between sorghum and groundnut. The level of aflatoxin in sorghum and groundnut grains was significantly higher than the level measured in maize and millet grains. Sorghum grains resulted in the highest levels of fumonisins, significantly higher than the levels measured in groundnut, maize, and millet (Fig. 1). For ochratoxins, there were no significant differences between the levels in sorghum and the groundnut grains. The level of ochratoxins in sorghum and groundnut grains was significantly higher than those measured in millet and maize grains. The level of deoxynivalenol in groundnut and millet did not significantly differ. Deoxynivalenol levels in maize and sorghum were higher than the level measured in groundnut and millet. Generally, millet resulted in the lowest toxin levels. The levels of aflatoxins, fumonisins, and ochratoxins observed in millet grains were significantly lower than those measured in the other grain types (Fig. 1 and Table S1).

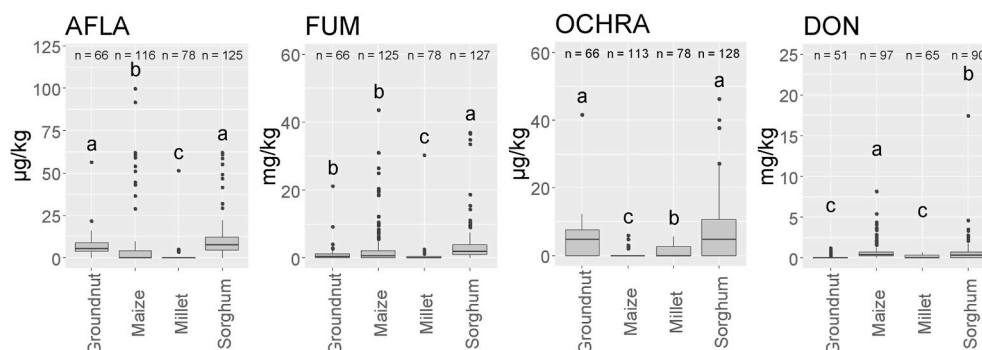


Fig. 1. Boxplots showing the variation in toxin content (AFLA: aflatoxins, FUM: fumonisins, OCHRA: ochratoxins, DON: deoxynivalenol) for the different grain types. Different letters above the boxes point to significant differences between grain types.

Sorghum had the highest frequency of contamination with aflatoxins (80%), fumonisins (93%), and ochratoxins (67%). For the other grain types, the frequency of positive samples depends on the toxin type (Fig. 2). Maize had the highest number of positive samples contaminated with deoxynivalenol (77%) but was least contaminated with ochratoxins (12%). As well, groundnut had low contamination with deoxynivalenol as only (20%) samples were positive (Fig. 2 and Table S2). Contamination of groundnut with aflatoxin was the second highest (79%).

3.2. Mycotoxins in the surveyed districts

The four mycotoxins were found to occur widely in grains from all nine districts. For each toxin, there were significant differences in toxin levels between districts (Fig. 3). For aflatoxins, fumonisins, and ochratoxins, grains from Kole resulted in the highest toxin levels, whereas the samples from Omoro showed a low contamination level for these toxins. For the other districts, the contamination level was toxin dependent. For example, grains from Kitgum had low levels of ochratoxins, whereas the deoxynivalenol levels were highest in this district. A similar phenomenon was observed for the Lamwo district. In the Nwoya district, the opposite was true as DON concentration was low whereas ochratoxins concentration was high (Fig. 3).

More than 50% of the grains collected from the districts of Kitgum, Kole, Lamwo, Oyam, and Pader were contaminated with aflatoxins (Fig. 4). In the other four districts (Gulu, Nwoya, Omoro, and Lira) less than 50% of the grains had aflatoxins contamination (Table S4). Fumonisin were widely spread. The frequency of grains with fumonisins contamination in each district exceeds the frequency of grains without fumonisins (Fig. 4). In Gulu, Kole, Nwoya, and Pader district, the frequency of samples positive of ochratoxins exceeds the frequency of samples negative of ochratoxins. However, contamination with ochratoxins in Kitgum, Lamwo, Lira, Omoro, and Oyam districts was low. For deoxynivalenol the variation between districts was highest, ranging from 1% negative samples in Kitgum district to 73% negative

samples in Nwoya district.

3.3. Mycotoxin content of maize and sorghum collected from the same household

A comparison of mycotoxin between sorghum and maize collected from the same household indicates variation in concentration and differential proportion of mycotoxin contamination. The aflatoxins concentration was significantly ($p = 0.0012$) higher in sorghum than in maize grains that were collected from the same households (Fig. 5). As well, the proportion of sorghum grains 77% with aflatoxins was higher than the proportion of maize grains 53% with aflatoxins (Fig. S1). Similarly, there was a significant variation in ochratoxins concentration between maize and sorghum. The concentration of ochratoxins in sorghum grains was high compared to the concentration observed in maize. Test for equality of proportions indicates more sorghum 58% were contaminated with ochratoxins than maize 2% (Fig. S1). The fumonisins concentration in sorghum was significantly ($p = 0.00099$) higher than the concentration observed in maize. As well more sorghum grains 94% had fumonisins contamination compared to maize 72% (Fig. S1). There was no significant difference ($p = 0.47$) in the concentration of DON observed in maize and sorghum grains that were collected from the same households (Fig. 5). However, the proportion of maize with mycotoxin DON was higher than the proportion of sorghum contaminated with DON.

3.4. Co-occurrence of mycotoxin in grains

Most grain samples were contaminated with a mixture of toxins. Aflatoxins were most frequently detected in association with other toxins, less than 0.35% of the samples solely contained this toxin (Fig. 6). Fumonisin, ochratoxins, and deoxynivalenol occurred as a single toxin in 4.59%, 6.00%, and 6.36% of the samples, respectively. Concerning the samples contaminated with two different toxins, the

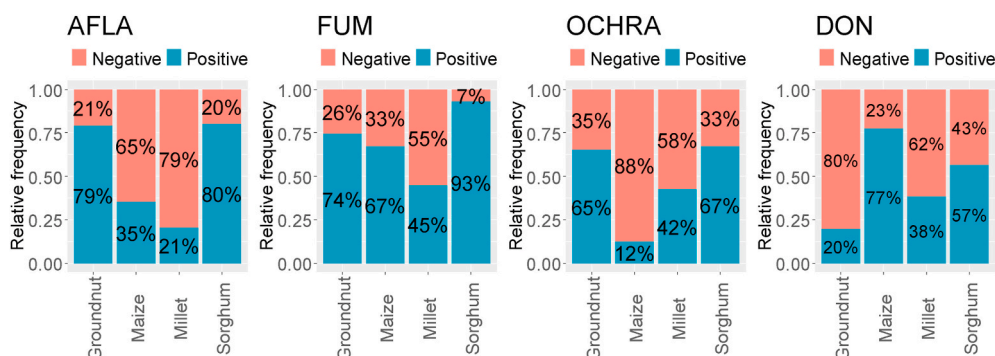


Fig. 2. Proportion of grains with mycotoxin contamination (AFLA: aflatoxins, FUM: fumonisins, OCHRA: ochratoxins, DON: deoxynivalenol).

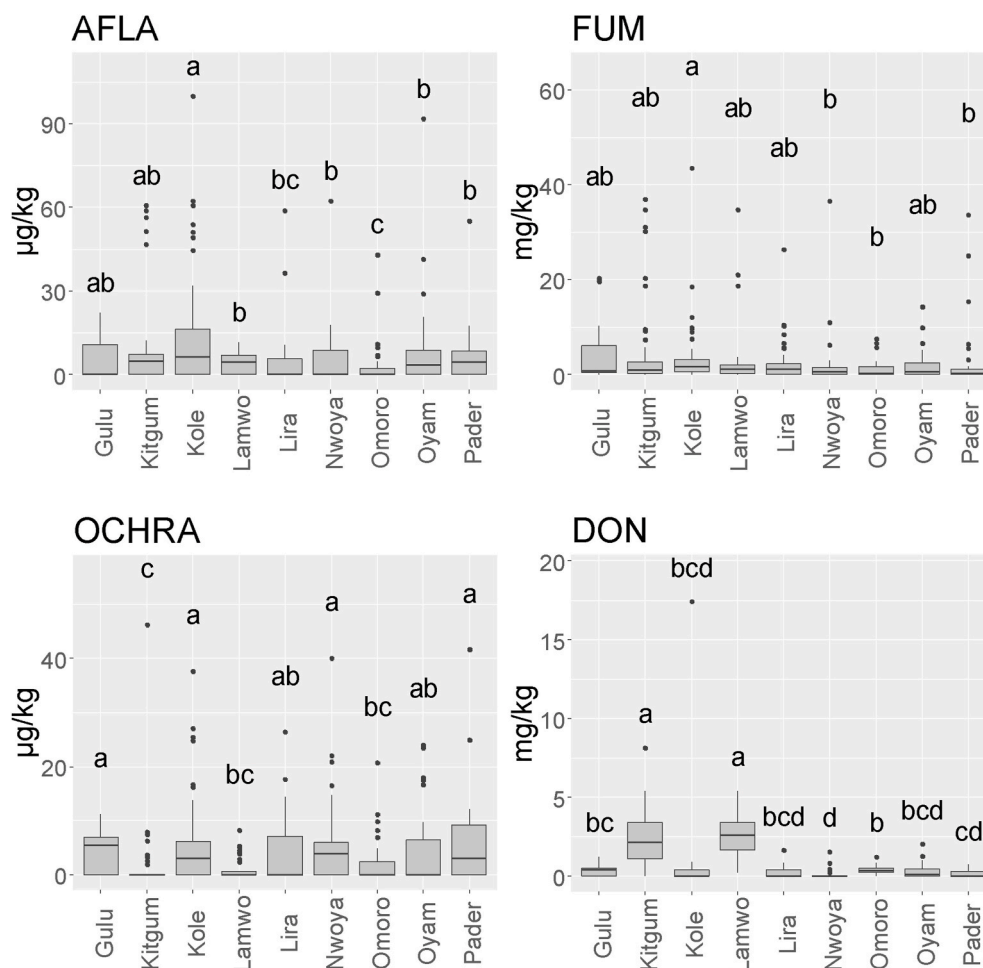


Fig. 3. Boxplots showing the variation in toxin levels for the different districts (AFLA: aflatoxins, FUM: fumonisins, OCHRA: ochratoxins, DON: deoxynivalenol). Different letters above the boxes point to significant differences between districts.

association between the *Fusarium* toxins DON + FUM was the most frequent (14.49%), and DON + AFLA was the least frequent dual association (0.70%). Among the four possible triple associations, AFLA + FUM + OCHRA was mostly detected (21.55%). A quadruple occurrence of the four toxins was found in 12.72% of the samples (Fig. 6 and Table S6).

Multiple toxins were mostly detected in sorghum grains followed by maize and millet. Contamination with all the four mycotoxins mainly occurred in sorghum grains (40.70%). None of the groundnut and millet samples had complexes of the four mycotoxins. Among the possible triple combinations, AFLA + FUM + DON was the most frequently detected, in the groundnut, sorghum, millet, and maize samples with frequencies of 50.98%, 37.21%, 4.62%, and 0.00%, respectively (Table 1). The triple combination AFLA + DON + OCHRA was the least frequently detected, it only occurred in 1.96% of the groundnut samples. Concerning the dual combination of toxins, DON + FUM was mostly detected (groundnut 7.84%, maize 27.16%, millet 16.92%, sorghum 4.65%), whereas DON + AFLA was only detected in maize with a frequency of 2.47% (Table 1).

3.5. Comparison of mycotoxin concentrations with regulatory standards

This section examined the proportion of samples with mycotoxin concentrations exceeding the regulatory standards. For all-grain types, the proportion with aflatoxin concentration exceeding Uganda regulatory limit of 10 µg/kg was less than grain samples with aflatoxin concentration below threshold (10 µg/kg). The proportion of groundnut and

sorghum grains with aflatoxins concentrations exceeding European regulatory limits of 4 µg/kg was higher than the proportion of grains with a concentration below the limit. Sorghum had the highest proportion of grains with fumonisins concentration exceeding the threshold value of 1 mg/kg compared to all the grain types (Fig. 7). For both ochratoxins (limit = 5 µg/kg) and deoxynivalenol (limit = 0.75 mg/kg), none of the grain types had more than 50% samples with a concentration exceeding the threshold. In general, the concentration of mycotoxins in millet grain samples least exceeded the regulatory standards, whereas sorghum samples exceeded the thresholds the most (Fig. 7 and Table S7).

3.6. Estimate of daily intake of mycotoxins in grains

Exposure to aflatoxin was higher in sorghum and maize grains compared to the exposure level obtained by consuming millet and groundnut. Daily exposure to fumonisins in maize grains was six-folds higher than the TDI value of 1 µg/kg. bw among adults and 33 folds higher than TDI in the infant. For sorghum grains, exposure to fumonisin was 8-folds higher than the TDI in adults and 46-folds higher than TDI in the infant (Table 2). The observed fumonisins exposure level in millet and groundnut were below the TDI for both adults and infants. Exposure to ochratoxins was below the TDI (0.017 µg/kg. bw) in all the grain types for adults and infants. For the infant, ochratoxins exposure was higher than TDI in sorghum grains (Table 2). Exposure to DON among adults exceeds the TDI (1 µg/kg. bw) in both maize and sorghum grains. Exposure to DON for infants was only low in millet but was 8 folds

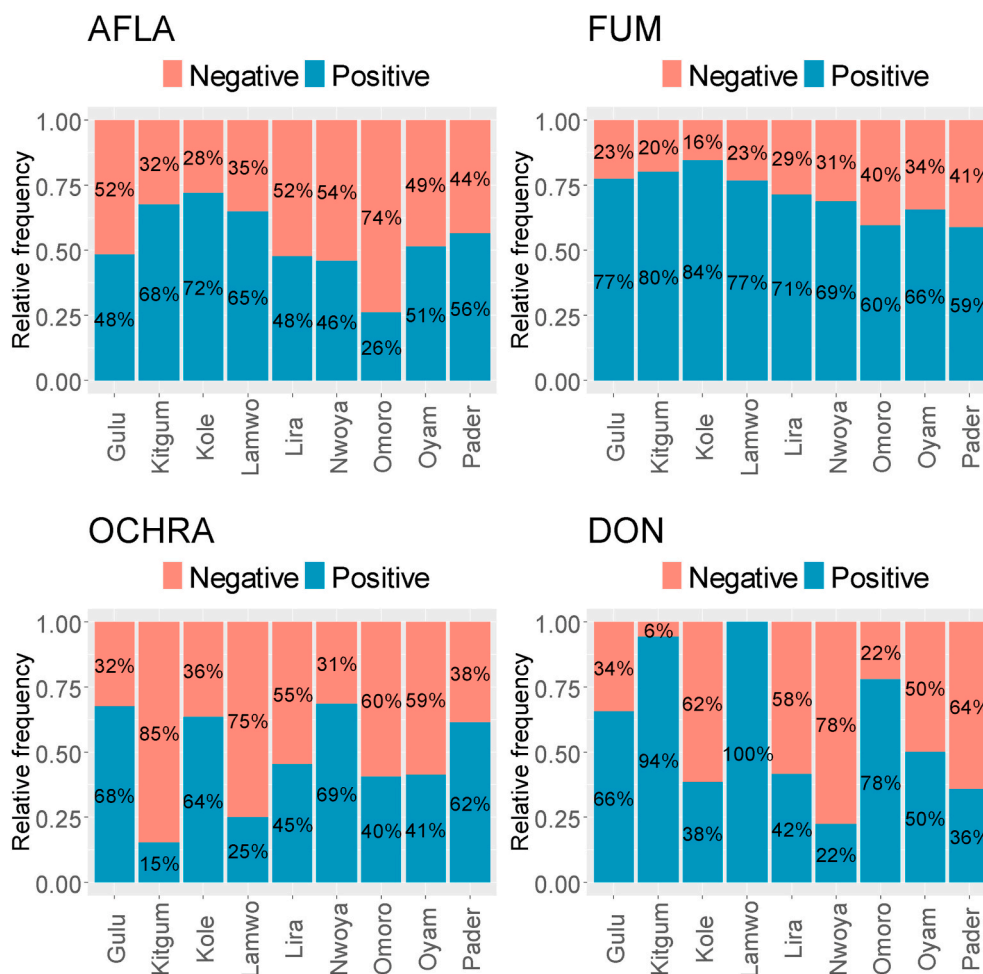


Fig. 4. Proportion of grain contamination with mycotoxins. (AFLA: aflatoxins, FUM: fumonisins, OCHRA: ochratoxins, DON: deoxynivalenol).

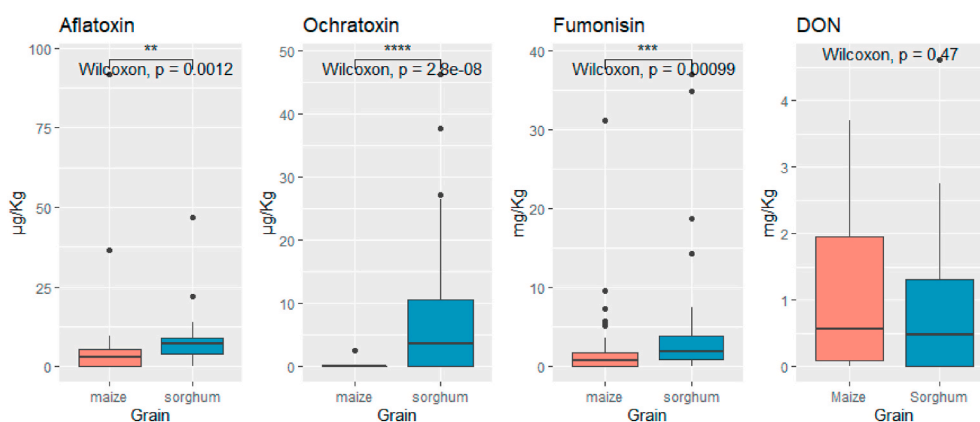


Fig. 5. Mycotoxin concentration of maize and sorghum grains that were collected from the same households. The double and triple asterisk (**) and (***) indicate significant p-value.

higher than TDI in both maize and sorghum grains (Table 2).

The hazard quotients were greater than one for all the grain types except for millet. Fumonisin had the highest HQ both in adults and in infants followed by DON and ochratoxins. In fumonisins, the HQ for the infant was five times higher than the HQ for adults both in maize and sorghum grains (Table 3). A similar trend also occurs for the mycotoxins DON and ochratoxins. The margin of exposure (MOE) of aflatoxins was high in adults compared to MOE observed in the infant for all the grain types. Millet had the highest value of MOE exceeding 10,000 whereas all

other grain types (sorghum, groundnut, and millet) had a margin of exposure to aflatoxin below the value of 10,000. (Table S8).

4. Discussion

Mycotoxins are important food/feed contaminants that severely affect grain quality and thus human and animal health worldwide. In Uganda, information about mycotoxin contamination is very scanty, particularly in northern Uganda that experienced a decade's long war.

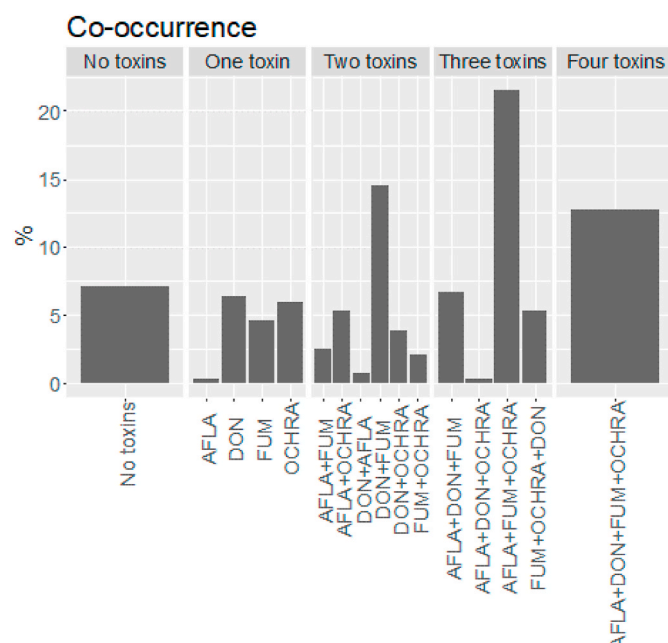


Fig. 6. Co-occurrence of mycotoxin in grains, proportion of samples contaminated with several toxin combinations (AFLA: aflatoxins, FUM: fumonisins, OCHRA: ochratoxins, DON: deoxynivalenol).

Table 1

Mycotoxin co-contamination of grains (n = the number of samples in which the toxins were found and the corresponding frequency (%)).

	Groundnut		Maize		Millet		Sorghum	
	n	%	n	%	n	%	n	%
AFLA	0	0.00	0	0.00	1	1.54	0	0.00
DON	1	1.96	11	13.58	6	9.23	0	0.00
FUM	0	0.00	10	12.35	2	3.08	1	1.16
POCHRA	0	0.00	0	0.00	16	24.62	1	1.16
DON + POCHRA	0	0.00	7	8.64	4	6.15	0	0.00
DON + FUM	4	7.84	22	27.16	11	16.92	4	4.65
DON + AFLA	0	0.00	2	2.47	0	0.00	0	0.00
AFLA + POCHRA	12	23.53	0	0.00	3	4.62	0	0.00
AFLA + FUM	0	0.00	5	6.17	1	1.54	1	1.16
FUM + POCHRA	1	1.96	0	0.00	3	4.62	2	2.33
FUM + POCHRA + DON	2	3.92	5	6.17	4	6.15	4	4.65
AFLA + DON + FUM	2	3.92	13	16.05	0	0.00	4	4.65
AFLA + FUM + POCHRA	26	50.98	0	0.00	3	4.62	32	37.21
AFLA + DON + POCHRA	1	1.96	0	0.00	0	0.00	0	0.00
AFLA + DON + FUM + POCHRA	0	0.00	1	1.23	0	0.00	35	40.70
No toxins	2	3.92	5	6.17	11	16.92	2	2.33

AFLA: aflatoxins, FUM: fumonisins, OCHRA: ochratoxins, DON: deoxynivalenol.

This information is very important in improving grain quality, good health, and economic prosperity which is a key aspect of peace recovery for the community of northern Uganda who depends on agriculture as the premier source of livelihood.

The toxins generally occur in all grain types which reflects the severity of contamination and the overall silent health burden among the people whose diet is primarily based on grains. In particular, the concentration of aflatoxins, ochratoxins, and fumonisins was high in groundnut and sorghum. These grain types are more susceptible to contamination compared to millet grains that had an overall low concentration and low prevalence of mycotoxins. The high occurrence of the field-mycotoxin fumonisins highlights that the region could be a

hotspot for *Fusarium verticillioides*, *F. proliferatum*, and *F. andiyazi* which are the main producers of fumonisins. This is mainly because resource-poor farmers in the area hardly afford technologies such as fungicide, irrigation, clean plant materials, and fertilizer application that improve plant vigor to overcome infection by the field base fungi to bring them under control. Such technologies should be highly prioritized and costs associated with adoption should be supplemented to resource-poor farmers who are mostly employed in the agricultural sector. As well, the occurrence of DON and fumonisins in groundnut indicates an equal susceptibility of the crop to infection by *Fusarium* species as well as by *Aspergillus niger* (Frisvad, Smedsgaard, Samson, Larsen, & Thrane, 2007; McDonald, 1970; Noonim, Mahakarnchanakul, Nielsen, Frisvad, & Samson, 2009; Odunfa, 1979; Xu, Yang, J.X., Chi, & Xie, 2015), which is similar to cereal crops.

The high occurrence of mycotoxins in grains from some districts still reflects a limited understanding of factors associated with mycotoxin contamination of grains among many local farmers just like in other parts of sub-Saharan Africa. To address the problem of mycotoxin contamination in Uganda, the government roll out a nationwide sensitization program in the year 2018 to reduce knowledge gaps among farmers. For some districts, the sensitization and knowledge reached the majority of farmers and were adopted which accounts for the relatively low prevalence of mycotoxins contamination of grains in contrast to districts with inadequate sensitization. Generally, in northern Uganda, the harvest of most grains occurs in the period between July to September (FEWS NET, 2017) during which rainfall and humidity are high which often affect proper drying of grains before storage (Lindblad & Druben, 1976). Partially dried grains are associated with fungal contamination and subsequent contamination of grains with mycotoxins in storage. In most cases, the storage facilities are characterized by pest damage of grains, poor aeration, poor moisture control, and poor temperature which further promotes fungal activities. As such in sub-Saharan Africa, contamination of grains with aflatoxins and ochratoxins tends to increase with the time the grains take within storage facilities (Manjula, Hell, Fandohan, Abass, & Bandyopadhyay, 2009). The use of grains resistant to storage pests and hermetic storage containers would greatly reduce the contamination of grains with mycotoxins in the store (Kumar & Kalita, 2017). However, most farmers are resource-constrained which reduces adoption and implementation of some good technologies that would improve grain quality.

Overall, the prevalence of the mycotoxins was highest in sorghum compared to other grain types. The high occurrence of mycotoxins in sorghum compared to maize from common households under the same storage conditions shows differences in susceptibility of the two main cereal grains in northern Uganda. The finding indicates that the millet and maize grown in the area are much more resilient to mycotoxin contamination compared to the sorghum. Although the production and consumption of millet among Ugandan had declined (Revoredo-giha, Akaichi, & Toma, 2018), this study indicates that millet grain can provide a better alternative shift to reduce high exposure to mycotoxins among the people of northern Uganda.

Sorghum, maize, and groundnut are the most important staple foods consumed by most populations in northern Uganda and by most people in East Africa (Cosmas, 2018; De Groote et al., 2014; Mboya, Tongona, Derera, Mudhara, & Langyintuo, 2011). Annual maize production in Uganda is estimated at 2.9 million metric tons, making it the most produced cereal followed by sorghum with a production capacity of 0.29 million metric tons (Food and Agriculture organisation of the United Nation, 2020). However, grains suffer from high contamination with mycotoxins, and a greater proportion does not meet the regulatory limits for export. With a high contamination level, rejection of grains becomes unavoidable, which narrows down the already limited market. For a community whose livelihood depends on agriculture, the ability to sell their products is critical to attaining economic prosperity. Mycotoxin contamination is one of the factors that contribute to poverty associated with the farming community in sub-Saharan Africa. Losses of grains

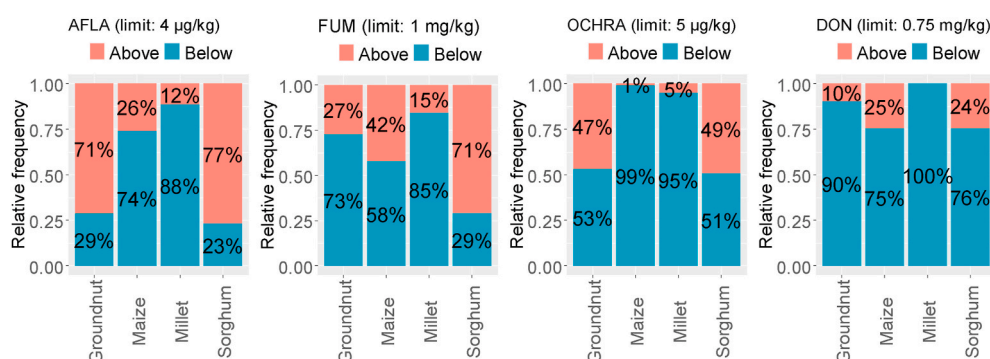


Fig. 7. Proportion of grains with mycotoxin concentrations higher than regulatory threshold (AFLA: aflatoxins, FUM: fumonisins, OCHRA: ochratoxins, DON: deoxynivalenol).

Table 2

EDI (µg/kg.bw) for the selected mycotoxins among adults and children.

Grain type		Aflatoxins	Fumonisin	Ochratoxins	Deoxynivalenol
Adults	Groundnut	0.002	0.285	0.001	0.035
	Maize	0.012	6.006	0.001	1.597
	Millet	0.000	0.002	0.000	0.000
	Sorghum	0.019	8.219	0.014	1.531
Infants	Groundnut	0.009	1.593	0.007	0.197
	Maize	0.065	33.633	0.004	8.942
	Millet	0.000	0.012	0.000	0.002
	Sorghum	0.107	46.024	0.078	8.576

Table 3

Hazard quotient for the mycotoxin in the selected grains.

Grain	Fumonisin		Ochratoxin		DON	
	Adult	children	Adult	children	Adult	children
Groundnut	0.28	1.59	0.067	0.38	0.04	0.20
Maize	6.01	33.63	0.037	0.20	1.60	8.94
Millet	0.00	0.01	0.000	0.00	0.00	0.00
Sorghum	8.22	46.02	0.798	4.47	1.53	8.58

because of mycotoxin contamination amount up to US\$577 million every year in Uganda (Lukwago, Mukisa, Atukwase, Kaaya, & Tumwe-baze, 2019).

The analysis also revealed that the majority of the grain samples were contaminated with multiple mycotoxins. The most surprising is the frequent co-occurrence of all the four mycotoxins in sorghum grains. Therefore, individuals whose diet heavily depends on sorghum are at risk of taking all the four mycotoxins in a single diet. The dependency of the majority of people on sorghum grain coupled with co-contamination with aflatoxins and fumonisins presents an imminent risk of elevating the prevalence of hepatocellular carcinoma and other associated health burdens in northern Uganda that already has high prevalence of hepatitis B infection. Co-occurrence may present different effects on the host which may be synergetic, additive, or antagonistic (Allassane-Kpembi et al., 2013; Kouadio, Dano, Moukha, Mobio, & Creppy, 2007; Lu, Fernández-Franzón, Font, & Ruiz, 2013; Wan, Turner, & El-Nezami, 2013). Co-occurrence of aflatoxins and fumonisins elevated serum cholesterol level, dysplasia, and apoptosis in rat models (Qian et al., 2016). The same observation may as well occur to humans exposed to both aflatoxins and fumonisins. Evaluation of the combined effect of all the four toxins would be equally important since the occurrence is very common especially in sorghum grains which is a staple food in this region. The high exposure to mycotoxins either singly or in combination elevates the health risk of individuals in northern Uganda. Exposure among children can be severe as their body system could not easily detoxify the high levels of mycotoxins. Aflatoxins have been implicated

in stunted growth among children and stunting occurs in one-third of children below the age of five in Uganda (USAID-DHS, 2018). As well aflatoxins are linked to the development of Edema and kwashiorkor in undernourished children.

The aflatoxins estimated intake in sorghum grains for the infant was higher than the value of 78 ng/kg b. w. per day which is considered as the benchmark dose for a 1% increase in cancer incidence in experimental animals in sub-Sahara Africa. The current level of aflatoxin intake in sorghum among infants from northern Uganda is at the worst level. However, for millet, the exposure estimates, indicate consumption of millet among all age groups in northern Uganda does not offer any significant risk of cancer development. As well, the MOE (<10,000) derived from sorghum and maize further raise health warning as low MOE is associated with an increased risk of cancer development among people who depend on the grains. Whereas in millet grain the MOE values were greater than 10,000 for both adult and infants which further support that consumption of millet among the community of northern Uganda offers a reduced risk of cancer development compared to sorghum and maize grains.

5. Conclusion

Mycotoxin contamination of grains was very high, and in most samples multiple toxins were present. The co-occurrence shows that the local people are exposed to a combination of mycotoxins in their diet. The problem even becomes worse if we take into account the high estimated daily exposure rates both in children and adults for some grain types. Sorghum grains was the prime and common source of dietary exposure to mycotoxins among people of northern Uganda. There is a need to explore in detail factors responsible for the higher contamination of sorghum with mycotoxins compared to other grain types grown in northern Uganda. As for now, the community of northern Uganda needs to work progressively towards improving the quality of grains through adopting best agricultural practices to increase the value of their grains in terms of toxins reduction and minimize exposure to the high mycotoxin levels.

CRediT authorship contribution statement

Godfrey Wokorach: Formal analysis, Writing - original draft, Writing - review & editing, Data curation, Investigation. **Sofie Land-schoot:** Formal analysis, Writing - review & editing, Data curation, Visualization. **Juliet Anena:** Investigation, Writing - review & editing. **Kris Audenaert:** Conceptualization, Funding acquisition, Resources, Supervision, Project administration. **Richard Echodu:** Conceptualization, Funding acquisition, Resources, Supervision, Project administration. **Geert Haesaert:** Conceptualization, Resources, Methodology, Project administration, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2020.107813>.

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