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Negligible Levels of Mycotoxin Contamination in Durum Wheat and Groundnuts from Non-Intensive Rainfed Production Systems

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Citation: De Santis, P.; Mengistu, D.K.; Kidane, Y.G.; Nankya, R.; De Santis, B.; Moracci, G.; Debegnach, F.; Marsiglia, R.; Reverberi, M. Negligible Levels of Mycotoxin Contamination in Durum Wheat and Groundnuts from Non-Intensive Rainfed Production Systems. *Sustainability* **2021**, *13*, 10309. https://doi.org/10.3390/su131810309

Academic Editor: Filippo Giarratana

Received: 21 July 2021
Accepted: 11 September 2021
Published: 15 September 2021

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Abstract: Mycotoxins are chemical contaminants that are invisible, tasteless, chemically stable and survive food processing. Contamination along the agri-food chain is difficult to control since their production and spreading are due to numerous factors including temperature, relative humidity, insect infestation, and susceptibility of the host plant. This is a pilot study which aims at assessing the contamination level of deoxynivalenol (DON), and its plant metabolites (3AcDON, 15 AcDON, DON 3G), nivalenol, T-2 and HT-2 toxins, and ochratoxin A in thirty-seven traditional varieties of Ethiopian durum wheat, and aflatoxins B1, B2, G1, and G2 in thirty-one varieties of Ugandan groundnuts grown in non-intensive rainfed production systems. Results indicate absence of mycotoxin contamination in all durum wheat samples and negligible levels of contamination (below the maximum levels tolerated by international standards) in groundnut samples. Further studies are required to assess if non-intensive production systems and varieties have a role in preventing and/or reducing mycotoxin contamination of the crops.

Keywords: deoxynivalenol; ochratoxin; nivalenol; aflatoxins; Ethiopia; Uganda; durum wheat; peanuts; non-intensive production system; modern variety; traditional variety; intraspecific diversity; genetic resources

1. Introduction

Mycotoxins are a group of chemical contaminants that are invisible, tasteless, chemically stable, and resistant to common domestic processing. They are produced on a wide range of crops (especially cereals and oil seeds) as secondary metabolites by ubiquitous fungi, mainly belonging to *Aspergillus*, *Fusarium*, *Alternaria,* and *Penicillium* genera. These fungi can be divided into (a) field fungi (e.g., *Fusarium*, *Alternaria*), which mainly activate mycotoxin production at pre-harvest level and (b) storage fungi (e.g., *Penicillium*), which proliferate after harvest under special conditions of temperature and moisture, although other fungi (e.g., *Aspergillus*, *Alternaria*) can occur during both pre- and post-harvest stages.

Because of their toxicity, mycotoxins are of serious human and animal health concern. Toxicity of mycotoxins can be acute (effects appear after a short exposure, often to high doses) but more frequently they exert a chronic effect (weaker symptoms that can occur

with long-term exposure to low doses of mycotoxins). Exposure to mycotoxins occurs through direct ingestion of contaminated crops or products deriving from animals that have been fed with contaminated feed (e.g., milk and meat), but can also occur through inhalation of dust when handling contaminated plants. Different strains of fungi are capable of producing more than one mycotoxin; thus, an agricultural product may be contaminated by more than one mycotoxin resulting in co-occurrence of contaminants that can have a synergistic effect on human and animal health.

There are approximately 400 mycotoxins worldwide [1]. The most studied, with chemical and toxicological characterization, include aflatoxins, fumonisins, trichothecenes, zearalenone, ergot alkaloids, and ochratoxin A, and for these groups of mycotoxins several countries worldwide have set maximum limits on agricultural products and derived foods. Among mycotoxins, two of the most relevant of food concern are aflatoxins (AFs) and deoxynivalenol (DON). Aflatoxins have received special attention in research due to their effect on human health, being among the most important genotoxic carcinogens of food origin also classified by IARC on the group 1 [2]. They are produced by several strains of *Aspergillus*, the most important being *A. flavus*, and *A. parasiticus.* Aflatoxins appear more frequently in tropical and subtropical regions, but can be found in several staple crops such as groundnuts, maize, sorghum [3]. Deoxynivalenol is one of the most important mycotoxins in cereal produced by fungi of the genus *Fusarium* (e.g., *F. graminearum* and *F. culmorum)* in predominantly warm-temperate regions. Given the importance of cereals in human and animal diets, exposure to this mycotoxin is very frequent. Furthermore, since cereals represent an important component in infant diets particularly during weaning, infants and children are particularly vulnerable to exposure, due to their body weight, limited diet and reduced detoxification capacity.

Mycotoxin contamination is recognized as an unavoidable worldwide problem, worsened by the trends in global climatic changes that are affecting the amount and distribution of temperature and precipitation patterns [4,5]. Increased risk of mycotoxin contamination is foreseen particularly between 40° N and 40° S, where environmental conditions favor fungi proliferation. Mycotoxin contamination is influenced by the climatic conditions, its concentration is highly heterogeneous and may be carried over into processed food and, depending on feed stuff contamination and the animal metabolism, in animal products such as milk, meat or eggs [6,7]. Contamination along the agri-food chain is difficult to control since numerous factors affect mycotoxins production and spreading. These include environmental conditions conducive to fungal colonization and mycotoxin production, such as temperature, relative humidity, insect infestation, and genetic susceptibility of the host plant. The growth of fungi usually occurs at temperatures of 10 °C–40 °C and water activity (a_w) of above 0.70, but these can vary significantly depending on the strain [8]. High values of moisture content in grains during storage, combined with high temperatures of storage environment also favor mycotoxin production. During plant cycle, stress factors that weaken plants, such as drought, inadequate fertilization and high plant densities, favor fungi proliferation and consequently mycotoxin contamination [9–11]. Good agricultural practices (e.g., good soil preparation, timely planting, adequate space among plants, and crop rotation with crops that don't share susceptibility to the same fungi) are deemed to be helpful to prevent and reduce the presence of mycotoxins.

The presence of mycotoxins as well as having important health consequences, also have an effect on economic performance of agricultural production. It is estimated that 25% of the global food crop production is contaminated with mycotoxins [12]. Yielding capacity of crops and grain quality are reduced resulting in lower market prices or total exclusion from trade; impacts on livestock fed on contaminated food are also relevant, resulting in reduction of growth, performance, and reproduction capacities.

In Africa, in addition to the physical factors (i.e., conducive climatic conditions), control of mycotoxin contamination is further hindered by poor agricultural practices and inadequate pre- and post-harvest management, such as poor facilities for drying, storage, and transportation of agricultural production. Moreover, a general lack of farmers' aware-

ness of mycotoxins contamination effects may have an impact both from an economic and a health point of view (see [13,14] regarding farmers' awareness in Uganda). Furthermore, while many countries have developed national mycotoxin legislations, which set standards on maximum levels of contamination, the absence of proper local legislations and inadequate regulations in most African countries results in a limited, if not absent, monitoring of the occurrence of mycotoxins [15,16]. In addition, in many cases rules and regulations are not binding or statutory and only provide a guidance for risk management. Governments in Africa often lack infrastructure, capacity, and resources required to apply efficient analytical methods (especially those capable of detecting contaminations at low concentrations). In most cases, the monitoring systems and limits to mycotoxins which are established to regulate trade and occurrence are checked only by private companies which need to comply with regulations for accessing the international market. Monitoring of mycotoxins, therefore, predominantly excludes food and feed produced by smallholder farmers who practice subsistence farming, which represents 80% of the food production in Africa [17,18].

The toxicity of mycotoxins represents an important food safety issue in Africa [19–21]. During the last 20 years, the number of mycotoxin outbreaks in several African countries, such as Kenya and Tanzania [22,23], has highlighted the seriousness of the problem, especially in those low-income countries where low diversity in diet exacerbates the issue of consumption of contaminated staple food [24].

Few studies have been conducted to understand the different levels of susceptibility within crops to mycotoxin contamination. Studies on the prevalence of mycotoxins do not usually distinguish between varieties, rather they stop at crop species and do not consider differences among commercial varieties (modern). Still less is known regarding the susceptibility of traditional varieties (locally adapted varieties characterized by genetic variability and conserved by farmers, also known as landraces) to toxigenic fungi. This pilot study aims at testing the contamination level of DON, 3AcDON, 15 AcDON, DON 3G, NIV, T-2/HT-2, and for OTA in traditional varieties of Ethiopian durum wheat and aflatoxins B1, B2, G1, and G2 in modern and traditional varieties in Ugandan groundnuts.

2. Materials and Methods

2.1. Durum Wheat

2.1.1. Description of Ethiopian Sites

The study was conducted in four sites in two regions in Northern Ethiopia (Tigray and Amhara) (Figure 1) characterized by different climatic conditions. Hagreselam, Tigray Region, is located at 13.65° N latitude, 39.12° E longitude and elevation of 2650 m.a.s.l. Temperatures range from 10 and 30 °C and an average rainfall of up to 760 mm yearly precipitation, with 500 mm reported during 2018. Its soil type is clay loam with a rooting depth of less than a meter. Ayba, Tigray Region, is located at 12.89° N latitude, 39.30° E longitude and elevation of 2730 m.a.s.l. Temperatures range from 10 and 30 °C and an average rainfall of up to 960 mm yearly precipitation. The soil type of this location is deep clay loam with good water holding capacity after rainfall ceases. Dinglet, Tigray Region, is located at 13.91° N and 39.51° E and at elevation of 2530 m.a.s.l. Average rainfall up to 716 mm with a clay loam soil type. Geregera, Meket wereda, Amhara Region, is located 11.40° N, 38.52° E at 2876 m.a.s.l. Average rainfall up to 1100 mm, the soil is characterized as brown Lithosol with PH of 6.0. The maximum and minimum temperature is 26 °C and 1 °C, respectively. The relative humidity varies between 100% in July and 10% in May [25,26].

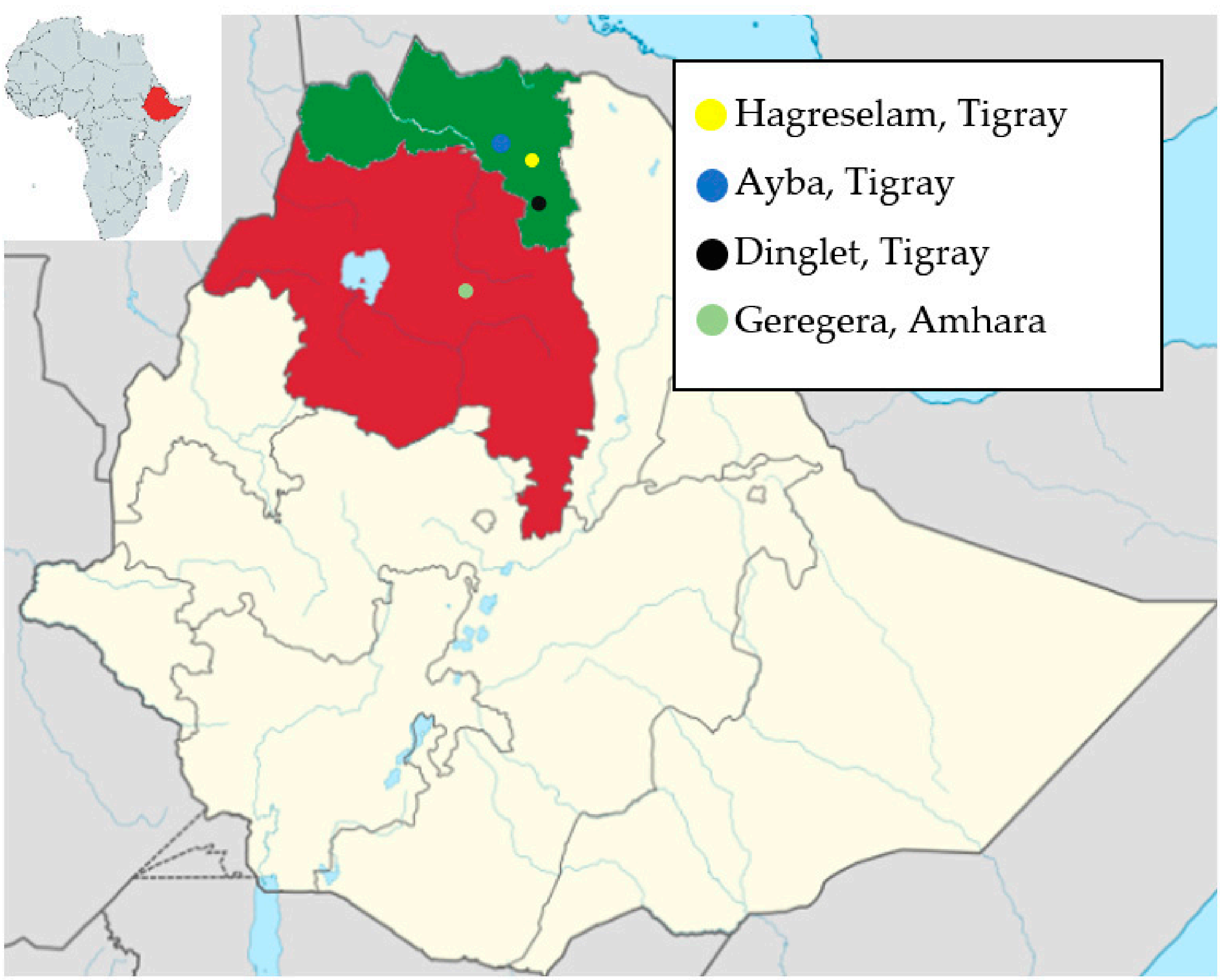

Figure 1. Map of Ethiopia showing the location of collection sites of durum wheat in the Amhara and Tigray regions.

2.1.2. Durum Wheat Samples

Durum wheat grains were made available by Bioversity International Ethiopia in collaboration with national partners: Mekelle University and Sirinka agricultural research center. These were derived from three sources:

1. small seed multiplication plots (3 m^2)
2. experimental plots (100 m^2) from Ayba research station
3. farmers' field (plot average size of 0.25 ha)

Seeds in the multiplication and experimental plots were grown under fertilizer application of full doses of P (100 kg/ha DAP) and 1/2 of N (50 kg/ha urea) applied during planting while the remaining half-dose of N was applied at the beginning of the tillering stage. After harvesting, approximately 250 g of grains were taken for each variety for this study. Samples from 2017 and 2018 were collected and conserved at room temperature with no special treatment, while 2019 samples were collected immediately after threshing, or manually collected from three different spots in the field, stored in plastic bags and grinded. For each variety the samples analyzed were collected as follows: 1 kg of grains was taken from the harvest bulk, laid on a sheet of paper and distributed in a rectangle, divided it into 8 different parts. Seeds were randomly taken from each part, so as to reach 0.5 kg. After carefully mixing the sub-bulk, 0.250 kg were ground and put into plastic bags. Durum wheat flour was mailed to laboratories of the Italian National Institute of Health,

Department of Food Safety, Nutrition and Veterinary Public Health, and stored at −20 °C until the analysis.

Fifty-six samples for a total of thirty-seven traditional varieties (including repetitions for 9 varieties over site and/or years and/or fields) were analyzed (Table 1): 63% were collected in multiplication plots in Ayba (2017: N = 24) and Dinglet (2017: N = 11); 5% were collected in experimental plots in Ayba (2017: N = 3); 32% were collected in farmers' fields in Geregera (2018: N = 3; 2019 N = 6), Ayba (2019: N = 6) and Hagreselam (2019: N = 3). Table 1 also includes information regarding days to flowering (DF, ranging from 77 to 101), days to maturity (DM, 117–148), and moisture content of mature grains (9.2 to 11.1%) of the varieties tested. These traits were identified as relevant for the association with fungi proliferation and consequent mycotoxin formation: for instance, contamination in early planted wheat crops is often lower than in crops of the same varieties planted later [27–29].

Table 1. Complete list of durum wheat varieties from Ethiopia, sampling locality, year and agromorphological traits of samples tested for mytoxins. Highlighted in grey the varieties analyzed from different sites and/or years.

Variety ID	Site	Year	Seed Source [a]	Sampling [b]	Days to Flowering	Days to Maturity	Moisture Content (%)
5654	Ayba	2017	1	1	93	138	10.1
5679	Ayba	2017	1	1	100	144	10.4
8208	Ayba	2017	1	1	87	136	9.9
8210	Ayba	2017	1	1	N/A	N/A	N/A
206551	Ayba	2017	1	1	88	142	9.6
206556	**Ayba**	**2017**	**1**	**1**	**96**	**148**	**9.3**
	Dinglet	**2017**	**1**	**1**			
208167	Ayba	2017	1	1	93	144	10.4
208228	Ayba	2017	1	1	91	146	10.5
208286	**Ayba**	**2017**	**1**	**1**	**101**	**147**	**10.2**
	Dinglet	**2017**	**1**	**1**			
208304	Ayba	2017	1	1	96	138	10.1
208315	**Ayba**	**2017**	**1**	**1**	**88**	**140**	**10.4**
	Dinglet	**2017**	**1**	**1**			
208317	Ayba	2017	1	1	84	138	11
208332	Dinglet	2017	1	1	N/A	N/A	N/A
208474	Ayba	2017	2	1	87	138	9.4
208479	Ayba	2017	1	1	N/A	N/A	N/A
208873	Ayba	2017	1	1	83	138	10.6
210803	Dinglet	2017	1	1	85	138	10.4
212564	Ayba	2017	1	1	84	142	10
213310	**Ayba**	**2017**	**1**	**1**	**89**	**140**	**11.1**
	Dinglet	**2017**	**1**	**1**	**89**	**140**	**11.1**
214357	**Ayba**	**2017**	**1**	**1**	**92**	**143**	**11**
	Dinglet	**2017**	**1**	**1**			
214571	Ayba	2017	1	1	91	143	9.2
222197	**Ayba**	**2017**	**1**	**1**	**N/A**	**N/A**	**N/A**
	Dinglet	**2017**	**1**	**1**			
222854	**Geregera**	**2018**	**3**	**1**	**86**	**134**	**10.1**
	Geregera	**2019**	**3**	**3**			
	Geregera	**2019**	**3**	**4**			
	Geregera	**2019**	**3**	**4**			
227056	Ayba	2017	1	1	77	119	10.4
227061	Ayba	2017	1	1	91	117	10.7

Table 1. *Cont.*

Variety ID	Site	Year	Seed Source [a]	Sampling [b]	Days to Flowering	Days to Maturity	Moisture Content (%)
234498	Dinglet	2017	1	1	90	143	10.8
236271	Ayba	2017	1	1	95	142	10.5
236282	Ayba	2017	1	1	80	148	10.2
236300	Geregera	2018	3	1	90	141	10.1
238573	**Geregera**	**2019**	**3**	**3**	**85**	**137**	**9.9**
	Geregera	**2019**	**3**	**3**			
	Geregera	**2019**	**3**	**5**			
238576	Geregera	2018	3	1	85	135	10.4
Chiraferas	Hagreselam	2019	3	1			
R2B6P31	Ayba	2017	1	1	N/A	N/A	N/A
Rigeat	**Ayba**	**2017**	**2**	**1**	**N/A**	**N/A**	**N/A**
	Ayba	**2019**	**3**	**1**			
	Ayba	**2019**	**3**	**1**			
	Hagreselam	**2019**	**3**	**1**			
SSD14	Dinglet	2017	1	1	N/A	N/A	N/A
Wehabit	**Ayba**	**2017**	**2**	**1**	**N/A**	**N/A**	**N/A**
	Ayba	**2019**	**3**	**1**			
	Ayba	**2019**	**3**	**1**			
	Ayba	**2019**	**3**	**1**			
	Ayba	**2019**	**3**	**1**			
	Hagreselam	**2019**	**3**	**1**			
Yerer	Dinglet	2017	1	1	87	140	10

[a] Seed source: 1 multiplication plot; 2 experimental plot; 3 farmers' field [b] Sampling method: 1. Stored in plastic bags at room temperature > 12 months; 2. Stored in plastic bags at room temperature < 12 months; 3. Samples collected after threshing; 4. Samples collected from 3 spots in the field; 5. Samples collected form single spot in the field.

Due to security reasons, which made travel within the country unsafe, it was not possible to collect samples from two different years for all varieties, as planned. In fact, the sampling scheme could only be completed for 10 out of the 37 varieties: three variety samples were collected from two years (2017, 2019), and seven were collected from different farmers/sites in the same year (Table 1).

2.2. Groundnut

2.2.1. Description of Ugandan Sites

The study was conducted in two sites in the Central Uganda: Nakaseke and Nakasongola (Figure 2). Nakaseke is located in the Central Wooded Savannah agro-ecological zone (0.72° N, 32.91° E) with an altitudinal range of 1086–1280 m.a.s.l., with an average rainfall of up to 1100 mm and temperature ranging between 16 and 30 Celsius. The soils in Nakaseke are sandy clay loam with low to medium productivity [30]. Nakasongola site is also located in the central plateau of Uganda (1.31° N, 32.46° E) that ranges between 616 and 1157 m.a.s.l. The annual rainfall received varies from 875–1120 mm/annum with two distinct rainy seasons, but it is reported to experience severe drought events. The average temperature ranges between 5 and 30 °C. The most dominant soil types include Gleyic Arenosols, Histosols, and Petric Plinthosols [31,32].

Figure 2. Map of Uganda showing the location of Nakaseke and Nakasongola sites.

2.2.2. Groundnut Samples

The groundnut samples were obtained from the harvests of 2019 and 2020. The 2019 samples were obtained from two Community Seed Banks (CSB) in Nakaseke and Nakasongola, while the 2020 samples were obtained from the Nakaseke CSB and from a local market in Kampala. Community seed banks are collective-action institutions locally governed and managed, whose core function is to collect, store, maintain, and distribute seeds for local use. The seeds maintained in the CSBs include commercial varieties, but they are mainly from traditional varieties, adapted to local environmental conditions, produced by members who at the end of the planting season deposit a given amount of seeds produced in their fields. CSB also include commercial varieties. The number of participants depositing their seeds in the CSB responds to the random sampling criteria necessary to guarantee representativity of the sample and avoid bias due to seed selection from a single source. All members receive training in good management practices and all CSBs have a quality committee who checks on the quality of the seeds. Storage facilities are basic, seeds are stored in sisal sacks of the capacity of approximately 100 kg, but precautions to prevent spreading of seed-borne diseases are taken. The average size of a farmer's field is 0.40 ha. Ugandan farmers adopt crop rotation with sweet potatoes, vegetables, cassava, beans, and maize, but rotation crops as well as the plot size are not systematic. Land

preparation is usually done manually in Nakaseke using hoes, while in Nakasongola, oxen are used as well as hoes. Farmers do not treat the fields before planting. The samples used for the study were grown without applying pesticides. Approximately 200 g of seeds were taken randomly from different parts of the sisal sacks, ground and stored in plastic bags in the CSB. Groundnut flour was taken to the Italian National Institute of Health, Department of Food Safety, Nutrition and Veterinary Public Health, and stored at −20° until the analyses.

Sixty samples for a total of thirty-one varieties (Table 2) were collected and analyzed over two consecutive years. In 2019, groundnut samples were collected from the Community Seed Banks (CSB) in Nakaseke (N = 18) and Nakasongola (N = 5); in 2020, samples for 24 varieties were collected from the Nakaseke CSB. Overall, the varieties analyzed were landraces (N = 12), modern (N = 15) and 5 unknown ones. Analyses of some varieties were repeated over time in the same site (12 samples from Nakaseke collected in 2019 and 2020) and over sites and time (N = 3). Additionally, in 2020, 13 samples were bought in Abaita Aababiri (N = 6) and Entebbe Central (N = 7) markets in Entebbe. For these it was not possible to have the names of the varieties, as in the market no differences were made.

Table 2. List of groundnut varieties from two sites in Uganda tested for aflatoxin contamination. Highlighted in grey the varieties analyzed from different site and/or year. Seed source: Nakaseke Community Seed Banks, Nakasongola Community Seed Banks, Abaita Aababiri market, Entebbe Central market in Entebbe, Uganda.

Groundnut Variety Name	Modern/Landrace	Seed Source	Year
Amuria	Unknown	Nakaseke	2020
Black	Unknown	Nakasongola	2019
Dok red	Landrace	Nakaseke	2020
Dok Tan	**Landrace**	**Nakaseke**	**2019**
			2020
Egoromoit	**Landrace**	**Nakaseke**	**2020**
		Nakasongola	**2019**
Emoi red Beauty	Landrace	Nakaseke	2019
Emoi red	Landrace	Nakaseke	2020
Etesoti	**Landrace**	**Nakaseke**	**2019**
			2020
India	**Unknown**	**Nakaseke**	**2019**
			2020
Kabonge red	Landrace	Nakaseke	2019
Kabonge white	**Landrace**	**Nakaseke**	**2019**
			2020
Kagoogwa Omutono	Unknown	Nakaseke	2019
Kawanda	**Landrace**	**Nakaseke**	**2019**
			2020
Mudugavu	Landrace	Nakaseke	2020
Ogwara	**Landrace**	**Nakaseke**	**2019**
			2020
		Nakasongola	**2019**

Table 2. *Cont.*

Groundnut Variety Name	Modern/Landrace	Seed Source	Year
Otira	**Landrace**	**Nakaseke**	**2019**
			2020
Serenut 2	**Modern**	**Nakaseke**	**2019**
			2020
Serenut 4	Modern	Nakaseke	2019
Serenut 5	**Modern**	**Nakaseke**	**2019**
			2020
Serenut 6 Tan	Modern	Nakaseke	2020
Serenut 7	Modern	Nakaseke	2020
Serenut 8	Modern	Nakaseke	2020
Serenut 9	Modern	Nakaseke	2020
Serenut 10	**Modern**	**Nakaseke**	**2019**
			2020
Serenut 11	Modern	Nakaseke	2020
Serenut 11 Tan	**Modern**	**Nakaseke**	**2019**
			2020
		Nakasongola	**2019**
Serenut 12	Modern	Nakaseke	2020
Serenut 14	**Modern**	**Nakaseke**	**2019**
			2020
Serenut 14 red	Modern	Nakaseke	2019
Tendo	**Modern**	**Nakaseke**	**2019**
			2020
Unknown	Unknown	Nakasongola	2019
Abaita Aababiri market	Unknown	Entebbe market	2020
Entebbe Central market	Unknown	Entebbe market	2020

2.3. Analytical Methods

2.3.1. Determination of DON, 3AcDON, 15 AcDON, DON 3G, NIV, T-2/HT-2, and OTA in Durum Wheat Flour by LC-MS/MS

Approximately two grams from each ground durum wheat flour sample was weighed in a 50 mL test tube and initially mixed with 2 mL of water. After addition of 8 mL of extraction solution (CH_3CN:H_2O:HCOOH 79:20:1, *v*/*v*/*v*) the test tube was quickly vortexed and placed for 45 min in a tumbler. After this time, 2 g of dispersive sample mixture DisQuE (Waters) was added in the test tube and thoroughly mixed for 1 min. The tube was then centrifuged at 10,000 rpm for 10 min. Two mL of the organic phase extract was dried under a gentle stream of N_2 and then the sample was recovered with 500 µL of injection solvent (Mobile Phase A and MP B, 50:50 *v*/*v*; MP A: H_2O, 0.3% HCOOH, 5 mM NH_4COOH; MPB MeOH, 0.3% HCOOH, 5 mM di 5 mM NH_4COOH). After filtration through cellulose acetate filter (PTFE, 0.2 µm), a 10 µL injection volume of the filtered sample was directly used for instrumental analysis. Mycotoxin determination was performed by LC-MS/MS. The screening method had been set up and validated for cereals with satisfactory performances [33] by a Waters UPLC system coupled with a Waters

Quattro Premier XE TQ mass spectrometer (Waters, Manchester, UK) equipped with an ESI source operating in positive ionization mode (ESI+). The acquisition was performed in multiple reaction monitoring (MRM). The chromatographic column used was a HSS T3 2.1 × 100 mm, 1.8 µm (Waters). Flow rate was 0.3 mL/min. Limit of quantification (LOQ) for DON, 3-AcDON, 15-AcDON, DON-3G, and NIV at 20 µg/kg, for T-2/HT-2 at 4 and 20 µg/kg respectively, and for OTA at 0.5 µg/kg.

2.3.2. Determination of Aflatoxins in Groundnuts by HPLC-FL

Aflatoxins determination was carried out according to ISO 14123:2008 method, briefly, 50 g from each homogenized grounded groundnut sample was mixed in a blender with a 5 g sodium chloride, 200 mL of extraction solution (MeOH:H_2O, 8:2, *v*/*v*), and 100 mL of n-hexane. After 3 min of high-speed blending, the extract was filtered through Whatman No. 4 paper and 10 mL of filtrated extract was transferred and diluted with phosphate buffered saline (PBS) in a clean conical flask. The diluted sample extract was applied to the conditioned immunoaffinity column (IAC) for the clean-up step. The IAC was washed with 15 mL of water and then the aflatoxins were eluted in a two-step procedure applying 0.50 mL + 0.75 mL of MeOH to the column, then allowed to pass through gravity. The eluate was collected in a 5 mL calibrated volumetric flask and filled to the mark with water. After shaking and adjusting to the given volume, the clear solution was used directly for HPLC analysis. The quantitative method was satisfactorily verified by using a liquid chromatographic determination carried out by reversed phase HPLC equipped with a spectrofluorometric detector, set to the wavelengths 365 nm for excitation and 450 nm for emission (JascoCorporation, Tokyo, Japan). Aflatoxins were separated with a C18 column (Symmetry 250 cm, 4.6 mm, 5 mm, Waters) and the mobile phase, set at a flow rate of 1 mL/min, consisted of a mixture of H_2O:AcCN:MeOH (54:17:29, *v*/*v*/*v*). Aflatoxin post column derivatization was performed with a 0.005% aqueous solution of Pyridine Hydrobromide Perbromide (PBPB) by using a post-column LC pump (zero-dead volume T-piece, reaction tubing minimum 450 × 0.5 mm id in PTFE) (LC pump Labflow 2000, Labservice Analytica, Bologna, Italy) at a flow rate of 0.4 mL/min. LOQ for AFB1 and AFG1 was 0.08 µg/kg and AFB2 and AFG2 0.05 µg/kg.

3. Results and Discussion

3.1. Mycotoxin Detection in Durum Wheat from Ethiopia

Cereal production constitutes the largest sub-sector in the Ethiopian economy. According to the Ethiopian Central Statistical Agency [34] records, during the 2017/18 planting season, cereal production represented almost 90% of the country's grain production, with wheat (including both bread and durum wheat) occupying the fourth place in terms of area covered [35], after maize, teff, and sorghum. Ethiopia is the largest producer of durum wheat in sub-Saharan Africa, planted on approximately 0.6 million hectares and accounting for 40% of wheat production [36]. Wheat in Ethiopia is mostly cultivated in the highlands by smallholder farmers under rainfed conditions, and is mainly grown for self-consumption, with only 20–30% of the production sold through the market [36,37]. This pilot study represents the first assessment of mycotoxin contamination in traditional varieties cultivated by smallholder farmers.

The multi-mycotoxin screening carried out on the 56 samples to assess the presence of deoxynivalenol (DON) and metabolites (3AcDON, 15 AcDON, DON 3Glucoside), Nivalenol (NIV), T-2 and HT-2 toxins and ochratoxin A (OTA) revealed lack of contaminants (<LOQ) in all analyzed samples. Notably, the result is confirmed on samples of the same varieties harvested from different farmers in the same year (Rigeat, Wehabit, 222854, 238573), or different years and locations (Rigeat and Wehabit).

The few studies so far published on mycotoxins contamination in durum wheat in Ethiopia (Table 3) reported scarce mycotoxin contamination [38,39], although reports of mycotoxins in food and food commodities [40] and of the fungi responsible for the production of mycotoxins [41] have recently raised a concern. Ayalew et al. [38] report a

low presence of DON, OTA, and NIV contaminants in durum wheat samples from Central and South Ethiopia. Worku et al. [39] report medium to low incidence of contamination for OTA (20.1%) and DON (9.5%), with a contamination range of 2.5–148 µg/kg, in samples from six wheat-growing districts from four regions of the country (Amhara, Oromia, Southern Nations Nationalities and Peoples Region (SNNP), and Tigray).

Table 3. Occurrence of DON, OTA, and NIV reported in past studies in Ethiopia in durum wheat.

	N. of Samples	Incidence	Range (µg/kg)	Study
DON	179	9.5%	0.35–1.14	Worku et al., 2017 [39]
OTA A	179	20.1%	2.5–148.8	
DON	23	14.3%	0.05–0.11	Ayalew et al., 2006 [38]
OTA A	107	23.4%	19.6 (average)	
NIV	23	4.3%	0.04	

Within the limitation of this study, related to the number of samples analyzed and the design of the research (one-year evaluation for the majority of samples) the total absence of mycotoxins in the varieties tested is an interesting result that deserves further attention. Even if not tested in this study, we can argue that the agroclimatic conditions are not conducive for the proliferation of these fungi. In fact, many other factors and specific climatic conditions may have contributed to reducing fungi proliferation: (i) altitude (>2500 m.a.s.l.), (ii) temperature (15–25 °C), (iii) precipitation (rainfall concentrated in the first two months after sowing (200–300 mm) in the months of July and August and drastically reduced from flowering to harvest -between 0–80 mm), and (iv) exposure to moderate/strong winds (contributing to humidity reduction). Moreover, the agronomic practices adopted in all four sites follow the recommendations to reduce mycotoxin proliferation [42], including tillage of the land (20–30 cm deep), management of residues, rotation in the fields with crops other than those that share the same disease responsible for the proliferation of toxigenic fungi (e.g., barley, maize, or sorghum). Indeed, the adoption of good management practices has been demonstrated to be effective in the control of deoxynivalenol contamination [9] and their effect is stronger when more practices are applied simultaneously. Furthermore, the non-intensive production system may have had a role in preventing the contamination.

3.2. Mycotoxin Detection in Groundnuts from Uganda

Groundnut is the second most important legume crop after common bean produced in Uganda, planted over an area of approximately 420,000 ha over the past 10 years [35] and produced mainly by smallholder farmers under low-input management practices [43]. Groundnuts are an important cash crop for domestic market, consumed in several different ways both raw and cooked, and a significant source of calories and nutrients in the country. However, this important staple crop is prone to aflatoxin contamination, which can occur in the field during postharvest, drying and storage, and transportation. Studies on mycotoxin occurrence in groundnut in Uganda concentrate on post-harvest contamination. Only recently, exposure studies have been carried out to examine the levels of aflatoxin in people living in rural areas [44].

The 60 samples collected in 2019 (N = 23) and 2020 (N = 37) were tested for AFB1, AFB2, AFG1, and AFG2, and results are reported in Table 4. In general, very low levels were detected in both years and sites, with the exception of two samples from Entebbe Central market where alarming contents were detected (456.04 µg/kg for AFB1, 13.56 µg/kg for AFB2, 224.83 µg/kg for AFG1). In 2019, all samples originated from the Community Seed Banks; of the 23 samples analyzed only 22% (N = 5) reported at least one of the four aflatoxins at low levels, as follows: 17% (N = 4) were positive for AFB1 (range 0.08–0.80 µg/kg); 9% (N = 2) for AFB2 (range 0.06–0.13 µg/kg); 17% (N = 4) for AFG1 (range 0.08–0.94 µg/kg); 0% for AFG2. In 2020 samples (N = 37) analyzed originated both from the Community Seed Banks and the two markets in Entebbe. Of the 37 samples analyzed, 41% (N = 15) of the varieties reported at least one of the four contaminants at

low levels, as follows: 35% (N = 13) were positive for AFB1 (range 0.14–456.04 µg/kg); 11% (N = 4) for AFB2 (range 0.06–13.56 µg/kg); 32% (*n* = 12) for AFG1 (range 0.09–224.83 µg/kg); 5% (N = 2) for AFG2 (range 1.06–7.29 µg/kg). The contamination values detected in 2020 were slightly lower than those detected in 2019 (≤0.50 µg/kg). With the exclusion of the two highly contaminated samples, the general low values of aflatoxin contamination in samples, both from the Community Seed Banks and from the markets, are somewhat unexpected.

Table 4. Levels of aflatoxin contamination in groundnut varieties collected in Uganda in 2019 and 2020. LOD = contamination ≤ the Level of Detection (i.e., 0.02 µg/kg for AFB1 and AFG1; 0.01 µg/kg for AFB2 and AFG2). LOQ = contamination above LOD and below the Level of Quantification (i.e., 0.08 µg/kg for AFB1 and AFG1; 0.05 µg/kg for AFB2 and AFG2). (-) = samples not available. * 2019 samples from Nakasongola, 2020 samples from Nakaseke; [1] = samples from Nakaseke, [2] = samples from Nakasongola.

Groundnut Variety Name	2019 *n* = 23 Samples				2020 *n* = 37 Samples			
	AFB1 µg/kg	AFB2 µg/kg	AFG1 µg/kg	AFG2 µg/kg	AFB1 µg/kg	AFB2 µg/kg	AFG1 µg/kg	AFG2 µg/kg
Amuria	-	-	-	-	**0.17**	≤LOD	**0.13**	≤LOD
Black	**0.80**	**0.13**	**0.94**	<LOQ	-	-	-	-
Dok red	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
Dok Tan	**0.08**	<LOQ	≤LOD	<LOQ	**0.14**	≤LOD	**0.14**	≤LOD
Egolomoit *	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
Emoi red Beauty	≤LOD	≤LOD	≤LOD	≤LOD	-	-	-	-
Etemoit	-	-	-	-	**0.14**	≤LOD	≤LOD	≤LOD
Etesoti	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
India	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
Kabonge red	≤LOD	≤LOD	≤LOD	≤LOD	-	-	-	-
Kabonge white	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	**0.11**	≤LOD
Kagoogwa Omutono	≤LOD	≤LOD	≤LOD	≤LOD	-	-	-	-
Kawanda	**0.16**	<LOQ	**0.28**	<LOQ	≤LOD	<LOD	≤LOD	≤LOD
Mubugavu	-	-	-	-	≤LOD	≤LOD	**0.13**	≤LOD
Ongwara [1]	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
Ongwara [2]	≤LOD	≤LOD	≤LOD	≤LOD	-	-	-	-
Otira	≤LOD	≤LOD	**0.08**	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 2	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 4	≤LOD	≤LOD	≤LOD	≤LOD	-	-	-	-
Serenut 5	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 6 Tan	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 7	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 8	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 9	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 10	**0.60**	**0.06**	**0.61**	<LOQ	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 11	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 11 Tan [1]	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 11 Tan [2]	≤LOD	≤LOD	≤LOD	≤LOD	-	-	-	-
Serenut 12	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
Serenut 14	≤LOD	≤LOD	≤LOD	≤LOD	**0.14**	≤LOD	≤LOD	≤LOD
Serenut 14 Red	≤LOD	≤LOD	≤LOD	≤LOD	-	-	-	-
Tendo	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD	≤LOD
Unknown	≤LOD	≤LOD	≤LOD	≤LOD	-	-	-	-
AA market#1	-	-	-	-	**0.17**	≤LOD	≤LOD	≤LOD
AA market#2	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
AA market#3	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD

Table 4. *Cont.*

Groundnut Variety Name	2019 *n* = 23 Samples				2020 *n* = 37 Samples			
	AFB1 µg/kg	AFB2 µg/kg	AFG1 µg/kg	AFG2 µg/kg	AFB1 µg/kg	AFB2 µg/kg	AFG1 µg/kg	AFG2 µg/kg
AA market#4	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
AA market#5	-	-	-	-	**0.50**	**0.06**	**0.18**	<LOQ
AA market#6	-	-	-	-	**0.25**	<LOQ	**0.11**	≤LOD
EC market#1	-	-	-	-	**0.41**	**0.06**	**0.16**	<LOQ
EC market#2	-	-	-	-	**0.19**	<LOQ	**0.09**	≤LOD
EC market#3	-	-	-	-	≤LOD	≤LOD	≤LOD	≤LOD
EC market#4	-	-	-	-	**456.04**	**5.58**	**224.83**	**1.06**
EC market#5	-	-	-	-	**13.19**	**13.56**	**42.39**	**7.29**
EC market#6	-	-	-	-	**0.66**	≤LOD	**1.52**	≤LOD
EC market#7	-	-	-	-	**0.48**	≤LOD	**1.95**	≤LOD

The studies on aflatoxin contamination in groundnuts in Uganda are not numerous, but those that do exist report an occurrence that deserves attention for the presence and for the concentration levels. These are often above the limits established by the East African Community and recognized in Uganda (Ugandan Standard East African Standard 57-1 on groundnut) (Table 5). According to the Partnership for Aflatoxin Control in Africa (PACA), groundnuts represent the third source of aflatoxin contamination in Africa and an analysis conducted in Uganda [45] reports that 20% of the groundnuts from the second largest district for groundnut production in the country (Soroti and Iganga in the Kiogo plains) contain aflatoxin levels above the national maximum level (10 µg/kg). A pilot study conducted by Asiki et al. [44] in rural south-western Uganda to assess the exposure to aflatoxin (aflatoxin-albumin) among the population revealed the presence of relatively high levels of aflatoxins. Of the 100 adults and 96 children included in the study only 4 children were found with undetectable levels of AF albumin adduct in their blood, with 75% of the participants had levels of 7.1 pg/mg albumin. It should be noted that the sources of exposure are multiple and include different foods other than groundnuts.

Table 5. Aflatoxin levels detected in different years and agroecological zones. Values in bold indicate contamination above 10 µg/kg; for PACA 2017 and present work data values in brackets indicate percentage of samples above 10 µg/kg (Ugandan Standard East African Standard 57-1 on groundnut).

Source of Samples	Region	Year of Collection	Groundnut Type of Sample	N of Samples	% of Contaminated Samples	Reference
Kiboyo	E Uganda	2003–2005	grains	25	**80**	Kaaya et al., 2006 [13]
Bugodi	E Uganda	2003–2005	grains	20	**75**	
Gayaza	E Uganda	2003–2005	grains	15	60	
Kabulamuliro	E Uganda	2003–2005	grains	12	67	
SW Uganda		NA	flour	3	**100**	Kitya et al., 2009 [46]
Kampala Markets (5)		2014	grains	20	**60**	Osuret et al., 2016 [47]
		2014	groundnut paste	20	**100**	
Kampala Market		NA	groundnut paste	33	**66**	Baluka et al., 2017 [48]
Mubende	Western Savannah Grasslands	NA	-	-	25 (**40**)	PACA 2017 [45]
Kamwenge		NA	-	-	30 (**33**)	
Masindi		NA	-	-	20 (**50**)	
Iganga	Kioga plains	NA	-	-	40 (**50**)	
Soroti		NA	-	-	60 (**33**)	
Tororo		2017	-	-	20 (**50**)	
Gulu	North Eastern Savannah Grasslands	2017	-	-	20 (0)	
Amuria			-	-	60 (**17**)	
Lira		2017	-	-	20 (**50**)	

Table 5. *Cont.*

Source of Samples	Region	Year of Collection	Groundnut Type of Sample	N of Samples	% of Contaminated Samples	Reference
Nakaseke	Central Wooded Savannah	2019	grains	18	0	Present work
		2020	grains	24	22	
Nakasongola	Kioga plains	2019	grains	5	1	
Entebbe Markets		2020	grains	13	9 (**22**)	

The samples analyzed within the framework of this pilot study were produced by the members of the Community Seed Banks (CSB, 30 farmers), smallholder producers who practice traditional agriculture under rainfed conditions. As already mentioned, all CSB members have been trained in good management practices (not specifically designed to control mycotoxin contamination). The non-intensive production system may have had a role in control of the contamination. It should be highlighted that the low/negative contamination levels have been consistent in all the samples collected in the same site in both years of the study (i.e., 13 varieties from Nakaseke: Dok Tan, Etesoti, India, Kabonge white, Kawanda, Ogwara, Otira, Serenut 2, Serenut 5, Serenut 10, Serenut Tan 11, Serenut 14, Tendo). The same applies to the varieties collected in the same year but from different sites (i.e., Ogwara and Serenut Tan 11). Although a study involving multiple sites over a prolonged period might provide more generalizable information, these results nonetheless underline some important considerations on the impact of the production system on mycotoxin contamination. Given the absence/negligible levels of contamination on the samples, the study does not allow to derive any conclusion on possible differences in susceptibility to the toxigenic fungi, and the consequent mycotoxin production, at varietal level.

What is however surprising is the number of contaminated samples from the market, for which no background information is available (e.g., origin, variety, source, storage system), and the average level of contamination. Except for two samples where alarming contamination levels were found, the remaining 11 showed levels way below the national maximum level (10 μg/kg).

Acur et al. [49] conducted a study on genetic diversity of aflatoxin-producing *Aspergillus flavus* in groundnut from three different agroecological zones in Uganda. Sixty-seven *A. flavus* isolates were identified, and subsequently characterized for the presence of aflatoxin biosynthesis gene cluster. The isolates were divided into two main clusters, a first one comprising aflatoxigenic strains and a second one comprising non-aflatoxigenic isolates. This study reports the existence of atoxigenic strains in Uganda, and the absence/low contamination of the samples collected in Nakaseke and Nakasongola could be also linked to the prevalence of non-toxigenic strains in the area, for which further studies are needed.

4. Conclusions

To our knowledge, this is the first preliminary study assessing mycotoxin contamination at varietal level in durum wheat and groundnut in Africa produced mainly for self-consumption. Multi-mycotoxin analyses on samples deriving from rainfed non-intensive production systems represented by small seed multiplication plots and smallholder plots of durum wheat from Northern Ethiopia, did not reveal any contamination, while low levels of contaminations were detected on groundnuts from Central Uganda, with the exception of two highly contaminated samples.

For durum wheat in Ethiopia, we can hypothesize that climatic conditions (scarce rains and mild temperature during crop cycle and more specifically during flowering and harvest) were not conducive to fungal proliferation and mycotoxin production. The absence of contamination on any of the samples analyzed makes it impossible to reach any conclusion on possible differences in susceptibility to the toxigenic fungi at varietal level and requires further studies. However, the negative results obtained from the samples of

the same varieties harvested from different farmers in the same year and different years and location are encouraging and deserve further investigation.

For groundnut in Uganda, the non-intensive agricultural practices together with the good management practices adopted by the farmers and the presence of non-toxigenic strains may have prevented mycotoxin development. Moreover, in this case, no hypothesis can be formulated regarding differences in susceptibility among groundnut varieties.

Multiple year evaluations of the varieties should be carried out to obtain more evidence on the role of non-intensive production systems in keeping the mycotoxin contamination at minimum levels, and to assess possible differences in susceptibility to fungi at the varietal level. Analyses on samples of the same varieties coming from both intensive and non-intensive production systems should be performed to compare contamination levels. Furthermore, building on the results presented by Acur et al., on the presence of atoxigenic strains, in the case of groundnut, fungal analyses should be performed to identify the fungi strains present in the field and elucidate on which strain niches colonize the soil and host plants to understand whether the low presence of aflatoxins can also be related to the presence of atoxigenic strains of the fungi, competing with the toxigenic strains and acting as a biocontrol.

Understanding the role of the non-intensive production system in reducing contamination levels and assessing differences in susceptibility at varietal level will contribute to improving food safety in those countries that lack of adequate technological advancement, resulting in poor monitoring of the occurrence of mycotoxins. Furthermore, the obtained information may be of relevance for industrialized countries where the sustainability of the productions should be restored and would provide a possible way forward.

Author Contributions: Conceptualization, P.D.S., M.R. and B.D.S.; methodology, P.D.S., M.R. and B.D.S.; formal analysis, G.M., F.D., B.D.S. and R.M.; resources, R.N., D.K.M., Y.G.K. and G.M.; writing—original draft preparation, P.D.S.; writing—review and editing, M.R., B.D.S. and F.D.; funding acquisition, P.D.S. and D.K.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the International Fund for Agricultural Development (IFAD) Grant number 2000001629, “Use of genetic diversity and evolutionary plant breeding for enhanced farmer resilience”, and the CGIAR Research Program on Water, Land and Ecosystems (WLE).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank the site teams and the farmers of Ayba, Dinglet, Geregera, and Hagreselam in Ethiopia and Nakaseke and Nakasongola sites in Uganda for their collaboration.

Conflicts of Interest: The authors declare no conflict of interest.

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